Updates in Dermatology

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Reykjavik, 7th April 1989

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Topical Corticosteroids Today

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Topical Corticosteroids Today: Chairman's Introduction

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We dermatologists are all familiar with corticosteroids and we use topical steroid preparations daily. In fact, we could hardly carry out our daily work without these medicaments. We have come to this point gradually during the last 30-40 years.

The story of topical steroid therapy starts with the observation of Sulzberger in 1952 that the anti-inflammatory activity of hydrocortisone is superior to all other dermatological medicaments available at that time. Since that discovery the hydrocortisone molecule has been modified in several ways and a series of new derivatives have been designed, synthetized, tested experimentally in clinics and finally became available for general use. The potency of the latest preparations can hardly be compared with that of hydrocortisone.

This fast development has been possible due to advances in the synthetic chemistry itself, and to the development of practical assay systems for screening purposes. A decisive step was the invention of the vasoconstrictor assay by Stoughton and McKenzie in the 1960s.

We have learned that, while modifications of the steroid molecule itself are important, the vehicle used to carry the active compound into the skin, is equally decisive. A prerequisite for steroid activity is its transfer into the skin and further to the deeper layers. This depends largely on the characteristics of the vehicle.

In the skin, the exact site of steroid activity is still open for discussion, but very much has been learned during the last years. The apparent anti-inflammatory, immunosuppressive and anti-proliferative actions can now be analyzed at the level of organs but also as a phenomenon in single cells, in cell organelles and finally as chemical events.

Practical clinical experience has taught us in which diseases it is wise to use topical steroids. Similarly it became clear in which disease conditions steroids should not be used. Sometimes, the initial success in treatment may eventually result in disappointments: the preparation may appear to have lost its potency or it may even start to directly irritate the skin condition. A longer use may cause quite undesirable effects.

While we clinicians are working with our patients and are collecting experiences, research on corticosteroids is continuing in laboratories and in various types of research set-ups. It is therefore wise to stop for a moment, to come together and exchange views, experiences and knowledge because we may have to learn from each other. There may be questions which can be answered, and it could also be worthwhile to know which questions can not be answered today. They are valuable in guiding further research efforts.

We have been lucky to obtain excellent speakers for this symposium who have an extensive knowledge and experience in the field. I welcome you all to this symposium on corticosteroids and hope that you enjoy it.

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International Symposium, Reykjavik, April 1989

The History of Corticosteroids

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The development from the discovery of the structure of cholesterol, the precursor of all steroids, to the first application of a topically effective steroid in 1952 took about 20 years. Thereafter the development of more potent topically effective steroids accelerated and the third generation of potent steroids with a topical activity of about 10 to 20 times that of hydrocortisone was already available about 10 years later. Today the newly developed non-fluorinated non-symmetric 16α,17α-acetal derivate budesonide could perhaps be considered as the sixth generation.

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The importance in biology of cholesterol and its homologues, the steroids, cannot be over stressed. Cholesterol-like structures have been discovered in pre-Cambrian shales formed some 2.7 billion years ago and the amazing versatility of this class of compounds was obviously essential to even the most primitive of early life forms. Steroids are components of cell membranes, fat metabolizers, cardioactive poisons, hormones and therapeutic drugs. All have the same steroidal skeleton but differ in the substituents linked to the 17 carbon atoms or in the directions these project into space.

Chevreul, a young chemist in Napoleonic France, discovered the 'non-saponifiable fats' and in 1812 was surprised to identify one such fat from gallstones. This fat he named 'cholesterol'. A hundred years later, Adolf Windaus realised the great biological importance of the steroids, previously almost ignored by chemists. After a further 20 years, in 1932, Windaus discovered the structural formula of cholesterol (Fig. 1), but before that his work on Vitamin D gained him the Nobel Prize.

The adrenal glands had not been discovered until the Renaissance. Anatomical studies had previously been carried out on corpses some time after burial when the delicate adrenals had already partially decomposed. In 1849 the Scot Addison realised the connection between the adrenals and the bronzed

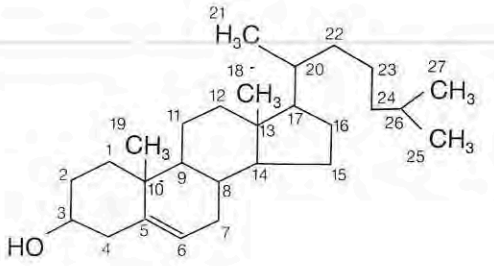


Fig. 1. Structure of cholesterol.

skin disease that was later to bear his name. This relationship was confirmed by Brown Séquard in 1855. On June 1st 1889, at the age of 72, Brown Séquard really put hormones on the map when he declared to an astonished meeting of eminent physicians that his apparent youthfulness was due to testicular extract therapy! In 1932 Thorn successfully demonstrated the activity of his 'cortin' on 2 adrenalectomized cats, winning him the AMA gold medal. In the same year Cushing described his syndrome related to excessive adrenal activity.

Kendall, working for the Mayo Foundation but in conjunction with Parke-Davis, processed 150 tons of adrenal glands between 1934 and 1949. However, Reichstein in Switzerland was at the same time working with a much smaller supply of adrenals from the Dutch company Organon. The rivalry between these 2 scientists was heightened by the rumour that vast supplies of adrenal glands were being shipped from Argentina to war-time Germany. It was concluded that all German pilots were receiving hormone injections. This caused near panic in Allied circles. The fact that it would have been physically impossible to have produced sufficient injectable material escaped those who should have known better.

After World War 2, Kendall, now working with U.S. Merck, had a lucky break. Mistakenly believing that rheumatoid arthritis was an adrenal insufficiency problem, Hench, a clinical friend of Kendall, purchased his entire supply of 'Substance E' in 1948. A miracle appeared to have occurred when a second patient was given 'Substance E' (later named corti-

sone). On Christmas Day the previously despondent patient, a shipping clerk, got out of bed, walked around and started to read again.

Merck set up large scale production of cortisone using bile as a supply of starting materials. Kendall, Hench and Reichstein were awarded the Nobel Prize in 1950 for discovering the hormones of the adrenal cortex, their structure and biological effects.

Thus, the history of the use of corticosteroid therapy is one of barely 40 years duration. Yet, during that period the management of a large number of diseases including skin conditions have been revolutionized.

It is to the Croatian Ružička working in Switzerland that we look for the characteristic, dedicated synthetic steroid chemist. His Nobel Prize was awarded in 1939 for work relating to terpenes and steroids. Russel Maher in the 1940s made frequent forays into Mexico which culminated in a break with Parke-Davis, the setting up of his own 'Laboratorios Hormonas', the production of plant-derived progesterone and the incorporation of a new firm called Syntex.

As previously mentioned, bile was used by Merck as a supply of raw materials but shortage of supply was a real problem. In addition, to produce 50 g of cortisone Upjohn required the adrenals from 2 million pigs. The race was on to find a more reasonable supply route. The breakthrough came from the bacteriologists.

However, cortisone when applied to the skin gave very disappointing results. Hydrocortisone derived from fermentation processes was first used topically by Sulzberger and Witten in 1952 and was indeed an enormous therapeutic breakthrough. Later work on terpenes and squalene led to the discovery of mevalonic acid, a precursor of important biochemical compounds including corticosteroids, by Folkers in 1961. Bloch in the US and Lynen in Munich were awarded the Nobel Prize for their elucidation of steroid biosynthesis and cholesterol metabolism in 1964.

The next step was the amplification of the effect of corticosteroids. Naturally occurring steroids tend to have low oral activity and topical activity is also limited. The relatively water soluble phosphate or succinate esters are generally used for systemic activity as injections or solutions. However, topical activity on the skin is generally poor. Double bonding between the first and second carbon atoms, esterification of corticosteroids at the C-17 or C-21 positions with fatty acids generally increases potency of the steroid. Cyclic acetonides formed at the C-16 and C-17 positions further increase topical activity (Fig. 2). Halogenation (fluoride or chlorine) at the C-6α (Fig. 3) or C-9α positions is also associated with increased potency.

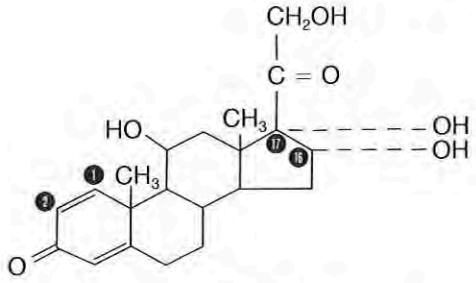


Fig. 2. Structure of 16α-hydroxyprednisolone.

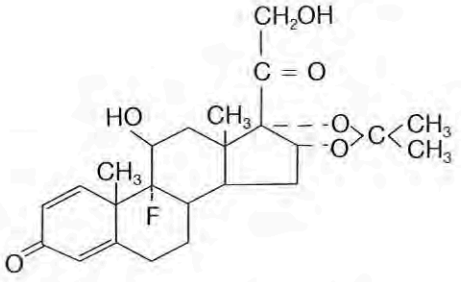


Fig. 3. Structure of triamcinolone acetonide.

Fluorination likewise tends to protect the steroid ring from metabolic conversion.

Triamcinolone acetonide was the first commercially successful of the halogenated topically active steroids. Vickers and Tighe in 1960 found the compound to have specific anti-inflammatory activity in treating psoriasis — a disease previously thought to be unresponsive to steroids. Flurandrenalone and flumethasone were also introduced at this time. Collectively they are often referred to as second generation steroids. They are about 4–6 times as active as hydrocortisone.

Betamethasone 17-valerate (Betnovate®, Glaxo and Celestoderm®, Schering AG) and fluocinolone, the so-called third generation steroids, followed. The latter emphasized the importance of esterification at the C-17 position, betamethasone free base itself being only as active as hydrocortisone. The selection of betamethasone 17-valerate from a variety of possible candidates was made using the Mackenzie-Stoughton vasoconstriction assay described by the pair in 1962.

This test was used to select the strongest steroids for later clinical evaluation. It is a screening technique that has served us well to the present day.

Research workers at Gist-brocades in Delft, The Netherlands, in the late 1960s were investigating the simple C-17 esters of hydrocortisone which finally led to the introduction of hydrocortisone 17-butyrate (Locoid®) (Fig. 4) and hydrocortisone 17-valerate (Westcort®). These esters were developed to mimic the efficacy of the medium potent second generation fluorinated steroids but to minimize the systemic and topical side-effects associated with halogenation. Hydrolysis of the esters, first in the skin and later in the blood to hydrocortisone and the free acid, was the rationale for separation of efficacy and side-effects. Locoid® was introduced in the early 1970s in Europe, later followed by the introduction in the USA. Westcort® registration in the USA preceded the introduction of Locoid®. Polano described these non-fluorinated medium potency steroids as fourth generation steroids.

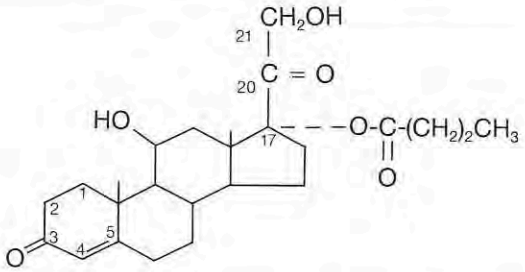


Fig. 4. Structure of hydrocortisone 17α-butyrate (Locoid®).

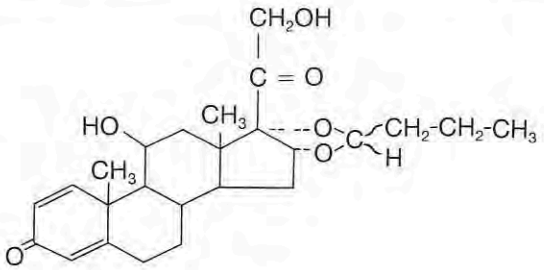


Fig. 5. Structure of budesonide.

Later introductions in the 1970s and early 1980s included the higher potency corticosteroids betamethasone diproprionate (Diprosone®) and clobetasol proprionate (Dermovate®), which can be regarded as fifth generation products. These very potent compounds are enormously valuable in the rapid control of steroid responsive dermatoses, but real risks of adverse reactions, both topical and systemic, accompany their use. Research undertaken in the late 1970s and throughout the eighties in Sweden has culminated in another development — budesonide, a prednisolone molecule with butyric acid side chains at C-16 and C-17. It is a potent, non-fluorinated steroid developed by the Astra group of companies and currently marketed as an inhalant for the treatment of asthma (Fig. 5). A topical formulation, Preferid®, will be introduced in Europe in the spring of 1989. This compound has a high local potency lying between that of clobetasol 17-proprionate and betamethasone-17,21-diproprionate, on vasoconstrictor assay. Systemic activity studies show Preferid® to have similar hypothalamic-pituitary-adrenocortical axis effects as hydrocortisone 17-butyrate. Should we regard this as a 'sixth generation steroid'?

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Topical Corticosteroids: Mechanisms of Action

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Corticosteroids modify the functions of epidermal and dermal cells and of leukocytes participating in proliferative and inflammatory skin diseases. After passage through the cell membrane corticosteroids react with receptor proteins in the cytoplasm to form a steroid-receptor complex. This complex moves into the nucleus, where it binds to DNA. The binding process then changes the transcription of messenger RNA (mRNA). Because mRNA acts as template for protein synthesis, corticosteroids can either stimulate or inhibit the synthesis of specific proteins. Thus corticosteroids are known to stimulate the production of a glycoprotein called lipocortin. The formed lipocortin inhibits the activity of phospholipase A_2, which releases arachidonic acid, the precursor of prostanoids and leukotrienes, from phospholipids. In contrast, corticosteroids inhibit mRNA responsible for interleukin-1 formation. These actions of corticosteroids on arachidonic acid metabolism and interleukin-1 formation produce anti-inflammatory, immunosuppressive and anti-mitogenic effects.

Although this theory based on protein synthesis may not explain all effects of corticosteroids, these examples illustrate that a specific action on the molecular level can explain some of the characteristic and typical pharmacological effects of topically applied corticosteroids. Additional studies of the mechanism of action of corticosteroids are warranted. Such studies will not only help to explain how corticosteroids work, but also create a background that is essential for the development of novel non-steroidal anti-inflammatory drugs.

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The beneficial effects of glucocorticoids in inflammatory and proliferative skin disease are thought to be pharmacological, i.e. they are exerted by concentrations higher than those pertaining physiologically and probably also by different mechanisms.

It is not the purpose of the present paper to review all the physiological and pharmacological properties of glucocorticosteroids. Hence, attention will be confined to those effects that have been suggested as possible mechanisms for their beneficial properties in skin disease. These may be distinguished as those active at the tissue level, those active at the cellular level, and the possible intracellular mechanisms. Some of the problems involved in the interpretation of this type of data will already be evident. For example, an observed phenomenon may not be a primary response, but it may be secondary to a different action at the same or at a different level. Also, specific hormonal properties must be distinguished from the many non-specific (physical) effects which high concentrations of glucocorticosteroids produce on numerous preparations ranging from whole organs to isolated enzymes. Final answers to these problems may never be forthcoming but the attempt to unravel mechanisms will continue to yield useful information.

In the following it will be described how the theory of glucocorticosteroid action based on protein synthesis can explain many of the effects of topically applied glucocorticosteroids although it can not serve as a single unifying concept of glucocorticosteroid actions.

RECEPTOR-BINDING AND TRANSCRIPTION

Glucocorticosteroids differ from other steroid hormones in that they produce effects in almost all tissues and cell types. However, on the molecular level all steroid systems have so many similarities that a general model of steroid action has been developed (Fig. 1). Being relatively small hydrophobic molecules, they cross the plasmamembrane by simple diffusion. Once inside the target cell the steroid binds reversibly to a specific receptor protein present in the cytoplasm. The binding of the hormone causes the receptor protein to undergo an allosteric change in its configuration, which increases its ability to bind to DNA. Because the receptor proteins are able to migrate through the nuclear pores, their increased binding affinity to DNA causes the hormone-receptor

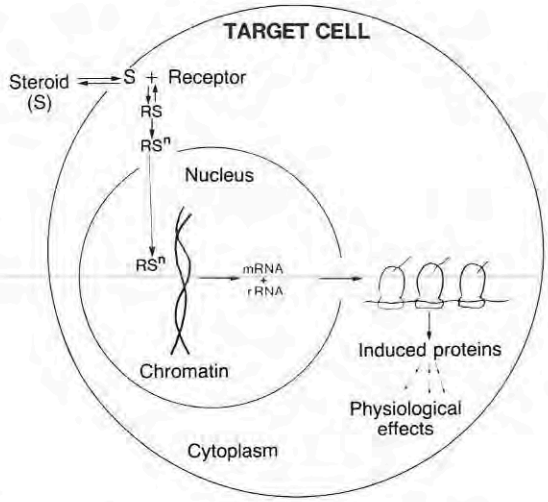


Fig. 1. Schematic drawing of steroid hormone signalling.

complexes to accumulate in the cell nucleus. Some of the activated hormone receptors bound to nuclear DNA can modulate gene transcription. This is the process by which RNA molecules are synthesized using DNA as template (Fig. 1). These RNA molecules then leave the nucleus to serve as mRNA molecules that direct the synthesis of new proteins in the cytoplasm. From each mRNA molecule many thousands of copies of a certain polypeptide chain can be made.

It is important to consider the question whether the specificity of steroid action can be explained by the effect on transcription and translation. It appears that only a small number of genes (i.e. DNA sequences) are directly influenced by steroid hormones. Furthermore, the same hormone-receptor complex is found to influence different sets of genes in different target cells. These characteristics may partially explain why steroids can affect different functions of different tissues and cell types. Finally, the products of these genes may in turn activate other genes and thereby produce a secondary response. The latter may result in a major amplification of the initial hormonal effect.

In this model of steroid action at least three important steps can be distinguished (Fig. 1): the receptor binding, the synthesis of specific mRNA (transcription) and the consequent new protein synthesis (translation). All these steps of steroid action have been demonstrated to be present in the skin. Thus, specific corticosteroid receptors have been identified in normal human epidermis (1) and in dermal fibroblasts (2). For the fibroblasts there is evidence that the steroid binding affinity correlates with the antiproliferative effect of corticosteroids. Thus, the reversible binding of the steroid to its receptor may explain steroid potency.

Although a complete catalogue of steroid-inducible proteins has not been established, the biosynthesis of several important proteins is thought to be regulated by corticosteroids. These proteins include lipocortin, interleukin-1 and lymphokines such as interleukin-2. In the following the effect of steroids on the synthesis of lipocortin and interleukin-1 will be described and it will also be discussed how these effects of steroids may partially explain the therapeutic effect of corticosteroids in skin diseases.

LIPOCORTIN

Lipocortin is a family of proteins that regulates the activity of phospholipase A_2 (3, 4). Phospholipase A_2 is one of the enzymes involved in the release of arachidonic acid from phospholipids. Because arachidonic acid is the precursor of prostaglandins and leukotrienes (all mediators of skin inflammation (5)) the regulation of phospholipase A_2 activity is of key importance in the regulation of inflammatory processes in the skin. Corticosteroids induce the production of lipocortin, which inhibits phospholipase A_2 and which will inhibit the release of arachidonic acid. The time course of this steroid effect is such that 30 minutes after adding hydrocortisone approximately half of the lipocortin is released (3). In contrast to nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit transformation of arachidonic acid into prostaglandins, corticosteroids block the formation of all arachidonate derivatives (eicosanoids), as they restrict the availability of free arachidonic acid. It has been an aim to develop NSAIDs that specifically inhibit lipoxygenase activity and thereby the formation of the potent leukotrienes. At present such compounds are not available for clinical use.

The results described above are all derived from in-vitro studies. It is a pivotal question whether corticosteroids act in this way when they are applied topically. This question was first considered by Hammarström et al. (6) who applied the potent steroid diflorasone diacetate 0.05% in a cream vehicle under occlusion for 24 hours to a psoriatic plaque. It was found that this steroid reduced the increased concentration of arachidonic acid in the psoriatic plaque. This find-

ing may be a molecular correlate of the therapeutic effect of steroids, which usually becomes visible after about 1 week.

Hammarström et al. speculated that the reduction of arachidonate concentration was secondary to modulation of phospholipase A_2 activity (6). This hypothesis was later confirmed by Norris et al. (7). They demonstrated that topical application of betamethasone valerate 0.1% to psoriatic lesions resulted in a reduction of the increased phospholipase A_2 activity within a few hours. These experimental results clearly demonstrate that corticosteroids directly affect the release of pro-inflammatory eicosanoids and suggest that this effect may be an important molecular mechanism for the therapeutic efficacy of steroids in psoriasis and similar diseases. It is, however, important to realize that steroids may also exert their effects by different mechanisms. Thus, steroids may alter effector cell sensitivity to leukotrienes (8).

INTERLEUKIN-1

Interleukin-1 is a family of polypeptides that mediates a wide variety of inflammatory responses. Interleukin-1 is present in the epidermis and can be released after for example ultraviolet irradiation. There is evidence that interleukin-1 is synthesized *in situ*, because it has been shown that cultured keratinocytes contain mRNA necessary for interleukin-1 biosynthesis (9).

When human keratinocytes are exposed to hydrocortisone in vitro, the release of interleukin-1 is reduced (10). This effect on interleukin-1 release involves both constitutive and ultraviolet-elicited interleukin-1 release. This reduction of interleukin-1 release might be secondary to reduced biosynthesis (gene transcription) or to inhibition of the transport of interleukin-1. These possibilities were studied by the same group of investigators who found that the reduced interleukin-1 release was caused by reduced transcription of the interleukin-1 gene by hydrocortisone (11). It is, therefore, possible that the ability of steroids to block interleukin-1 biosynthesis may account for some of the anti-inflammatory and antiproliferative effects of steroids.

The effect of steroids on isolated mediator systems have been described. It is, however, important to realize that the mediators work in concert. This is illustrated schematically in Fig. 2. In addition to arachidonic acid, lipocortin regulates the formation of platelet-activating factor, another phospholipid

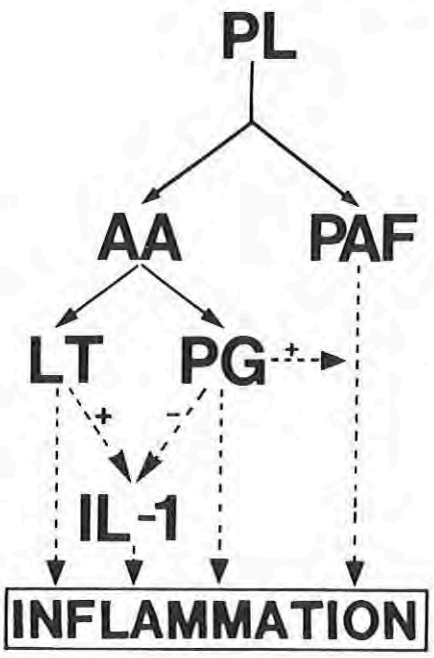


Fig. 2. Interaction between inflammatory mediators. PL = phospholipase, AA = arachidonic acid, PAF = platelet-activating factor, LT = leukotriene B_4, PG = prostaglandin E_2, IL-1 = interleukin-1.

product. Prostaglandin E_2 and leukotriene B_4 have opposite effects on interleukin-1 release, and prostaglandin E_2 can potentiate the inflammatory response to platelet-activating factor. Therefore, it becomes clear that the net effect of steroids on the inflammatory response is not a simple addition of the effects on the isolated mediator systems.

IMMUNE FUNCTION

While the potent effects of glucocorticosteroids on immune function are well recognized, the mechanisms of action of these drugs are unclear.

Glucocorticosteroids have been reported to affect B- and T-lymphocytes and monocytes/macrophages. Early reports on the effect of glucocorticoids on the immune system suggested that their major effect is on the presentation of antigen rather than antibody production. Later it was found that topical administration of various glucocorticosteroids to guinea pig skin caused a marked decrease in the number of detectable epidermal Langerhans cells (12). The mechanisms by which the density of Langerhans cells is

diminished is not clear. It appears that Langerhans cells may be composed of a heterogeneous population in terms of sensitivity to glucocorticosteroids, and that glucocorticosteroids may completely abolish the expression of surface Ia antigen on a subpopulation of Langerhans cells or that they may be cytolytic to this sensitive population (13).

Several types of T-lymphocyte activities are inhibited by glucocorticosteroids. These include interleukin-2 production and mitogen-induced T-cell proliferation. Interestingly these effects of glucocorticosteroids are reversed by leukotriene B_4 (14). These findings support the concept that glucocorticosteroids inhibit T-cells via phospholipase A_2 inhibition.

CONCLUSIONS

From observations made in a variety of experimental systems, a model of glucocorticosteroid action has emerged. The essential features of this model apply for all of the steroid hormones. It proposes that steroids act through modification of gene expression via changes in protein transcription and translation. Examples of key proteins regulated by glucocorticosteroids are lipocortin and interleukin-1. Because these two mediator systems can induce inflammation, these effects of glucocorticosteroids may partially explain their anti-inflammatory properties.

It should, however, be emphasized that the model of steroid hormone action presented here can not explain all effects of glucocorticosteroids. Further studies are, therefore, needed to unravel the pleiotropic nature of steroid action. The results of such studies may not only lay the foundation for the development of new and improved glucocorticosteroids, but also for the design of non-steroidal drugs with anti-inflammatory properties.

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Development of Glucocorticosteroids with Enhanced Ratio between Topical and Systemic Effects

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A high potency at the application site and a low incidence of glucocorticoid side-effects form the desired profile of glucocorticosteroids for anti-inflammatory therapy. A new type of glucocorticosteroid 16,17-acetals with an improved topical/systemic activity ratio has been developed. Non-symmetric 16,17-acetal substitution increased the topical anti-inflammatory activity more than the systemic activity in rodents, whereas fluorine substitution in 9α- or 6α,9α-positions increased the systemic more than the topical potency. The non-fluorinated, non-symmetric 16α,17α-acetal budesonide reached the highest ratio, which was five to ten times better than that of the earlier known 16,17-acetonides such as triamcinolone acetonide, or that of the 17α-esters such as beclomethasone 17α,21-dipropionate.

Although budesonide and betamethasone 17α,21-dipropionate have the same topical anti-inflammatory potency, the latter decreased plasma and urinary cortisol levels significantly more when ointment preparations were compared in volunteers.

Budesonide is efficiently biotransformed in the liver to metabolites such as 6β-hydroxybudesonide and 16α-hydroxyprednisolone, which are 50–100 times less potent than the parent steroid. In homogenates of rat or human adult livers budesonide is biotransformed two to five times more rapidly than desonide and triamcinolone acetonide.

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Since their introduction 40 years ago glucocorticosteroids have been the most efficacious drugs for relieving inflammation. The endogenous hydrocortisone (Fig. 1) was the first to be used for treatment of skin diseases (1). The disappointment arising from the observation of severe adverse effects on long-term therapy of rheumatoid arthritis with cortisone stimulated structure-activity studies of glucocorticosteroids to separate anti-inflammatory potency from adverse effects.

Early research was concentrated on the improvement of the systemic anti-inflammatory activity and the lowering of unwanted side-effects such as sodium retention. The systemic activity of hydrocortisone was enhanced by insertion of an extra double bond in the 1,2-position or by introduction of a fluorine atom in the 9α-position (Fig. 1) resulting in prednisolone and 9α-fluorohydrocortisone, respectively. The latter substitution resulted in the highest potentiation but this was accompanied by a disturbance of the electrolyte balance. However, this was effectively counteracted by further substitution at the 16-position with an α-hydroxy, α-methyl or β-methyl group resulting in triamcinolone, dexamethasone and betamethasone, respectively (2). These studies gave no clear indication on separation of anti-inflammatory activity from adverse effects other than the disturbance of electrolyte balance.

Adrenal suppression, osteoporosis and decreased resistance to infection continued to be a problem. One way of minimizing adverse effects involves the topical application of steroids to the inflamed tissue, thus allowing smaller systemic concentrations of the hormone. The skin is perhaps the easiest target for such a therapy.

To make systemically potent glucocorticosteroids suitable for topical use on the skin, their lipophilicity must be increased. This is achieved by masking one or more of the hydrophilic hydroxyl groups in the 16α-, 17α- or 21-positions with a lipophilic group (3). Examples of such structural changes are the modifications of triamcinolone to triamcinolone acetonide and hydrocortisone to hydrocortisone 17α-butyrate (Locoid®) (Fig. 1). There are two types of parent glucocorticosteroids which can be modified for topical use, namely 16-methyl and 16α-hydroxy steroids such as dexamethasone, betamethasone and triamcinolone. The 16-methyl steroids are made lipophilic by esterification of the 17α- or the 17α- simultaneously with the 21-hydroxy groups (Fig. 1), e.g. betamethasone 17α-valerate (Betnovate®) and betamethasone 17α,21-dipropionate (Diproderm®) (3). The 16α-hydroxy steroids are made topically active by masking the 16α- and 17α-hydroxy groups to form a 16,17-acetonide, e.g. desonide, triamcinolone aceto-

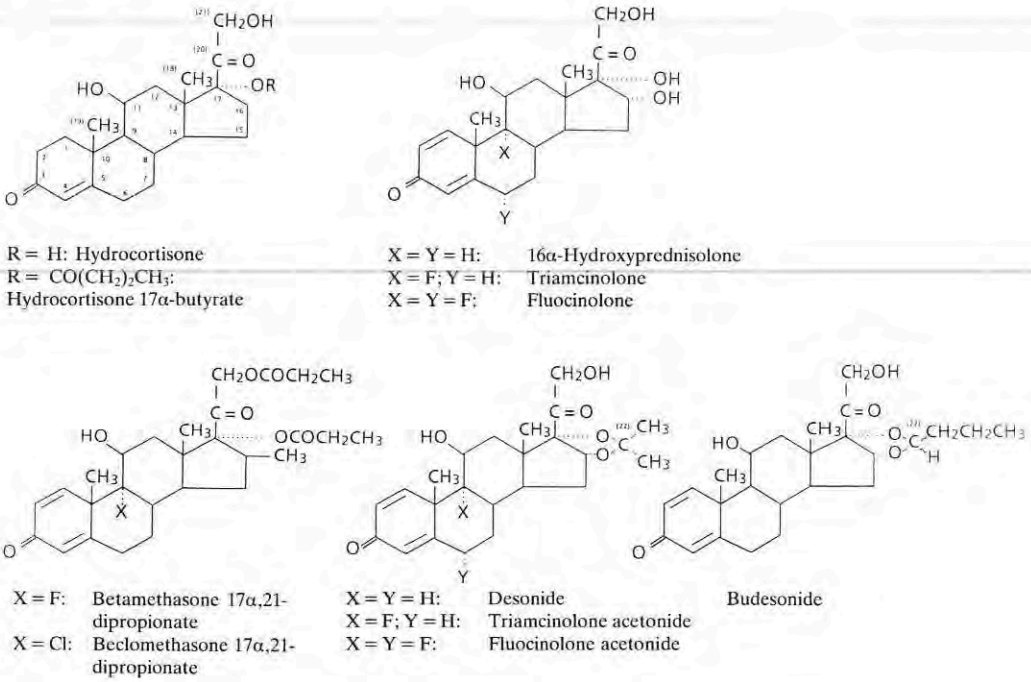


Fig. 1. Structures and generic names of the glucocorticosteroids discussed.

nide and fluocinolone acetonide (Fig. 1) (3).

The aim of the present investigation was to study how modifications in the structure of the 16,17-acetonide type of topical glucocorticosteroids would influence their effects and pharmacokinetics.

MATERIALS AND METHODS

Glucocorticosteroids

The structures and generic names of the 16α,17α-acetal glucocorticosteroids are shown in Fig. 1 and Table I. Two types of acetals were compared; the currently used 16,17-acetonides (acetal type A) and the new non-symmetric 16,17-acetals (acetal type B) (3–5).

Animals

The animal experiments were performed on young male Sprague Dawley rats weighing 90–100 g or male NMRI-mice weighing 20–25 g.

Human volunteers

Tests to determine the topical skin blanching potency of glucocorticosteroids were performed on the volar side of the arm.

The assessment of topical anti-inflammatory potency

The topical anti-inflammatory activity was measured as the potency to inhibit ear oedema formation in rats or mice using a model (6) which is a modification of the 'Tonelli test' (7). The glucocorticosteroids were applied topically as acetone solutions (20 μl/ear side in rats and 10 μl/ear side in mice) 16 hours before oedema induction. The topical 'blanching' potency was determined after the application of ethanol solutions of glucocorticosteroids on human skin. After evaporation of the solvent, the area was occluded under plastic wrapping for 18 hours (8).

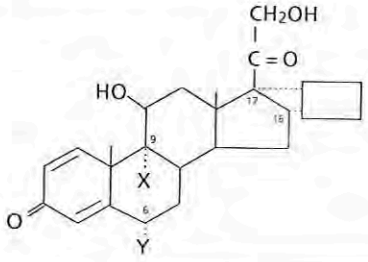
The assessment of systemic glucocorticoid potency

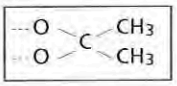
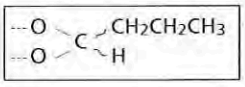
The systemic effects were assessed from the extent of thymus involution 4 days after epicutaneous application of the glucocorticosteroids in rats and 3 days after application in mice (6).

Relative binding affinity (RBA) for the glucocorticosteroid receptor

The binding affinity of the studied steroids for the glucocorticosteroid receptor of rat skeletal muscle was determined in relation to the affinity of dexamethasone (9).

Table I. *Topical anti-inflammatory activity measured as potency to inhibit rat ear oedema and systemic activity as thymus involution after epicutaneous application to rats. The activities are expressed in potency relative to budesonide. The ratio topical/systemic activity is calculated as the quotient between the relative potencies.*



Fluorine substitution in the 6- and 9-positions		Acetal in the 16,17-position Type A 		Type B 			
X	Y	Topical activity	Systemic activity	Topical activity	Systemic activity	Topical/ systemic activity	Generic name
H	H			1	1	1	Budesonide
F	H			2.2	2.5	0.88	S-1298
F	F			2.5	5.4	0.46	S-1314
H	H	0.2	2.2			0.11	Desonide
F	H	0.3	5.3			0.05	Triamcinolone acetonide
F	F	0.7	8.5			0.08	Fluocinolone acetonide

Biotransformation

The biotransformation has been studied extensively in vitro by incubation of tritium labelled glucocorticosteroids with the 9,000 g supernatant fraction of skin and liver from rat and man (10).

Effect on the hypothalamic-pituitary-adrenocortical (HPA) axis

Plasma and urine cortisol levels were assessed after treatment of volunteers on three consecutive nights with ointment preparations of Locoid® 0.1%, Diproderm® 0.05% and Preferid® 0.025%, the latter two equipotent preparations (11).

RESULTS

Topical anti-inflammatory potencies

The inhibition of ear oedema in rats by the 16α,17α-acetal substituted glucocorticosteroids is shown in Table I. The results indicate that it is more important to change the acetal substituent (from type A to type B) than to introduce fluorine atoms in the 6- and 9-positions in the steroid ring skeleton to achieve high topical anti-inflammatory potency.

The type B acetals were at least five times more potent than the corresponding type A compounds in inducing skin blanching and the change of the acetal type was more important than fluorine substitution in the steroid ring skeleton. Accordingly, there was a close correlation between the results obtained in the rat ear oedema model and the skin blanching test (6).

RBA for the glucocorticosteroid receptor

The RBA of the type B 16,17-acetal budesonide for the glucocorticosteroid receptor is approximately two, eight and 200 times higher than that of triamcinolone acetonide, dexamethasone and hydrocortisone, respectively (9). The correlation between RBA and topical anti-inflammatory potency in the rat ear oedema model (Fig. 2) is close, supporting the view that the topical activity at the application site is greatly dependent on the affinity for the glucocorticoster-

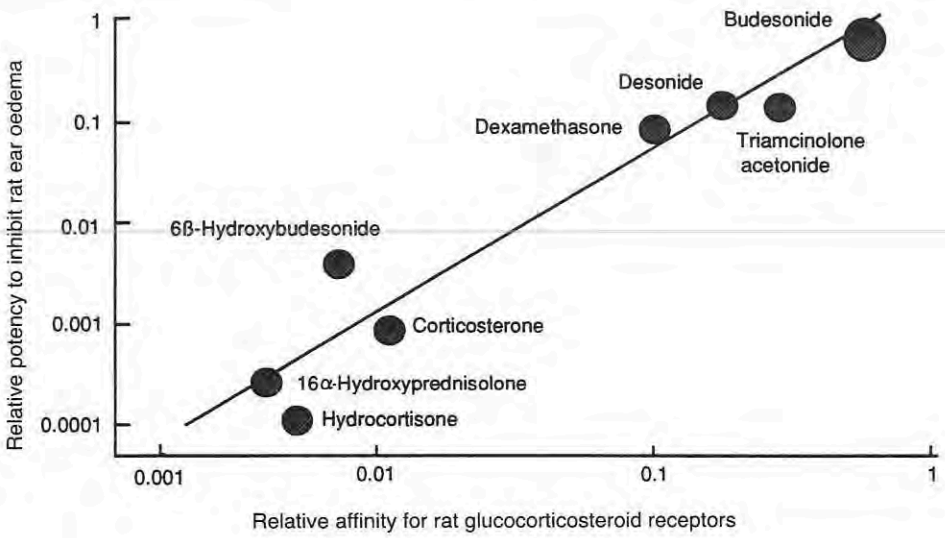


Fig. 2. Correlation between RBA for rat glucocorticosteroid receptors (determined in vitro) and topical anti-inflammatory potency in the rat ear oedema model. (Adapted from (9).)

oid receptor. On the other hand, the correlation between RBA and the thymus involution is low (9). This indicates that the pharmacokinetic properties of the individual glucocorticosteroids also contribute to the systemic effects.

Systemic glucocorticoid potency in rats

By increasing the topical doses it was also possible to induce systemic effects. The type B acetals had the same or lower systemic potencies than the corresponding type A glucocorticosteroids (Table I). Ring skeleton fluorination greatly enhanced systemic potency. For example, fluocinolone acetonide has eight to nine times the systemic activity of budesonide.

The relation between topical anti-inflammatory and systemic potencies in rats

The selectivity for topical activity can be expressed as a ratio between the potencies of topical and systemic activity (Table I). The new type B acetals attain approximately a tenfold better ratio than the earlier available 16α,17α-acetonides (type A). The highest ratio was reached for budesonide which accordingly is a more selective glucocorticosteroid for topical therapy than the 16α,17α-acetonides desonide, triamcinolone acetonide and fluocinolone acetonide (Table I).

Table II. *Potency of some glucocorticosteroids used in skin therapy compared with budesonide to induce skin blanching in man (8).*

Compound	ED_{50} (μg/ml)	Relative potency
Budesonide	0.9 (0.6– 1.2)	1
Betamethasone 17α,21-dipropionate	0.9 (0.6– 1.3)	1
Betamethasone 17α-valerate	1.8 (1.3– 2.5)	0.5
Fluocinolone acetonide	2.2 (1.5– 3.3)	0.4
Hydrocortisone 17α-butyrate	7.8 (4.8–12.3)	0.1
Desonide	10.2 (6.7–15.6)	0.1

Topical selectivity of budesonide in relation to the 16β-methyl 17α- and 17α,21-ester steroids

The topical selectivity of budesonide has been compared with those of betamethasone 17α-valerate and beclomethasone 17α,21-dipropionate using similar methods as described above. Assessment was carried out in mice as the latter two steroids had no topical anti-inflammatory effects in rats (12). Betamethasone 17α-valerate (Betnovate®) and beclomethasone 17α,21-dipropionate represent the other main type of topically active glucocorticosteroids (3). Beclomethasone 17α,21-dipropionate is the analog of betamethasone 17α,21-dipropionate (Diproderm®) in which the 9α-fluorine atom has been replaced by a 9α-chlorine atom (Fig. 1). Beclomethasone 17α,21-dipropionate (Becotide®) has been used preferably in asthma and rhinitis therapy. The activity of betamethasone 17α-valerate (unpublished results) and beclomethasone 17α,21-dipropionate (13) to inhibit ear oedema formation was approximately half that of budesonide, which is consistent with the skin blanching potency in man (Table II) (14). The ability to induce thymus involution in mice after topical application was three to four times higher for beclomethasone 17α,21-dipropionate than for budesonide. Betamethasone 17α-valerate and budesonide had a similar potency. Thus, budesonide also attained a twofold better ratio between the topical and systemic activities than betamethasone 17α-valerate (unpublished results) and a tenfold better ratio than beclomethasone 17α,21-dipropionate (13).

Human skin blanching test

The ability of budesonide to induce skin blanching in man was compared with some commonly used glucocorticosteroids (Table II). Budesonide and betamethasone 17α,21-dipropionate (Diproderm®) are of equal potency. Betamethasone 17α-valerate (Betnovate®) and fluocinolone acetonide (Synalar®) show medium potency, whereas hydrocortisone 17α-butyrate (Locoid®) and desonide (Apolar®) were the least active substances in this test (8).

Biotransformation in skin

Incubation experiments in human and rat skin homogenates with budesonide, hydrocortisone and triamcinolone acetonide showed that little or no biotransformation occurred (10).

Biotransformation in liver tissue

The biotransformation rate of budesonide, desonide and 16α-hydroxyprednisolone has been compared in vitro in human and rat liver preparations (15). Budesonide was biotransformed six times more rapidly than desonide, whereas 16α-hydroxyprednisolone was biotransformed very slowly, if at all, in human liver (Fig. 3). The rank order was the same in rat liver. The biotransformation rate of budesonide in human liver was also twice and six times as high as that of triamcinolone acetonide and hydrocortisone, respectively (Fig. 4) (10). In rat liver the order was hydrocortisone > budesonide > triamcinolone acetonide, and the biotransformation rate of budesonide was five times more rapid than that of triamcinolone acetonide (10).

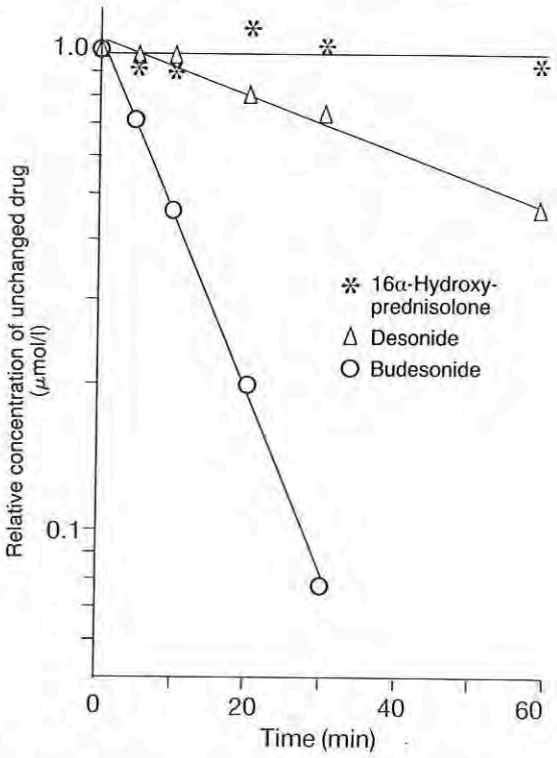


Fig. 3. The influence of 16,17-acetal substitution on the in vitro biotransformation rate in human liver preparation (pooled livers from six individuals) (16).

Effect on the HPA-axis

The systemic side-effects of budesonide, measured as an inhibition of the function of the HPA-axis, were compared with those of the ester structures hydrocortisone 17α-butyrate and betamethasone 17α,21-dipropionate in volunteers (11). Budesonide and hydrocortisone 17α-butyrate were not significantly dif-

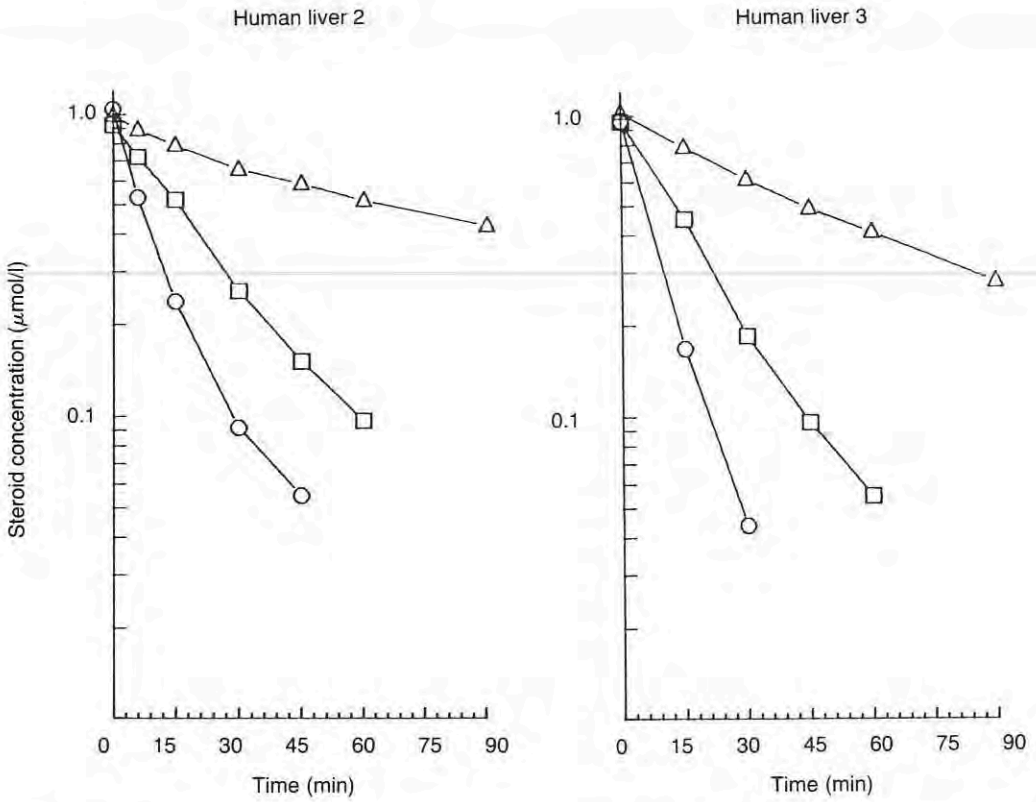


Fig. 4. Biotransformation rates of budesonide (○), triamcinolone acetonide (□) and hydrocortisone (△) in human liver preparation. The decrease in concentration of unchanged steroid in two out of five livers is shown. Initial glucocorticosteroid concentration was 10^{-6} mol/l (10).

ferent at any time and they caused at the most a 50% suppression of the plasma cortisol level. Betamethasone 17α,21-dipropionate reduced the plasma cortisol level at the most by 96% (Fig. 5). Six out of nine volunteers had to be withdrawn after two nights of treatment with the latter compound due to too low cortisol levels. These six subjects are shown separately in Fig. 5B. The variations in 24-hour urinary cortisol excretion show a similar behaviour as the plasma cortisol levels (11).

DISCUSSION

To increase the potency of glucocorticosteroids intended for systemic use, it has been common to reduce the biotransformation rate and the transcortin binding, e.g. triamcinolone, dexamethasone and betamethasone. Increased affinity for the glucocorticosteroid receptor may also be a contributory factor (9). The relative importance of each of these factors has not been assessed so far. Therefore, the aim of the present studies was to investigate how structural variations in the 16,17-acetal substituent as well as the fluorine substitutions in the 6- and 9-position of the steroid ring skeleton would influence the topical and systemic activities in relation to receptor affinity and biotransformation rate.

The results show that it is more important to optimize the 16,17-acetal substituent than to introduce fluorine atoms in the 6- and 9-positions if a potent topical anti-inflammatory glucocorticosteroid is desired. The highest activity was obtained when a hydrogen atom and a straight alkyl chain containing three carbons was substituted in the 22-position in the molecule (cf. budesonide in Fig. 1). Fluorine substitution in the ring skeleton, on the other hand, potentiated the systemic activity to a higher degree than the topical activity. Accordingly, budesonide reached a much better topical/systemic activity ratio

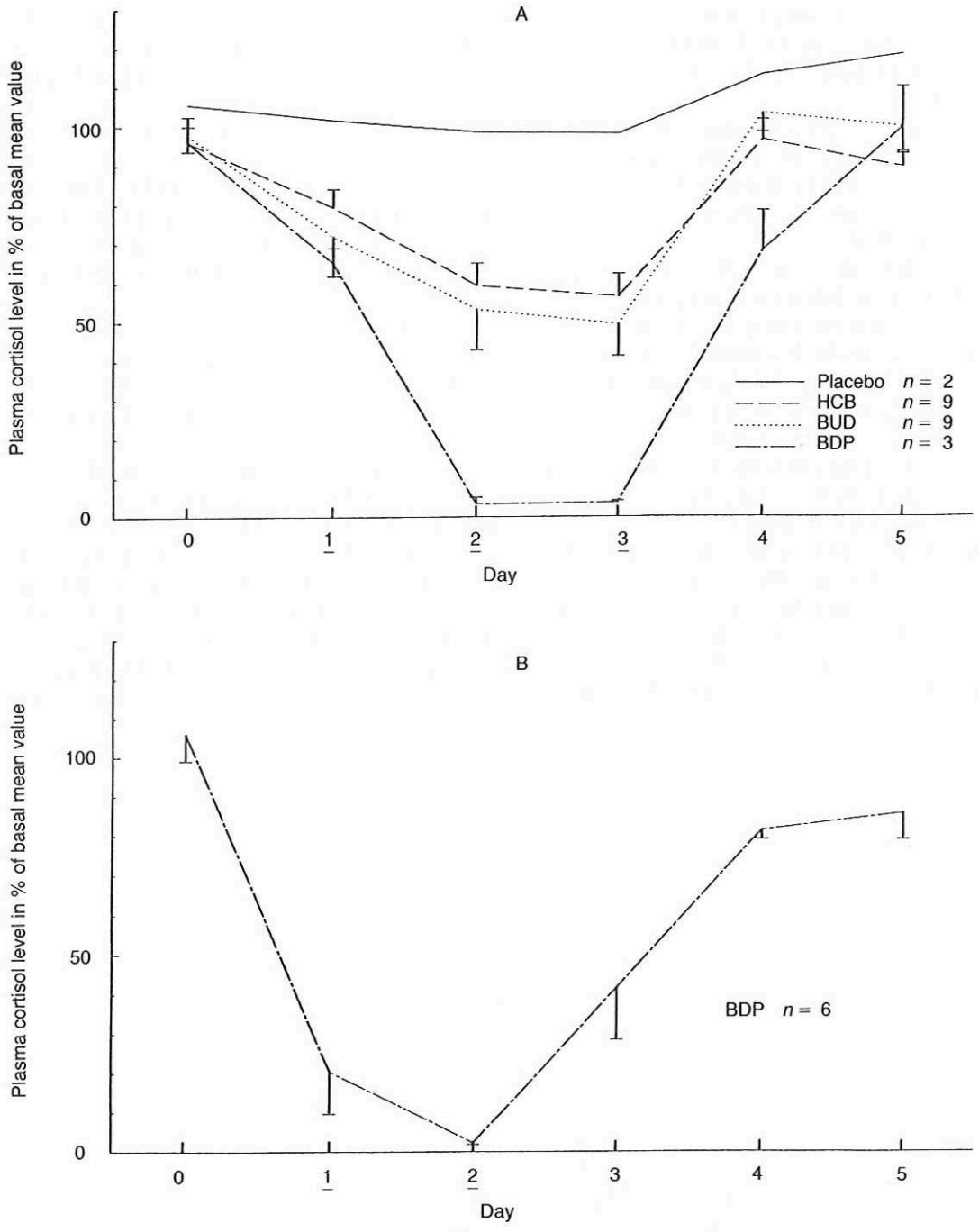


Fig. 5. Changes in plasma cortisol in volunteers of budesonide (BUD, Preferid®), hydrocortisone 17α-butyrate (HCB, Locoid®) and betamethasone 17α,21-dipropionate (BDP, Diproderm®) ointment preparations as percentage of basal mean value after three nights of occlusion (A) and after two nights of occlusion with Diproderm® (B). Starting values for plasma cortisol were taken and are marked in A and B with SEM values (day 0). Underlined days indicate treatment days (11).

halogenated 16,17-acetonides in rats or the 17α-ester compounds such as beclomethasone 17α,21-dipropionate and betamethasone 17α-valerate in mice (results not shown).

Budesonide is a very potent glucocorticosteroid at the site of application due to a high affinity for the receptor. The lack of biotransformation in the skin contributes to a high local concentration of the drug in the target organ.

When budesonide was given by systemic routes its anti-inflammatory potency was not greater than its other types of systemic glucocorticoid activities, e.g. thymus involution (4). This means that at the receptor level budesonide, like other glucocorticosteroids, cannot differentiate between anti-inflammatory and other types of glucocorticoid effects (17). Thus, inactivation by metabolic transformation in the liver after absorption from the sites of deposition explains the low systemic activity of budesonide.

Budesonide is oxidized or reduced by the liver enzymes into several metabolites. The oxidized products 6β-hydroxybudesonide and 16α-hydroxyprednisolone are the major metabolites in man (Fig. 6) (18–20). Both have negligible affinity for the glucocorticosteroid receptors (Fig. 2). The latter metabolite is formed by cleavage of the 16,17-acetal after incorporation of oxygen at the 22-carbon in the oxidation step (21). The 16,17-acetonides do not undergo this type of metabolic cleavage since they provide no site of oxygenation at the 22-carbon, probably due to the absence of a hydrogen atom at this position. Thus, the metabolism of budesonide follows two pathways, whereas the 16,17-acetonides follow only one. This contributes to the effective inactivation and favourable local/systemic activity ratio of budesonide.

To judge the clinical value of the selectivity obtained with budesonide, it was also necessary to make a comparison with the selectivity of the 17α- and 17α,21-esters, e.g. hydrocortisone 17α-butyrate and betamethasone 17α,21-dipropionate (11). Comparative studies of systemic side-effects are difficult to perform in patients due to gradual improvement of the skin barrier function during the healing process, and also due to intra- and inter-individual variations of the lesions. Therefore, the investigation was performed in healthy volunteers in whom a more reproducible defect of skin barrier function was induced by the standard occlusion technique. Although budesonide and betamethasone 17α,21-dipropionate have

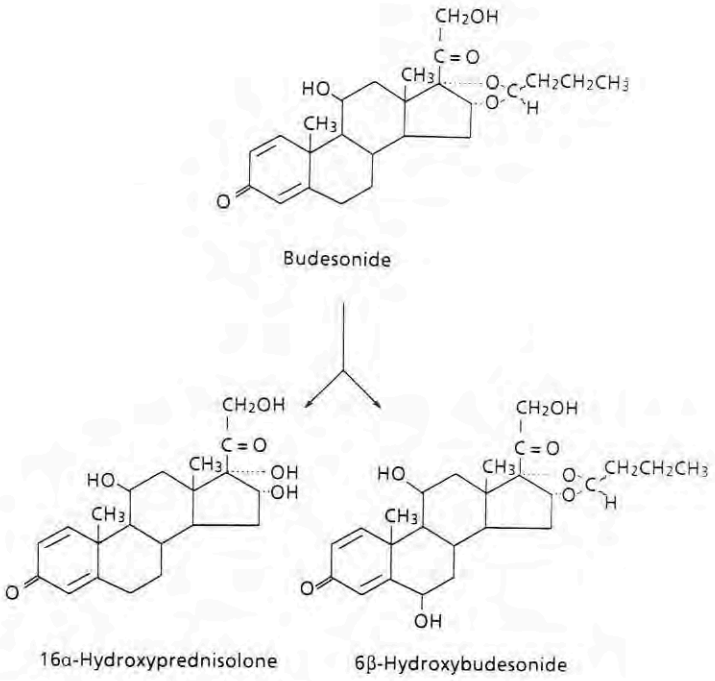


Fig. 6. Structures of 16α-hydroxyprednisolone and 6β-hydroxybudesonide, the two major metabolites of budesonide.

the same topical anti-inflammatory potency (8) the latter caused a significantly greater suppression of both plasma and urinary cortisol levels. No significant difference was found between budesonide and hydrocortisone 17α-butyrate (11) despite the large difference in topical anti-inflammatory potency (8).

In conclusion, the non-symmetrical (type B) 16,17-acetals (e.g. budesonide) are very potent topical glucocorticosteroids. Due to the inactivation by rapid and efficient liver metabolism they are more selective for the topical treatment of skin sites than the currently used 16,17-acetonides, and the 16β-methyl 17α-esters such as betamethasone 17α-valerate and betamethasone 17α,21-dipropionate.

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Potency Classification of Topical Corticosteroids: Modern Perspectives

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The effects of topical corticosteroid therapy upon human epidermal Langerhans cell surface markers were studied. The reduction in numbers of human leukocyte antigen-DR$^+$/T6$^+$ Langerhands cells proved to be a potency-related effect and Langerhans cell enumeration may therefore have some use as an adjunct in the assessment of steroid potency. Further studies showed that corticosteroid therapy also reduced the function of Langerhans cells in terms of epidermal cell alloantigen-presenting capacity. However, experimental variation and the high antigen-presenting potency of residual flow cytomterically sorted human leukocyte antigen-DR$^+$/T6$^+$ Langerhans cells following steroid application (1) mean that such functional assessments of epidermal cell alloantigen-presenting capacity cannot be recommended as an easily reproducible method for measuring steroid potency.

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The efficacy of a topical application of hydrocortisone (initially called 'compound F') was first described in 1952 (2). Since that time many different corticosteroid preparations have been introduced, establishing this form of treatment as the largest single class of dermatological therapy in modern practice with sales in the USA alone (as long ago as 1974) of 100 million dollars (3). Because there are now many different topical corticosteroid preparations available, it is important that the expected potency of a particular formulation be made clear to the prescribing clinician.

The local anti-inflammatory effects of topically applied corticosteroids are dependent upon at least four factors: 1. the intrinsic potency of the steroid molecule itself, 2. the concentration of the preparation, 3. the site of application and 4. the rate of percutaneous penetration (which is dependent upon the type of vehicle and the degree of occlusion). In order to assess the potency of different steroid preparations a variety of experimental models have been devised. These have included assessment of the anti-inflammatory action of selected topical steroids following the application of a controlled inflammatory insult, e.g. croton oil (4, 5) following controlled trauma (6) or after ultraviolet light-induced inflammation (7). Such methods have proved unsatisfactory due to lack of consistency or reproducibility and have never come into common practice. As an alternative approach, steroid potency has been assessed by the quantification of steroid-induced skin atrophy using various techniques including skin fold thickness measurement (8, 9), radiographic measurement of skin thickness (10, 11) and a pulsed ultrasound technique (11, 12).

However, the most simple, reproducible and cheap technique which remains the most widely used assay for the screening of topical corticosteroid potencies is the vasoconstrictor assay.

THE VASOCONSTRICTOR ASSAY

The introduction of the vasoconstrictor assay (13) provided a simple and reproducible technique by which the anti-inflammatory properties of a topically applied corticosteroid could be measured. This method of comparing the effects of topical corticosteroids initially involved the application of the steroid in a 95% alcohol solution. However, the vehicle in which a particular steroid is carried is known to be of vital importance in determining the overall potency of the preparation (3, 14), and the vasoconstrictor assay is now commonly performed with the pharmacological product actually used in clinical practice (i.e. the steroid–vehicle combination). In the vasoconstrictor assay, the potency of a formulation is related to the degree of blanching produced at the treated site. Measurement is usually dependent upon a subjective assessment of the response, though blood flow measurement by laser-Doppler velocimetry has been utilised by some workers in an attempt to make the test more objective (15). Extraneous factors, such as the ambient temperature and the blanching effect of the

vehicle, may influence the interpretation of the vasoconstrictor assay. This test remains a useful non-invasive screening procedure for the initial assessment of the anti-inflammatory properties of topical corticosteroids. In general, it provides a reasonably good correlation between the relative strengths of different topical preparations and their efficacy in clinical practice (16–18). For some steroids, however, the degree of correlation between potency measured by the vasoconstrictor assay and by an anti-inflammatory assay (modification of croton oil and kerosene-induced cutaneous inflammatory responses) was poor (5). Not only did the results from the vasoconstrictor assay differ from those of anti-inflammatory assays, but the results of the anti-inflammatory assays also varied depending on the irritant used.

Despite certain reservations, the vasoconstrictor assay remains the most widely used method for the classification of the relative potencies of topical corticosteroids. Together with clinical assessment, it forms the basis of the standard ranking order by which the different preparations are compared. Alternative methods for measuring steroid effects on the skin which are more objective and less subject to extraneous influences would be desirable.

Allergic contact dermatitis reactions provide a good in-vivo model of a cell-mediated immune response in the skin. Topical steroids are known to depress both the induction and the elicitation phases of these reactions (19, 20). Not only allergic contact dermatitis reactions but also a wide variety of other steroid-responsive inflammatory skin disorders are characterised by a lymphocyte-rich infiltrate. Moreover, since Langerhans cells are known to be critical to the development of allergic contact dermatitis reactions (20, 21), it seems reasonable to assume that topical corticosteroids act, at least in part, through effects upon Langerhans cell-dependent epidermal cell-induced T-lymphocyte activation. Possible sites of action for the immunosuppressive activity of steroids on this system include both the stimulator antigen-presenting cells and the effector lymphocytes. Modern methodology allows us to assess in more detail some of these effects and to quantify aspects of steroid-induced suppression of skin immune function.

EFFECTS OF CORTICOSTEROIDS UPON LANGERHANS CELL-DEPENDENT EPIDERMAL CELL IMMUNE FUNCTION

The skin immune system comprises intra-epidermal Langerhans cells which are dendritic, bone-marrow derived, antigen-presenting cells which express cell-surface Fc (crystallizable fragment of immunoglobulin) and C3 receptors, membrane adenosine triphosphatase, T6/CD1a antigen and the major histocompatibility complex class II molecule (also termed human leukocyte antigen-DR) (22–25); and keratinocytes which secrete a wide variety of immunomodulatory cytokines including a powerful immunostimulatory factor termed epidermal cell derived thymocyte activating factor, which is similar/identical to interleukin-1 (26, 27). These immunologically active epidermal cells interact with other components of the immune system both in the epidermis itself, where trafficking lymphocytes come into close contact with them, and also in regional lymph nodes, to where Langerhans cells migrate especially under certain circumstances, e.g. during allergic contact dermatitis reactions (28). This interactive system has been referred to as the skin associated lymphoid tissue system (29).

It is well recognised that topical corticosteroid therapy modifies a variety of epidermal Langerhans cell surface determinants including the major histocompatibility complex class II/human leukocyte antigen-DR and CD1a/T6 cell surface molecules. This effect was initially described in experimental animals where the epicutaneous application of corticosteroids was shown to reduce the number of the immune response associated antigen (equivalent to major histocompatibility complex class II/human leukocyte antigen-DR) positive Langerhans cell in both mice (30) and guinea pigs (31). Less extensive investigations with regard to Langerhans cell enumeration following steroid therapy have demonstrated similar effects in human skin (32, 33), where it has been suggested that the application of topical corticosteroid therapy selectively reduces major histocompatibility complex class II/human leukocyte antigen-DR expression to a greater extent than the reduction in CD1a/T6 expression (i.e. thereby inducing the presence of Langerhans cells which are positive for major histocompatibility complex class II/human leukocyte antigen-DR but negative for CD1a/T6).

Experiments in rodent systems have gone on to demonstrate that in addition to affecting Langerhans

cell surface determinants, topical corticosteroid therapy also suppresses Langerhans cell-dependent epidermal cell antigen-presenting capacity in the in-vitro epidermal cell–lymphocyte reaction (34).

It is clear that much of the currently available data relating to the effects of topical corticosteroid therapy on the skin immune system are murine-derived data. Extrapolation to man must be viewed with extreme caution since the two species exhibit fundamental differences in their susceptibility to the effects of corticosteroids (35). Further investigation into possible effects of corticosteroids in human skin, including modifications of Langerhans cell surface markers and alterations in Langerhans cell antigen-presenting function, is therefore needed.

EXPERIMENTS

The aims of the experiments described here were to assess the effects of topical corticosteroid therapy upon epidermal Langerhans cell expression of human leukocyte antigen-DR and T6/CD1a surface molecules in human skin, and also to study the suppression of epidermal cell antigen-presenting capacity. These experiments were performed with topical corticosteroid preparations known to be of differing potencies in order to determine whether any observed steroid-induced changes were potency-related.

Three commonly used topical corticosteroids were used throughout and were chosen because of their different potency ratings in the British National Formulary. They were the moderately potent clobetasone butyrate 0.05%, the potent betamethasone valerate 0.1% and the very potent clobetasol propionate 0.05%. In all cases the ointment application forms were used and Glaxo UK kindly provided the active preparations as well as the appropriate ointment vehicle alone for use as control.

Subjects applied the corticosteroid to clinically normal skin on the flexor aspect of one forearm and the inactive control vehicle alone on the contralateral forearm twice daily for 7 days. Two circular sites, each of approximately 4 cm diameter, were treated on each arm and the tubes containing the corticosteroid were weighed at the beginning and end of the 7 day treatment period. The mean total quantity of steroid ointment applied was 1.25 g per application site per week.

Details of the experimental methods are given elsewhere (36). In brief the procedure was as follows. At the end of the 7 days' treatment period, epidermal sheets were raised by a standard suction blister technique and were then divided. A portion was retained for in-situ identification of human leukocyte antigen-DR$^+$/T6$^+$ epidermal Langerhans cells using a standard double-labelling immunofluorescence technique. Human leukocyte antigen-DR$^+$/T6$^+$ epidermal Langerhans cells were quantified per mm^2 and expressed, for steroid treated skin, as a percentage of the count obtained from the contralateral control forearm. The remainder of the blister roof was processed to yield a single cell suspension following trypsinisation. Responder allogeneic peripheral blood mononuclear cells were isolated by centrifugation over Ficoll Hypaque in a standard fashion. Epidermal cell–lymphocyte reactions were set up and Langerhans cell-dependent T-cell proliferation was measured at 7 days by the incorporation of tritiated thymidine. Results for steroid-treated sites were again expressed as a percentage of those for control skin.

Immunofluorescence examination of epidermal sheets stained with anti-human leukocyte antigen-DR and/or OKT6 revealed a marked decrease in the number of human leukocyte antigen-DR$^+$/T6$^+$ Lan-

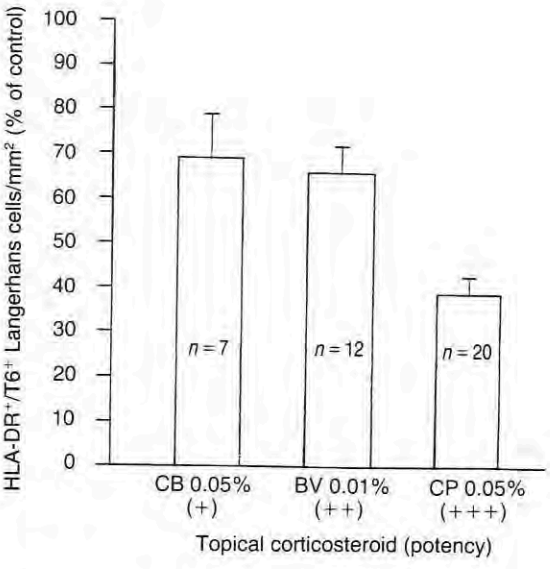


Fig. 1. Density of HLA-DR$^+$/T6$^+$ Langerhans cells in epidermal sheets expressed as a percentage of control (mean ± SEM) following application of clobetasone butyrate (CB) 0.05%, betamethasone valerate (BV) 0.1% or clobetasol propionate (CP) 0.05%. Reproduced by kind permission of the editor from (36).

gerhans cells following topical corticosteroid therapy, which was statistically significant for all three steroids ($P < 0.05$). Langerhans cell density in control skin ranged from 530–937 Langerhans cells/mm^2 (mean 672). The steroid-induced reductions occurred in a potency-dependent fashion (Fig. 1). The greatest decrease in human leukocyte antigen-DR$^+$/T6$^+$ Langerhans cells/mm^2 expressed as a percentage of control (mean ± SEM) was induced by clobetasol propionate (39.1 ± 3.0%), followed by betamethasone valerate (66.1 ± 5.4%) and clobetasone butyrate (69.2 ± 8.8%). Statistical analysis showed that there was no significant difference between the reductions in human leukocyte antigen-DR$^+$/T6$^+$ Langerhans cells following clobetasone butyrate and betamethasone valerate therapy, but the reduction after clobetasol propionate therapy was significantly greater than that following the other two therapies ($P < 0.001$). In experiments with the double immunofluorescence staining technique, it was determined that, in both control and steroid-treated skin, all human leukocyte antigen-DR$^+$ Langerhans cells were also T6$^+$ and vice versa. Cells which were either human leukocyte antigen-DR$^+$/T6$^-$, or -DR$^-$/T6$^+$ were not observed.

In individual experiments, the alloantigen-presenting capacity of equivalent numbers of epidermal cells

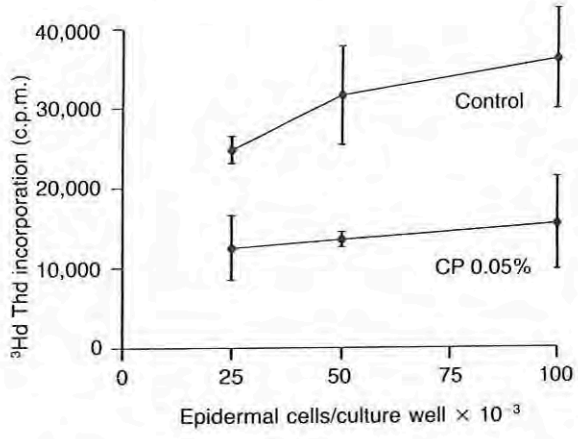


Fig. 2. Effect of topical therapy with clobetasol propionate (CP) 0.05% on the allostimulatory capacity of epidermal cells in the allogeneic epidermal cell–lymphocyte reaction. A representative experiment showing epidermal cell–lymphocyte reaction curves with results expressed as mean ± SEM (^{3}Hd Thd = tritiated thymidine). Topical corticosteroid therapy substantially reduces epidermal cell antigen-presenting capacity. Reproduced by kind permission of the editor from (36).

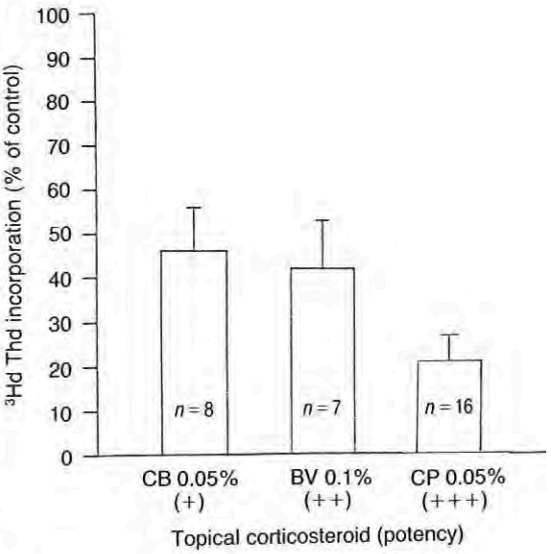


Fig. 3. Effect of topical corticosteroid therapy on the Langerhans cell-dependent allostimulatory capacity of epidermal cell suspensions in the allogeneic epidermal cell–lymphocyte reaction. Results are derived by expressing the amount of tritiated thymidine (^{3}Hd Thd) incorporation by epidermal cells from steroid-treated sites as a percentage (mean ± SEM) of ^{3}Hd Thd incorporation by equivalent numbers of epidermal cells from control sites. The steroids applied were clobetasone butyrate (CB) 0.05%, betamethasone valerate (BV) 0.1% or clobetasol propionate (CP) 0.05%. Reproduced by kind permission of the editor from (36).

from steroid-treated and control sites was compared (Fig. 2). The data from many such individual experiments were combined by expressing the allostimulation achieved by epidermal cells from steroid-treated skin as a percentage of the allostimulation achieved by control epidermal cells (Fig. 3). The alloantigen presenting capacity in the epidermal cell–lymphocyte reaction of epidermal cell suspensions from steroid-treated sites was significantly decreased compared with that of epidermal cells from control sites ($P < 0.01$ for all 3 steroids). Steroid therapy reduced the alloantigen-presenting capacity of epidermal cell suspensions to the following mean percentages ± SEM of control: clobetasone butyrate 45.1 ± 10.75%, betamethasone valerate 41.9 ± 12.0% and clobetasol propionate 20.6 ± 5.6%. Depressed epidermal cell allostimulatory capacity was not restored by the addition of exogenous interleukin-1 (36) (data not shown) indicating that this depression was not primarily due to a steroid-induced decrease in the production of

epidermal cell derived thymocyte activating factor/interleukin-1 by keratinocytes. Analysis and comparison of the effects of the three different preparations on the allogeneic epidermal cell–lymphocyte reaction in a large number of subjects (Fig. 3) showed that topical corticosteroid therapy resulted in a marked, potency-related reduction in the capacity of epidermal cells to present alloantigen.

There was a trend suggesting that clobetasol propionate 0.05% therapy depressed allostimulation to a greater extent than did either of the other two preparations but this difference reached statistical significance only for clobetasone butyrate 0.05% ($P < 0.05$).

The reduction in the numbers of human leukocyte antigen-$DR^+/T6^+$ Langerhans cells proved to be a potency-related effect and Langerhans cell enumeration may therefore have some use as an adjunct in the assessment of steroid potency. Further studies showed that corticosteroid therapy also reduced the function of Langerhans cells in terms of epidermal cell alloantigen-presenting capacity. However, inter-subject and inter-experiment variation together with the fact that Langerhans cells are extremely potent at antigen presentation and residual flow cytometrically sorted human leukocyte antigen-$DR^+/T6^+$ Langerhans cells from steroid-treated skin retain normal alloantigen-presenting function (1) (data not shown) mean that such assessments of epidermal cell alloantigen-presenting capacity cannot be recommended as an easily reproducible method for measuring steroid potency.

DISCUSSION

A variety of methods have been proposed for the measurement of the anti-inflammatory actions of topically applied corticosteroids. The different methods each have their own advantages and disadvantages, but overall the method of choice remains the vasoconstrictor assay. This assay itself suffers from a variety of drawbacks, including the effects of extraneous influences such as the ambient temperature and the blanching effects of the vehicle. In addition, unless laser-Doppler flow velocimetry is used (15), interpretation of the effects is subjective thereby making quantification difficult. The development of an alternative method of measuring steroid potency independent of such unwanted influences and more objective methods would be desirable.

Topical corticosteroids have well-recognised immunosuppressive actions as evidenced by suppression of the induction and elicitation phases of contact sensitisation. These actions are related to effects on the skin immune system including effects upon intra-epidermal Langerhans cells and also possible effects upon cytokine production by keratinocytes.

Corticosteroids have been shown to decrease the expression of Langerhans cell surface markers in rodents (30, 31) and to reduce the antigen-presenting capacity of epidermal cells in mice (34). In an attempt to further elucidate corticosteroid effects on Langerhans cells in human skin following the application of steroids in a manner approaching therapeutic use, Langerhans cell numbers and function following the application of three corticosteroid preparations of differing potencies were studied. It was also of interest to see whether the observed effects correlated with steroid potency.

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Adverse Effects of Topical Corticosteroids

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Topical corticosteroids may induce local and systemic adverse reactions. Some of the corticosteroids are at low risk to induce several manifestations, but gross overdosage or misuse of the most potent steroids are responsible for a number of adverse reactions. An overview of the effects of topical corticosteroids upon the different structures of human skin is presented here.

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Local and systemic adverse reactions of topical corticosteroids were noticed clinically since the mid sixties. They are now well documented and characterized by a series of biological features.

It was often considered an established fact that the therapeutic potency of topical corticosteroids and the risk of developing adverse reactions were correlated. In clinical practice, such a view is probably too dogmatic for two main reasons. On the one hand, the patient's inclination for overusage and erratic usage of the preparation increases the risk of developing unwanted effects irrespective of the potency of the steroid. On the other hand, the pharmaceutical industry tries to develop a new generation of topical corticosteroids combining a potent topical efficacy with relatively low systemic effects (1–3). There have been claims in the past for such a separation of local and systemic effects, but convincing proof was not always provided, so that in our opinion this aim has not been realized yet.

CLINICALLY RECOGNIZABLE ADVERSE REACTIONS

If most of the structures of the skin are rapidly altered by topical corticosteroids, not all of them can be noticed clinically, and it takes several weeks of treatment before the changes can be observed (4–7).

The local adverse reactions include skin atrophy, striae, telangiectasias, purpura, easy bruising, stellate scars, hypopigmentation and hirsutism. There may be dissemination of various types of viral, bacterial and mycotic infections, and development of miscellaneous disorders such as steroid acne, perioral dermatitis and gluteal granulomas.

In addition to local alterations, evidence of systemic effects may also be present. Failure to thrive, retardation of growth, Cushing's syndrome and pituitary-adrenal suppression have been reported (1, 8–12). These alterations are usually mild and transient. They may, however, be more pronounced and persistent in children treated with the most potent halogenated steroids. It has been reported that some topical corticosteroids can suppress the pituitary-adrenal axis after only 2 weeks of use, with a return of the plasma cortisol levels to normal in 2–3 days after discontinuation of treatment.

There are many factors modulating the expression of clinically recognizable adverse reactions. They include the overall potency and metabolism of the steroid, and the quality of the steroid delivery system implicating the nature of the base, the quality of the stratum corneum, and so many factors influencing the binding of the steroid in the different layers of the skin. The age of the patient also influences the pattern and type of alterations, owing to differences in pharmacokinetics of steroids and to changes in the barrier and reservoir functions of the stratum corneum. Some of these variations could be responsible for the occurrence of distinct effects such as striae distensae, which are a feature of puberty and early adult life, while diffuse thinning of the skin is more susceptible to develop rapidly in the old age. It must be remembered that most of the local adverse reactions of corticosteroids mimick the aspect of chronological aging (13, 14).

ASSESSMENT OF ADVERSE REACTIONS

The clinical changes of steroid-induced atrophy go hand in hand with evidence of histological atrophy (15). Specific glucocorticoid receptors are present in

human skin. As a general rule, the epidermal and dermal receptors bind corticosteroids in a similar manner. Furthermore, there is an overall relationship between the binding of corticosteroids to these receptors and their potency (16).

Corticosteroids have several characteristic effects on the epidermis. They can be demonstrated histologically quite early during treatment (15, 17–19). Epidermal atrophy becomes apparent as a thinning of the malpighian and horny layers. Rete ridges are flattened. The size of individual keratinocytes and corneocytes is decreased as assessed by morphometry. Langerhans cells are also altered. Apparently there are variations in their immunohistochemical characters, as well as cell death and/or emigration out of the epidermis (Fig. 1). The relationship between melanocytes and keratinocytes is also modified and becomes clear as a decreased amount of melanin released to the keratinocytes.

Atrophy and wasting of collagen bundles are the major end result of the effect of corticosteroids on the dermis (4, 20–22). Histologically, such changes are only discernable after several months of treatment because of the long half-life of collagen polymers. Therefore, other approaches are needed to establish atrophogenicity. Rapid changes can be measured within the first days of treatment consisting of variations in dermal thickness and in the response to extensional and compressional forces (22–29). These first critical changes could be explained by modifications in the viscoelastic function of glycoproteins and proteoglycans which insure the interfibre adhesion of collagen. These changes appear to parallel modifications in the resident cell population of the dermis which is normally heterogeneous (30). As an early event during corticotherapy, Factor XIIIa positive dendrocytes of the papillary dermis become smaller and less dendritic. Subsequently, they are reduced in number (Fig. 2).

In the course of time, the marked reduction of thickness of the reticular dermis becomes histologically observable. This is almost entirely due to the wasting of collagen bundles while reticulin fibres (Fig. 3) and elastic fibres appear unaffected. Vessels without their contention by dermal stroma enlarge (Fig. 4). This fact has been proposed as a predictive test method for atrophogenicity (31).

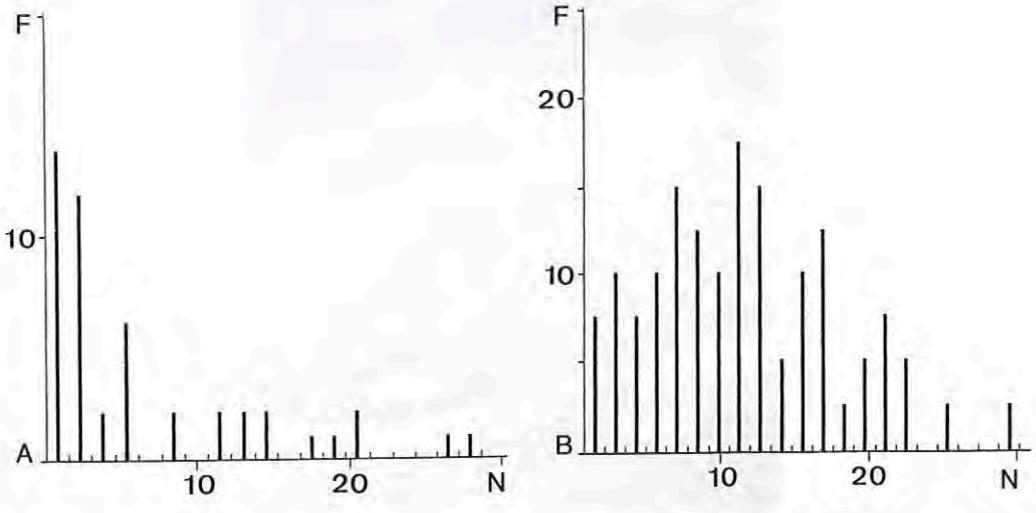


Fig. 1. Absolute frequency (F) of the number of Langerhans cells per arbitrary units of length of stratum corneum (N) after immunolabelling with S-100 protein. The number of Langerhans cells is markedly reduced after 2 weeks of topical corticotherapy (A) compared with controls (B).

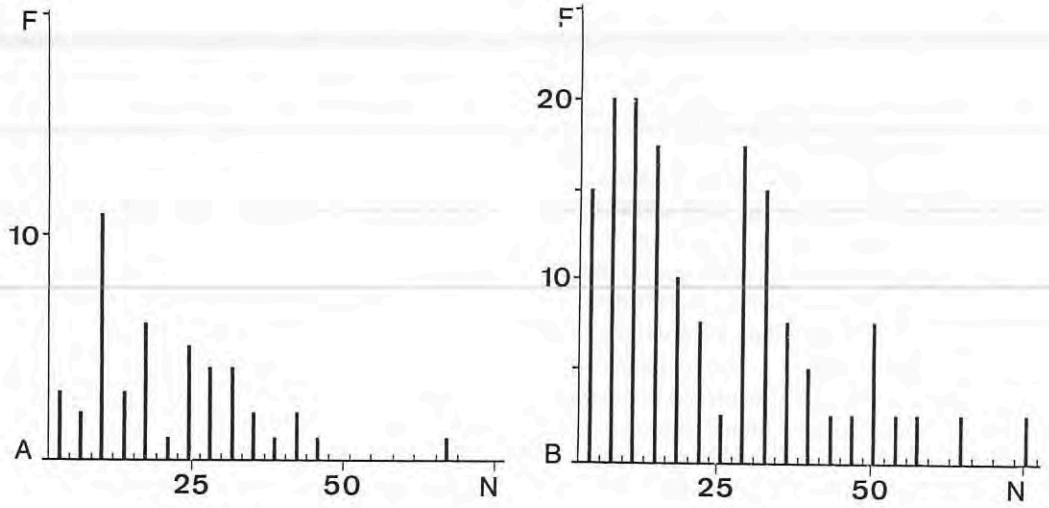


Fig. 2. Absolute frequency (F) of the number of Factor XIIIa positive dendrocytes in the papillary dermis (N). Results are expressed per arbitrary units of length of stratum corneum. The number of dendrocytes is reduced after 2 weeks of topical corticotherapy (A) compared with controls (B).

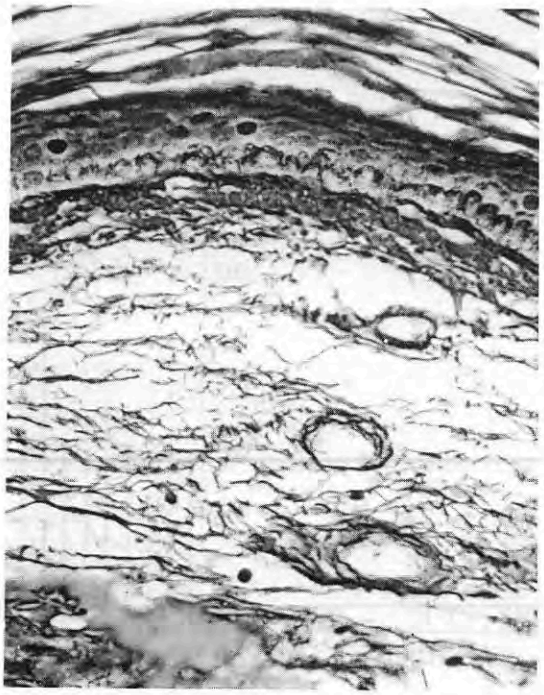


Fig. 3. Atrophy of the reticular dermis due to topical corticotherapy. Reticulin fibres remain numerous while collagen bundles have disappeared (reticulin stain).



Fig. 4. Dilation of the superficial vascular plexus during topical corticotherapy (alkaline phosphatase).

DISCUSSION

Due to the multiple effects of topical corticosteroids on the structures of the skin, it seems inappropriate to assess these changes solely by one single approach. An appropriate evaluation should combine histology with histometric techniques and non-invasive methods such as ultrasound and the determination of rheological properties of the skin.

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The Choice of Topical Steroids — A Personal View

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Topical steroids are the most commonly prescribed drugs for use on the skin. Various factors affecting the choice of such a steroid in the UK are considered and explained.

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The British National Formulary and MIMS, a monthly catalogue, list some 18 steroid compounds for topical use alone or in combination with any of 16 antimicrobial agents not to mention tar, urea, crotamiton, calamine and dimethicone. There are no less than 176 separate dermatological formulations available in the UK including foams, gels, aerosols, lotions, creams, oily creams, ointments, tape and even an impregnated bandage. How may one choose the most appropriate from such an 'embarras de richesse'? Some reasons for making a rational choice are obvious from Table I; others are personal and speculative. Rationally speaking, the criterion to decide between the many equivalents ought to be cost. But often this must not be the case.

Table I. *Considerations in choosing a topical steroid.*

1. Diagnosis
2. Potency
3. Safety (cutaneous and systemic)
4. Site to be treated
5. Age
6. Formulation
7. Combination with antibacterial agent
8. Avoidance of sensitizer
9. Previous choice of steroid being unsuccessful
10. Cost
11. Size of pack
12. Range of potency
13. Psychological factors

SELECTION OF A TOPICAL STEROID IN RELATION TO DIAGNOSIS

Eczema should be treated whenever possible with the weakest steroid capable of controlling the condition: an increase in potency of the steroid being indicated at times of exacerbation. Potent steroids are reserved for only the most severe eczema, both in the short and long term (1). Initial treatment should parallel the severity of the eczema. It is unreasonable to expect severe lichenification, for example, to respond to hydrocortisone.

In psoriasis the consensus view is that potent topical steroids should be used only for a limited period, perhaps 3 months. Psoriasis responds well to clobetasol and to potent agents used under occlusion. Unfortunately the early benefit is not maintained in any but the most trivial of cases. Recurrent disease is likely to be unstable, even pustular and more extensive than originally (2).

In the UK a view has emerged that potent topical steroids should be avoided if at all possible, although it may be hard to find a substitute for problem areas such as the face, hands and flexures. A formulation of clobetasol ointment and dithranol hastened clearance without compromising efficacy or the chance of relapse when compared with dithranol in Lassars paste (3). Topical hydrocortisone shortens the remission induced by the Goeckerman regimen (4). Skin lesions responding to potent topical steroids only are listed in Table II.

Combination products

Combination products with antibacterial agents are indicated for angular stomatitis, candidiasis, dermatophyte infection, infected eczema, kerion, napkin psoriasis, otitis externa and seborrhoeic eczema. Fungal and yeast infections heal faster and more certainly (5, 6) with the addition of a steroid to the formulation, without compromising the efficacy. Candidal infection may not clear at all with the use of a suitable anticandidal preparation alone.

The skin surface in eczema is more often than not colonized by *Staphylococcus aureus*. Leyden and

Table II. *Conditions responding to topical steroids.*

Disorder	Potency group (1 = max, 4 = weak)
Eczema and dermatitis	1–4
Psoriasis	1–2
Alopecia areata	1
Balanitis xerotica obliterans	1
Cheilitis	1
Cryotherapy reaction	1
Epidermal naevus, warty type	Impregnated tape
Granuloma annulare	1
Hypertrophic scarring	1
Juvenile plantar dermatosis	4
Lichen sclerosus et atrophicus vulvae	1 then 3–4
Lichen simplex	1–2
Lichen planus	1
Lupus erythematosus	1
Necrobiosis lipoidica	Impregnated tape
Palmoplantar pustulosis	1–2
Papular urticaria	1
Pemphigus foliaceus	1
Pretibial myxoedema	1 with occlusion
Pyogenic granuloma arising in retinoid therapy and acne fulminans	1–2
Sarcoidosis	1
Vitiligo	1

Kligman (7) found a combination of fluocinolone and neomycin more effective than fluocinolone alone in infected lesions. Many preparations have been elaborated to take advantage of this effect and they include neomycin, gentamicin, chlortetracycline, bacitracin, fucidin and soframycin. Combinations such as Adcortyl® (neomycin–triamcinolone acetonide–nystatin with ethylene diamine as the emulsifier) are an attempt to treat eczema, candidal infection and intertrigo with one agent. It carries a high risk of sensitisation. Many other similar preparations have been elaborated for use in otitis externa, but these should be avoided at all costs.

Salicylic acid has antiseptic and penetration enhancing properties. It is combined with Diprosone® as Diprosalic® and is useful for the scalp and vitiligo.

Urea enhances the penetration of hydrocortisone, it is keratolytic and because it is a humectant it causes the epidermis to retain water. Dermatitis of the fingertips and juvenile plantar dermatosis will sometimes respond to urea–hydrocortisone combinations when potent agents have failed. Almeyda and Fry found that urea–hydrocortisone was as effective as betamethasone 17-valerate in atopic eczema (8).

Potency

There is as yet no good evidence to contradict the claim that potency and toxicity go hand in hand. The intensity with which topical steroid therapy can be pursued is limited by the adverse effect of skin atrophy. In comparison, systemic effect seldom matters.

Topical steroids may be grouped into four categories of potency as shown in Table III.

Usage

No increase in effect can be obtained by increasing the frequency of application beyond once daily because effectiveness is governed by the intrinsic potency of the steroid molecule (9). Emollients may be used to reduce the frequency of steroid application in a sparing effect. Exceptionally a weekly application of clobetasol propionate may work better than daily betamethasone valerate without adverse effect. The thickness of the ointment layer does not seem to affect the therapeutic response (10). However, increasing the area of application increases systemic absorption (11). It seems best to encourage patients to undertake treatment as infrequently as is consistent with a reasonable response using the minimum quantity of ointment.

In general, topical steroids are formulated to give the maximum potency for that molecule.

Betamethasone valerate 0.1% and 0.025% are both ranked as potent. Hydrocortisone 1% seems as good as 2.5%. An exception is triamcinolone acetonide which used to be available as a forte 0.2% preparation that probably ranked with clobetasol propionate, while full strength at 0.1% is rated as potent. A quarter of this strength (0.025%) is mildly potent and a tenth strength (0.01%) is weak.

Dilution of commercially available formulations is practised but there are some theoretical and some real problems which may be encountered as a result. Efficacy, for example, may be in doubt. Pharmacological unsuitability of the diluent can destroy the steroid content especially when alkaline materials such as aqueous cream or tars are added (12). The diluent base may have a partition coefficient which favours retention of the steroid in the cream rather than the skin, or the mixture may separate. Sensitizers may be added unsuspected by the prescriber.

Safety

It is axiomatic that the intensity with which topical steroid therapy may be pursued is limited by resulting atrophy of the skin, which even moderately potent

Table III. *Grouping of topical steroids according to potency.*

Tradename	Generic name	%
Group 1: superpotent		
Dermovate®	Clobetasol propionate	0.05
Diprosone®	Betamethasone dipropionate	0.05
Nerisone® forte	Diflucortolone valerate	0.3
Group 2: potent		
Adcortyl®	Triamcinolone acetonide	0.1
Betnovate®	Betamethasone valerate	0.1
Betnovate® RD	Betamethasone valerate	0.025
Haelan® X	Flurandrenolone	0.05
Halciderm®	Halcinonide	0.01
Ledercort®	Triamcinolone acetonide	0.1
Locoid®	Hydrocortisone butyrate	0.1
Metosyn®	Fluocinonide	0.05
Nerisone®	Diflucortolone valerate	0.1
Preferid®	Budesonide	
Propaderm®	Beclomethasone dipropionate	0.1
Stiedex®	Desoxymetasone	0.25
Synalar®	Fluocinolone	0.025
Topilar®	Fluclorolone	0.025
Tridesilon®	Desonide	0.05
Group 3: moderately potent		
Eumovate®	Clobetasone butyrate	0.05
Haelan®	Flurandrenolone	0.0125
Modrasone®	Alclometasone dipropionate	0.05
Neomedrone®	Methylprednisolone	0.25
Stiedex® LP	Desoxymetasone	0.05
Synalar® 1:4	Fluocinolone	0.00625
1:10	Fluocinolone	0.0025
Ultradil®	Fluocortolone hexanoate	0.1
	pivalate	0.1
Ultralanum® plain	Fluocortolone hexanoate	0.25
	pivalate	0.25
Group 4: weak		
	Hydrocortisone preparations	0.1–2.5
Alphaderm®	Hydrocortisone with urea	1
Calmurid® HC		1
Sential®	Hydrocortisone with urea and NaCl	0.5

agents may cause after, for example, 2 years of treatment. However, it should be remembered that there is considerable individual susceptibility. Moreover, recovery occurs when treatment is stopped, except for striae. Marked solar elastosis and potent topical steroids are a particularly unhappy combination, producing a skin with the strength of wet paper.

Systemic effect may on occasion be important. The circumstances are given in Table IV. There has been recent concern about absorption of clioquinol causing subacute myelo-optic neuropathy but no case has resulted yet (14).

Contact dermatitis due to the steroid itself is rare

Table IV. *Circumstances producing pituitary adrenal suppression.*

Use of superpotent steroid on more than half the body surface (10)
Use of potent steroid with occlusion on most of the body surface
Liver disease
Infants and small children

and seldom recognized. However, it is suspected if the condition of the skin fails to improve or even deteriorates during treatment.

The rapid suppressive effect of clobetasol propionate on the pituitary-adrenal axis has been described many times. Data on 13 ambulant out-patients using quantities ranging from 10–300 g per week show a progressive fall in both resting and stress stimulated plasma cortisol levels. The graph in Fig. 1 shows that subnormal cortisol levels develop when more than 65 g per week are used (12). Children are much more vulnerable than adults so that widespread applications should not exceed mild potency on a sustained basis.

Site

The seborrhoeic complexion is inherently intolerant of all but the weakest topical steroids. Stronger agents should not be used as otherwise the face becomes insidiously redder over a few months and ultimately resembles rosacea. However, clobetasol propionate may be tolerated in the long term on normal or dry skins with only a little skin atrophy as in the treatment of lupus erythematosus of the face, which usually responds well.

Scalp eczema or psoriasis respond poorly to topical steroids which are therefore better avoided, although a few patients respond well to Diprosalic® lotion (betamethasone dipropionate with salicylic acid).

Use of the hands removes steroids from the skin so that here it is necessary to use stronger preparations than on other areas of the body. Paradoxically some urea–hydrocortisone preparations work well on finger tip dermatitis, presumably because of emollient and humectant effects.

The skin of the penis and scrotum absorb as much as 100% of applied corticosteroids and is in any event very thin (13). However, the general rule about hydrocortisone only for this area may reasonably be broken in the treatment of lichen sclerosus et atrophicus of the vulva and balanitis xerotica obliterans. All but the worst instances of the latter resolve completely. Absorption of clioquinol is a recently rediscovered problem as up to 40% may be absorbed under occlusion which compares with conditions within the napkin area (14).

Formulation

Propylene glycol dissolves corticosteroids to make more potent formulations than were previously possible. It is antibacterial (15), sensitization occurs occasionally and it is somewhat irritating so that dryness and asteatotic eczema may follow its use (16).

Cetomacrogol cream bases are rather drying when applied to broken dry skin, but they are well tolerated on raw surfaces. It is a welcome trend that more oily

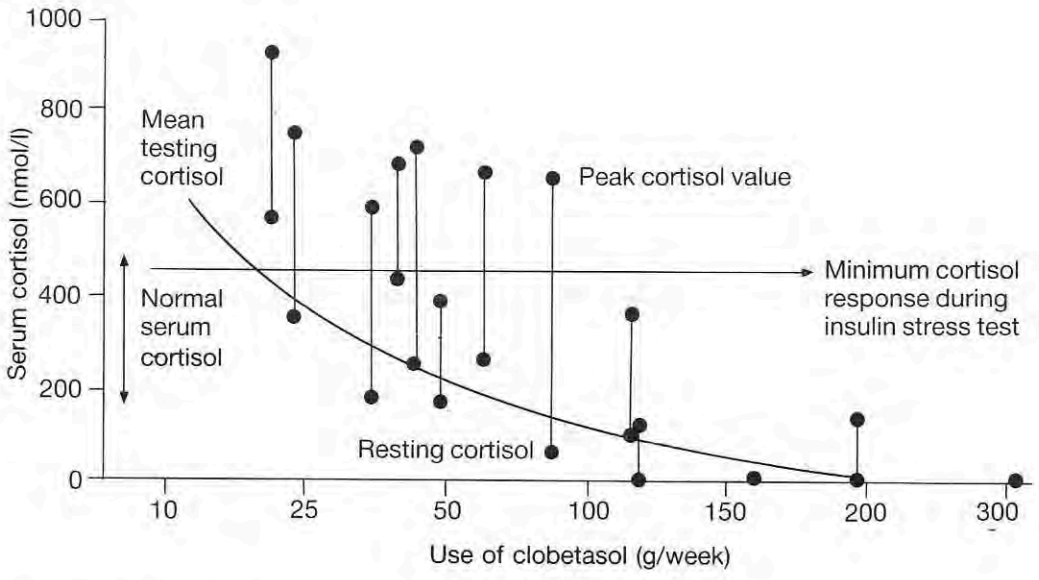


Fig. 1. The effect of clobetasol propionate on the insulin stress test in 13 patients.

creams are becoming available: they are more emollient than normal creams and much more pleasant to use than ointments.

Flurandrenolone tape is useful for necrobiosis lipoidica, lichen simplex and warty epidermal naevi.

Where allergic contact dermatitis is suspected an ointment base of white soft paraffin should be chosen. Lanolin, preservatives, the steroid itself, fragrance and rarely the waxes and emulsifiers are responsible for allergic reactions. MIMS lists the sensitizers in topical steroids.

Cost

The difference between the highest and lowest cost preparations in the potent group is around fourfold in the UK. Use of 100 g per week of the least compared with the most expensive preparation would save £400 per year, equivalent to 3 days in an NHS-hospital in the UK. Hydrocortisone is approximately half the price of the cheaper potent agents. Combination products are invariably expensive. Cost is a useful discriminator between equivalent topical steroids when other factors have been duly considered.

Psychological factors

The reasoning behind a therapeutic decision may well not be suited for precise analysis but some identifiable influences on dermatological thinking follow.

The pharmaceutical company has a high profile with the dermatologist. It supports research, meetings, travel and the literature has a good scientific ring. On the other hand, it may be counterproductive to be asked to entertain a change in a lifetimes' prescribing habits because there is a need to promote a new drug.

A preparation may be seen to be superior on theoretical grounds, for example improved topical to systemic effect (17).

'Buy British and put a Swede out of a job!' It is unknown how often this reason enters a serious therapeutic decision.

As in all branches of medicine it is preferable to have mastered the correct use of a few drugs than to be an apprentice with all of them.

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Prospects for Future Topical Glucocorticoid Development

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Current glucocorticoids are inactivated mainly in the liver. Results from studies of their catabolism; their concentration gradients in epidermis and upper and lower dermis after topical application to intact and injured skin; and the concentration needed to inhibit synthesis of human connective tissue by skin fibroblasts; suggest that their systemic and local adverse reactions would logically be reduced further by adding a rapid extrahepatic biotransformation. An ideal glucocorticoid should be locally inactivated during, or immediately after, absorption.

The data available for three glucocorticoids with some extrahepatic metabolism suggest that such relatively labile steroids may have a more autoregulating absorption than that of the conventional, more stable, steroids. This means that in skin areas with a damaged stratum corneum, the balance between steroid influx and inactivation may favour anti-inflammatory activity, while that balance is insufficient in intact skin for a triggering of glucocorticosteroid activity. When the skin lesions heal, and the high influx rate tapers off, corticosteroid activity in the epidermis and dermis may be better cut off than with the conventional, metabolically stable, corticosteroids.

The compounds subject to local metabolism available today appear to have only moderate topical corticosteroid activity. There are still no valid data to support a claim that their catabolic effects on the connective tissue of diseased skin are less than those of conventional topical steroids. However, novel glucocorticosteroids with a still better relation between high intrinsic glucocorticosteroid activity and rapid metabolic turnover in skin should be designed and tested.

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AIMS IN DEVELOPING NEW TOPICAL GLUCOCORTICOIDS

Currently no other topical therapy can match the broad anti-inflammatory efficacy of glucocorticoids. The main limiting factor in their use is not lack of efficacy. Therefore, the main aim for their further development is to reduce or at least delay the appearance of adverse reactions. Limiting factors to their clinical use include connective tissue atrophy, tachyphylaxis and systemic corticosteroid effects, features which, however, play different roles in skin and airway diseases (Table I).

Reduction of unwanted systemic glucocorticoid effects

Topical therapy is based on the concept that the site of application is the final target of the desired anti-inflammatory action. Through this mode of application the risks of most of the serious systemic, adverse reactions are avoided or minimized. The risk of provoking systemic reactions is greater with inhalation into airways than with topical skin application, because there is no absorption barrier in the mucosa of the airways like the epidermal stratum corneum.

Logically, systemic adverse reactions as a consequence of topical application of glucocorticoids should be reduced by enhancing their rate of catabolism in the systemic circulation. As inhalation carries a higher risk of provoking unwanted systemic effects

Table 1. *Topical glucocorticosteroid therapy — some limiting factors in the respective treatment of skin and airways diseases*

	Limiting factor	
	Skin disease	Airways disease
Local effects:		
connective tissue atrophy	+	−
tachyphylaxis to therapeutic efficacy	(+)	−
Systemic/glucocorticosteroid effects	(+)	+

than for topical application to skin, the development of glucocorticosteroids with a rapid inactivation by biotransformation has been governed to a large extent to fulfil the demands of inhalation therapy. An example is budesonide, as described by Thalén et al. (1).

Budesonide combines high anti-inflammatory potency at the site of application with a first-pass metabolism of 90% in human liver (2). When glucocorticosteroids are administered by inhalation a further enhancement of their liver biotransformation rate would not reduce their systemic activity much further, due to the fact that the major part (80–90%) of inhaled substance is deposited in the oropharynx and swallowed. This big swallowed fraction is efficiently inactivated in the liver with budesonide (2). Even if in topical skin therapy the absorption rate through skin is low, all absorbed glucocorticosteroid is transported via venous return directly to lung and heart, and is then widely distributed within the body. Only less than one third of the glucocorticosteroid will then go directly to the liver. The glucocorticosteroids in current clinical practice including budesonide, are inactivated by biotransformation mainly in the liver. This means that when current glucocorticosteroids are absorbed from skin only the minor fraction of them is subject to inactivation by first-pass metabolism during the first circulation. Therefore, with the aim to further diminish the risk of provoking systemic glucocorticosteroid actions in children and in adults undergoing intense topical therapy on large skin surfaces, one line of development would be to design new potent glucocorticosteroids, which are rapidly inactivated by extrahepatic biotransformation. From the biochemical point of view this is a similar aim as discussed below.

Reduction of catabolic actions on skin connective tissue

Table I suggests that one important medical advance in topical skin therapy would be to reduce the local catabolic effects seen in protracted therapy (3).

The disappearance of the fibrous components in the connective tissue of glucocorticoid-treated dermis depends on specific blockade of the *de novo* synthesis of these components by dermal fibroblasts: such inhibition is induced without reduction in general protein synthesis (4, 5). The inhibition affects collagen types I and III (6), hyaluronic acid (hyaluronan) (5, 7, 8), and the proteoglycans, demonstrated for their proteins (9, 10) as well as for their glycosaminoglycans (5).

Such reduced dermal connective tissue synthesis gradually leads to a condensed, partly transparent and finally fragile skin. The turnover times of the various fibrous components vary, so that the reduction is seen much earlier for hyaluronic acid and proteoglycan than for collagen. Therefore, in short-term experiments, assessment of the two former components is a better measure of catabolic activity.

As blocked synthesis and not enhanced breakdown is the main mechanism of glucocorticoid-induced dermal atrophy (11, 12), the catabolic process can at least be partly mimicked by metabolic experiments with cultured human skin fibroblasts such as those described by Saarni et al. (7, 8) and Särnstrand et al. (5).

Ponec (13) found for conventional glucocorticoids that their inhibition of connective tissue metabolism in vitro correlated with their anti-inflammatory activity in vivo. Even if the two effects are induced by different cell types, these cells contain the same glucocorticoid receptor, which seems to be uniform for all normal human cells (14, 15). There is no evidence today that these actions can be differentiated at the receptor level. The current knowledge of the chem-

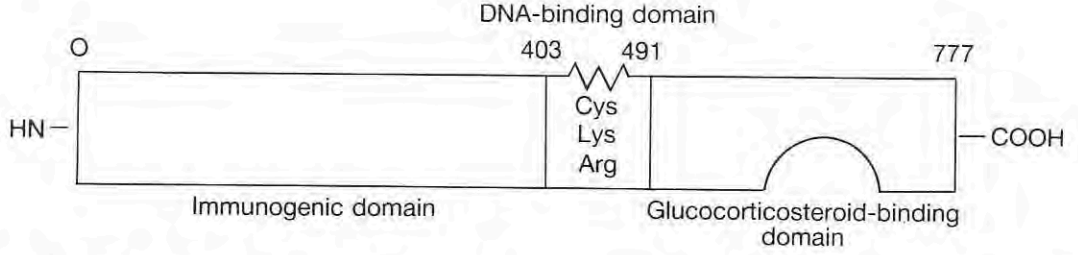


Fig. 1. Schematic structure of human glucocorticosteroid receptor. The figures refer to amino acid numbers from the N-terminal part. (Reproduced from (15).)

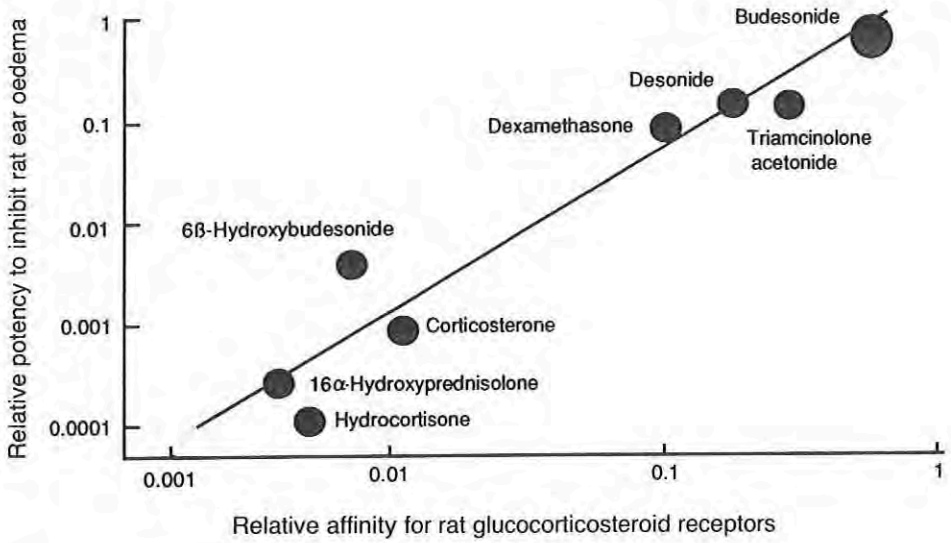


Fig. 2. Correlation between the relative binding affinity to rat glucocorticosteroid receptor determined in vitro (16) and the topical anti-inflammatory potency in the rat ear oedema model (18). (Reproduced from (16).)

istry of this uniform human glucocorticoid receptor has been summarized in Figure 1.

Pharmacokinetics and catabolic effects of budesonide in skin

Budesonide has high affinity for glucocorticoid receptors (16, 17) and potent anti-inflammatory effects on animal (18) and human (18, 19) skin. For budesonide and other glucocorticoids not inactivated locally, there is very close correlation (Fig. 2) between affinity to the glucocorticoid receptor and topical anti-inflammatory potency (16). This shows that at the application site, the steroid activity is greatly dependent on affinity to the receptor, which triggers the subsequent activity.

In binding assay studies using isolated cytosol at 4 °C the budesonide K_D (dose at which the dissociation rate from the receptor is 50%) is 5 nmol/l. This is in keeping with the dose which after prolonged incubation induces some glucocorticoid action in isolated cell systems such as blockade of human basophil histamine release (20), reduced human eosinophil granulation (21) and inhibition of proliferation of lectin-triggered human lymphocytes (22). Prolonged levels of budesonide in the low nanomolar range may also inhibit synthesis and secretion of hyaluronic acid and sulphated glycosaminoglycans (proteoglycans) by cultured human skin fibroblasts (Fig. 3). In this regard, budesonide is less potent than fluocinolone acetonide which blocks such secretion even at 10^{-11} mol/l.

Figure 4 shows the budesonide concentrations obtained in human skin by the in-vitro absorption model of Schaefer et al. (23). For some hours after application of a budesonide ointment 0.025% to intact skin budesonide levels remain stable at around 1 μmol in the epidermis and 100 and 10 nmol in the upper and lower dermis, respectively. The significance of stratum corneum in limiting the percutaneous absorption is shown in Table II. When budesonide is applied to skin from which the stratum corneum has been stripped, its concentrations are approximately one hundred-fold higher than when applied to corresponding intact skin. In most cases (Table II) the concentration difference between epidermis and dermis is also diminished.

This in vitro study shows firstly that in the dermal compartments of skin with damaged barrier budesonide concentrations are reached which would block the production of components of fibrous connective tissue. Secondly, due to the levelling of the concentration gradient in damaged skin, it would not be enough to compensate for the high dermal levels by applying a more diluted glucocorticosteroid preparation. Even then the dermal concentration can be anticipated to be well above the nanomolar level.

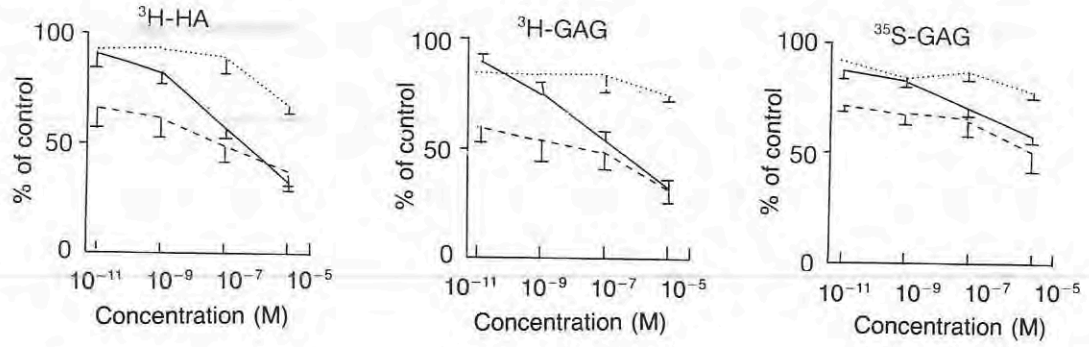


Fig. 3. Effects of budesonide, fluocinolone acetonide and hydrocortisone on synthesis of hyaluronic acid (HA) and sulphated glycosaminoglycans (GAG) from human skin fibroblasts. Fibroblasts in culture were pre-incubated with steroids. After 24 hours 35 SO_4- and 3H-labelled glucosamine were added, and after an additional 48 hours sulphated glucosaminoglycans and hyaluronic acid were isolated. (Reproduced from (5).) ..... Hydrocortisone; —— Budesonide; - - - - Fluocinolone acetonide.

These fibroblast function and skin absorption studies explain why ointment formulations of budesonide, applied under occlusion for 5 days a week, may cause thinning of the treated skin after about 3 weeks (27).

Pharmacokinetics and catabolic effects of budesonide in the airways and lung

Prolonged inhalation of budesonide or other potent glucocorticosteroids does not induce connective tissue atrophy in the airways of lungs (Table I). One reason for this difference between the airways and the skin may be that the fibroblasts in these two tissues may be inherently different in their sensitivity to glucocorticoids.

Skin and lung tissue samples for establishment of fibroblast culture were collected from three patients undergoing lung operations (28): the skin samples were taken from the dermal part of the wound. After several passages the fibroblast cultures were grown to confluency and metabolic experiments were started (Fig. 5). The fibroblasts were first exposed to budesonide for 24 hours and then tritiated glucosamine was added as a hyaluronic acid precursor. After a further

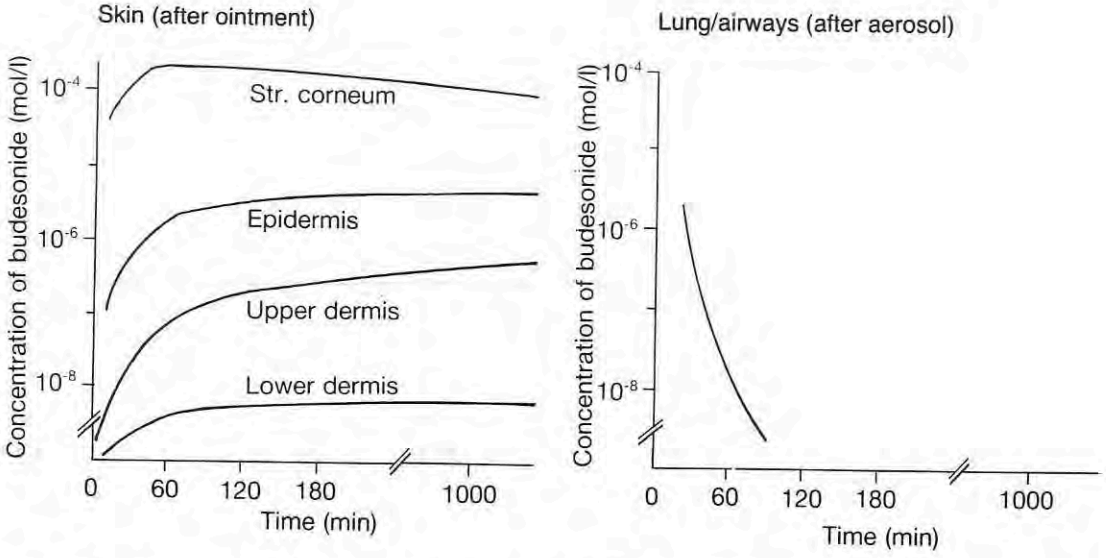


Fig. 4. Schematic presentation of time–response relationship for budesonide concentration in skin and lung tissue, respectively. (Based on (24, 25, 26).)

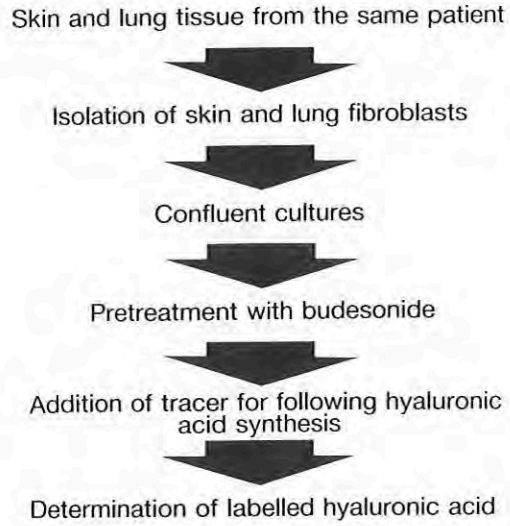


Fig. 5. Methodological basis for the comparison of the glucocorticosteroid sensitivity of skin and lung fibroblasts from the same patient. (For more details see (28).)

24 hours the accumulated labelled hyaluronic acid in the medium was measured. The results showed (Fig. 6) that inhibition of hyaluronic acid secretion by lung fibroblasts needs more than one hundred times the concentration of budesonide than is necessary to induce a corresponding effect in skin fibroblasts. The same difference in sensitivity between skin and lung fibroblasts has been reported from the human foetus (28).

In addition to this difference in inherent sensitivity to the catabolic effects of glucocorticoids, it is claimed that there is also an important pharmacokinetic difference when glucocorticosteroids are applied topically to skin and lung, respectively. As there is no structure in the lungs and airways which has a similar function as that of the stratum corneum reservoir and barrier in the skin, the bulk of steroids deposited in the airways is absorbed rapidly into the bronchial and pulmonary circulation. Figure 4 demonstrates this difference in a schematic way and the distinction has been confirmed by other study results as well (29). This suggests that immediately after inhalation the

Table II. *Budesonide concentrations in skin layers after topical application to intact and to artificially damaged (stripped) human skin. (Unpublished investigation (24).)*

A. *Abdominal skin*

Skin layer	Budesonide (μmol/l)			
	30 min after application		300 min after application	
	Intact	Damaged	Intact	Damaged
Epidermis	0.093	27.9	1.72	18.6
Upper dermis	0.008	7.86	0.047	8.89
	(0.09)	(0.28)	(0.03)	(0.48)
Lower dermis	0.003	0.098	0.006	2.70
	(0.03)	(0.003)	(0.003)	(0.15)

( ) = ratio upper or lower dermis to epidermis.

B. *Skin from lower arm*

Skin layer	Budesonide (μmol/l)			
	30 min after application		300 min after application	
	Intact	Damaged	Intact	Damaged
Epidermis	0.124	31.7	6.45	NI
Upper dermis	0.013	2.41	1.48	NI
	(0.10)	(0.08)	(0.23)	
Lower dermis	0.003	0.10	0.18	NI
	(0.02)	(0.003)	(0.03)	

( ) = ratio upper or lower dermis to epidermis.
NI = not investigated.

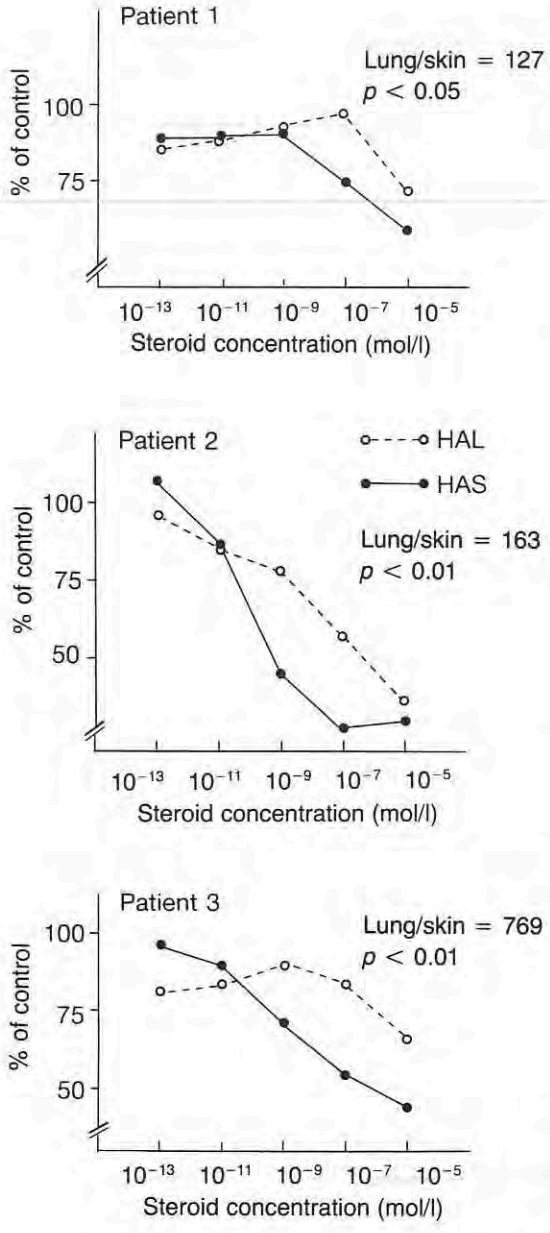


Fig. 6. Effect of budesonide on the secretion of labelled HA into the medium from cultured fibroblasts, established from adult lung (HAL) and adult skin (HAS) from three patients. Values are expressed as means from at least eight cultures per cell line and concentration, and as percentages of vehicle-treated control. The lung/skin ratio refers to the increase in budesonide concentration required to inhibit ^{3}H-HA secretion from lung fibroblasts to the same extent as in skin fibroblasts (calculated by linear regulation analyses). (Reproduced from (28).)

budesonide concentration in the airway and lung compartment is of a similar magnitude as that attained in epidermis of topically treated skin. However, the subsequent time–concentration relationships of the drug in lung and skin are quite different. While the skin concentration of budesonide remains high for many hours, there is a rapid and marked drop in the lungs and airways. Ninety minutes after inhalation of a very high single dose, the human lung budesonide concentration is approximately 5 nmol/l (26), a concentration lower than that needed to reduce markedly the synthesis of connective tissue by isolated lung fibroblasts in vitro (Fig. 6).

Induction of catabolic activity in connective tissue may depend not only on the strength but also on the duration of exposure to the glucocorticoid. Prolonged exposure to just the peak physiological cortisol levels can induce catabolic activity. Figure 7 shows the normal pattern of diurnal variation of plasma cortisol (hydrocortisone) with a morning peak of around 20 μg/100 ml or 500 nmol/l. As a comparison it can be mentioned that the receptor affinity of cortisol is approximately 200 times lower than that of budesonide (16). This cortisol peak, which lasts for only a few hours, does not trigger skin atrophy or osteoporosis. In Cushing's syndrome, which is often characterized by severe skin and bone catabolism, the main deviation is not so much that plasma cortisol rhythm shows an exaggerated peak but that the normal diurnal variation is lost. The lowest plasma cortisol

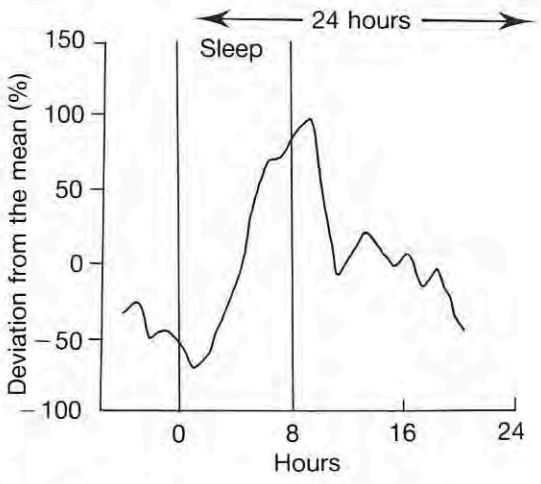


Fig. 7. Schematic presentation of the normal pattern of diurnal variation of plasma cortisol. (Reproduced from (30).)

level in patients with Cushing's syndrome is often of similar magnitude as the peak value in normal persons (31). This close relationship between continuous glucocorticoid exposure and catabolic activity suggests that the more transitory steroid exposure induced in lungs and airways by inhalation will have much less catabolic effect on fibroblasts in these sites than the very prolonged exposure which has to be endured by the fibroblasts in the skin.

Potential ways to reduce the catabolic activity of topical steroids in skin connective tissue

Before discussing potential modes, the first issue to address is whether the blockade of fibroblast function is a key mechanism behind the anti-inflammatory efficacy of glucocorticosteroids? If so, the problem would be insoluble even theoretically. The main anti-inflammatory mechanisms of steroids in skin diseases are poorly known and may differ from disease to disease. However, even if some fibroblast products may markedly contribute to prolonged inflammation and remodelling (especially in sclerotic skin diseases), it is proposed that the main anti-inflammatory actions of topical glucocorticosteroids depend much more on modulation of endothelial cells, keratinocytes, Langerhans cells and recruited inflammatory cells than of fibroblasts. Therefore, with regard to this proposed distinction of key cells behind the anti-inflammatory and catabolic actions, respectively, it is suggested that some separation *per se* might be possible.

This is unlikely to be achieved by trying to differentiate between the glucocorticoid receptors of fibroblasts and those of the other cell types: such receptors seem to be uniform for all normal human cell types (Fig. 1). Pharmacokinetics should provide a better approach. If it is assumed that the main target cells for anti-inflammatory efficacy are located in the epidermis or in the epidermal-dermal junction in the papillae, while the fibroblasts are located in the deeper skin compartment, then a sharp concentration gradient of drug through the skin layers may logically solve the problem. However, to reach a functional separation, the concentration gradient of glucocorticoids in the skin should be much steeper than that obtained with conventional agents (Fig. 4, Table II). As stated above, the glucocorticosteroids of current clinical practice are not inactivated by biotransformation during the absorption process. Thus, what remains to be scrutinized is whether a better profile would be reached in prolonged therapy by steroids, which are metabolically very 'labile' during the absorption process. For a glucocorticoid of that kind the balance between the rates of influx and metabolic inactivation may favour triggering of glucocorticosteroid receptors in epidermis of primarily skin with injured barrier. In intact skin, with its reduced influx, the inactivation rate might outweigh. Thus, theoretically a glucocorticoid of that profile may have a better autoregulating activity, inducing anti-inflammatory activity primarily in areas with injured barrier function.

However, the most important medical aim would be to reach a very steep glucocorticoid concentration gradient within inflamed skin from epidermis to the deeper dermal levels. This may be much more difficult to attain than the effect differentiation between skin areas of high and low absorption discussed above. An obstacle in obtaining improvement of the steroid concentration gradient within inflamed skin is that the transfer rate from epidermis to dermis is probably much faster than the normally slow diffusion rate through the stratum corneum into the 'living' epidermis.

Knowledge of such flux rates, of the metabolic capacities of the different skin layers and of the vascular extraction rates from the dermis is so poor, that the best test of the above pharmacokinetic hypothesis is to assess the results with novel glucocorticoids, which may be partly metabolized in the skin.

The 21-carboxylates

Development of these esters is based on the studies of Laurent et al. in the 1970s. They showed that 21-carboxylic acids are inactive metabolites of certain glucocorticoids: active steroids were designed from their esters (32). The proximity of the C21-carboxylate to the C20-keto groups makes this type of glucocorticoid ester labile. As a consequence the non-protein bound fraction can therefore easily be hydrolized to the inactive carboxylic acid, even by esterases in extrahepatic tissues (33, 34). Wendt, Kapp and colleagues described fluocortin butylester, a weak topical anti-inflammatory compound with a potency similar to that of hydrocortisone acetate, but virtually without systemic steroid effects (35) or skin thinning activity (36). Fluocortin butylester represented an interesting novel principle, but its affinity to the glucocorticoid receptor was too low to be a substitute for the more potent steroids, application of which is limited by their local and systemic adverse effects. However, 21-carboxylates with higher affinities for the

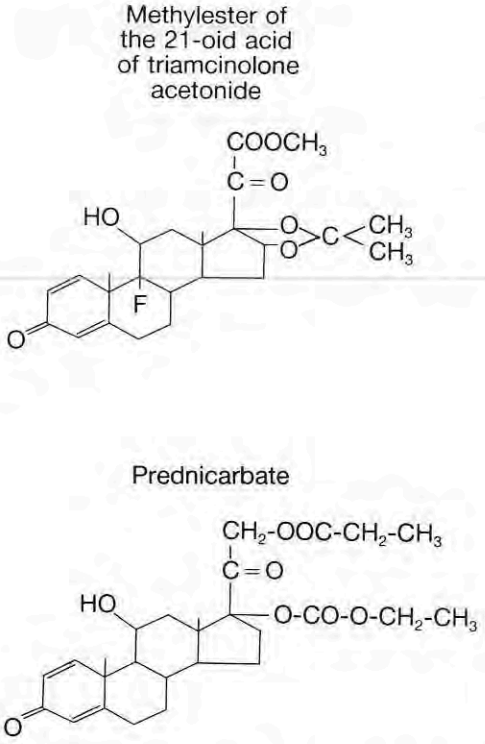


Fig. 8. Chemical structures of methylester of the 21-oic acid of triamcinolone acetonide and prednicarbate.

glucocorticoid receptor, such as the methylester of the 21-oic acid of triamcinolone acetonide, have now been reported (Fig. 8). This compound has about the same high affinity for the glucocorticoid receptor as triamcinolone acetonide (37). It is hydrolyzed in rat as well as in human serum. When tested topically in a mouse model of contact hypersensitivity, triamcinolone acetonide methylester had a much more localized anti-inflammatory efficacy than that reported for conventional glucocorticoids metabolized by the liver (38). This supports the view that the drug is rapidly inactivated during or after the absorption process.

D5115, a 21-carboxylate synthesized at AB Draco, has a 16α,17α-acetal structure. Its affinity to rat glucocorticoid receptor is approximately half that of budesonide (Fig. 2) and similar to that of triamcinolone acetonide methylester. D5115 is rapidly hydrolized in extrahepatic tissue such as lung and plasma. Although it has strong receptor affinity, even in high concentration D5115 lacks topical anti-inflammatory effect in the rat ear oedema model (Fig. 2) (16). The very close correlation in Figure 2 is obviously valid only for glucocorticoids which are not locally metabolized before or during absorption. However, there was a modest anti-inflammatory effect when D5115 was applied to ears with a partially stripped stratum corneum (39). This shows that the extent and/or rate of absorption through the horny layer of skin is the critical step in triggering the anti-inflammatory activity of this type of drug.

When glucocorticoids reach their target sites, they probably do not need to remain there in intact form for long periods of time to trigger some types of anti-inflammatory efficacy. Hamster cheek pouch (40) and rat tracheal studies (41) have shown that topical superfusion with budesonide for just 5–10 minutes is enough to trigger strong anti-permeability activity that starts after 30–60 minutes and lasts for several hours.

These findings suggest that proper comparison of topical anti-inflammatory potency of 21-carboxylates and conventional glucocorticoids should be performed on diseased, and not on intact skin, or on skin mimicking the defective barrier of diseased skin. Whether the local metabolism of 21-carboxylates is fast enough to reduce the catabolic action on connective tissue of diseased skin has still to be elucidated. Also such studies should be performed on inflamed skin.

Carbonate esters

Carbonates are also chemically and biochemically unstable esters. Prednicarbate (Fig. 8) is a prednisolone derivative with the conventional side chain in the 17β-position but with a 17α-ethylcarbonate, 21-propionate ester. In skin the 21-propionate is quickly cleaved off and the 17α-ethylcarbonate group is partially hydrolized (42). The final metabolite is prednisolone, which retains glucocorticoid activity. Prednicarbate has potent topical anti-inflammatory activity on mouse and rat ears (43). When incubated for 2 days with human skin fibroblasts it has little blocking effect on procollagen synthesis (44). However, it is not known whether this missing activity is due to its more marked metabolic inactivation during incubation compared with other glucocorticoids. A 0.25% cream of prednicarbate applied without occlusion to intact human skin for 8 weeks caused no skin thinning (45), but when applied in Finn occlusion chambers for 4 weeks it induced the same skin thinning as 0.1% betamethasone valerate. Thus, with a sufficient influx this compound blocks the connective tissue synthesis of skin.

CURRENT CONCLUSIONS

The above findings suggest that by designing glucocorticosteroids which have the capability to be biotransformed in skin, it may be possible to develop compounds with a more autoregulating anti-inflammatory activity exerted in skin areas with impaired barrier function. In such areas the enhanced influx velocity might overcome the inactivation and/or removal rate, while that balance is insufficient in intact skin for triggering a glucocorticosteroid activity. However, the reported findings have to be reproduced and supported by biochemical analyses of flux and biotransformation rates in intact and in inflamed human skin. If this principle works under clinical practice, such therapy may lead to a better autoregulation of the anti-inflammatory activity to the diseased sites and to an automatic cut off when these sites heal. However, probably only a moderate anti-inflammatory potency may be reached with glucocorticosteroids of that type.

There is still no support that glucocorticoids of that profile will have less catabolic activity on diseased skin than what is provoked by conventional glucocorticoids. However, more studies are warranted with the glucocorticosteroid of highest metabolic turnover and where the skin metabolism leads to inactive metabolites. Even if current as well as forthcoming model drugs of that type would fail to reach the main therapeutic goal requested (reduced catabolic activity in *diseased* skin), such studies will learn us much about the location of the target cells for triggering the anti-inflammatory efficacy and the flux rates into and within skin. This means that for the next years — and especially when a high topical anti-inflammatory efficacy is demanded — we have to learn to handle in the best way the gluocorticosteroids of current clinical practice.

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Discussion

CHAIRMAN: PROF. V.K. HAVU

Prof. V.K. Havu (Finland): Dr Murray, do you think that new steroids are needed? There are plenty of very effective steroids already available.

Dr J.R. Murray (UK): That is not only an interesting question but also difficult to answer, except of course if it is considered in relationship to Dr Brattsand's paper regarding the metabolic degradation of corticosteroids. If we look intellectually at Wilbur Wright's machine in which he first flew, and compare it with a Concorde, we may argue that perhaps a Concorde is not necessary — but nevertheless it is there and it does serve some purpose, albeit perhaps only the transportation of wealthier people. The differences between those two aircraft were accomplished only by many small steps in between. Each step, however small, was important because it represented an advancement. The same stepwise development applies to therapeutic medicine.

There is another factor. I am basically a first-line physician, a general practitioner (GP), with a considerable interest in dermatology. It is the GP who is the main prescriber of topical corticosteroids in Western Europe, and it is the GP who needs steroids with a high ratio between topical activity and adverse reactions — because he is less likely to have the control over patients that physicians in hospital practices will certainly have.

One of the policies that Gist-brocades itself has relied on is the sort of three-phase concept, in which hydrocortisone is always used in the initial phase. Treatment is carried out with Locoid® (hydrocortisone 17-butyrate) if the lesion requires a steroid of medium potency. Another hydrocortisone derivative for a potent steroid is used only if the skin lesion is refractory to the mild as well as to the medium-potent steroid.

To answer your question, yes, I think there is a considerable need for new steroids, certainly one that may be just as useful to your GP colleagues as it is to myself.

Prof. Havu: Dr Kragballe, is the correlation between receptor binding and potency of steroids sufficient to explain potency differences of steroids? Is it simply a question of affinity to receptors?

Dr K. Kragballe (Denmark): According to studies by Ponec in 1980, receptors for steroids have been demonstrated to exist both in human epidermis and in fibroblasts. Binding of the steroid to the receptor was shown to be correlated with the capacity of the steroid to inhibit the proliferation of fibroblasts.

To answer your question, we can certainly agree that at least in some systems there is a correlation between receptor binding and the effect on cell function.

Prof. Havu: Are the receptors present in all tissues?

Dr Kragballe: Yes, I would say in most tissues; there are receptors on all normal cells of the body, and they all respond to glucocorticosteroids.

Prof. Havu: The mechanism of action of corticosteroids covers a vast and complicated area. You told us something about lipocortins. They explain many of the actions of steroids. Are there steroid effects which cannot be explained by the mediation of the lipocortins?

Dr Kragballe: Lipocortin is only one example of a protein which is affected by steroids. As I mentioned, interleukin-1 (IL-1) and possibly many other mediators are also affected by them. It was just an example of an important protein. For example, the effect on leukocyte chemotaxis is indirectly also dependent upon lipocortin because its chemotactic activity which is determined by phospholipase A2 is mediated by lipocortins. I think these are important.

Question from the audience: How do the steroids affect eicosanoids, prostaglandins and leukotrienes?

Dr Kragballe: Eicosanoids, prostaglandins and leukotrienes are derivatives of arachidonic acid; in other words, arachidonic acid is their precursor. The corticosteroids affect the formation of these compounds by modulating the release of this precursor, arachidonic acid. This means that by modifying the release of arachidonic acid from phospholipids the steroids will also affect the formation of other less related

compounds. In addition to modifying the formation of these compounds, the steroids will also inhibit the sensitivity of certain tissues to these mediators. Corticosteroids have therefore a dual effect; it is not an effect only on the formation but also on the sensitivity of the tissues to these mediators.

Dr J. De Bersaques (Belgium): It has been stated that all the effects of steroids are due to one receptor, and that it is the same receptor for all steroids. Are the effects produced through this receptor identical for each steroid once the steroid has bound to the receptor?

Dr Kragballe: It is not a question of a common receptor. There are specific receptors for each type of tissue, and in addition there are only certain genes in one cell type that are affected by this activated receptor complex. The set of genes that are modified by steroids are different from tissue to tissue, so there is no question of an effect on the same receptors or the same genes in a different tissue.

Dr De Bersaques: Considering your statement, my question is now whether different genes in one tissue are influenced if the steroid is changed? [*Dr Kragballe:* What do you mean by changing the steroid?] If another steroid is given to the same receptor, will this affect other genes, or will some other genes possibly be spared? In other words, if another glucocorticoid is given which binds to the receptor, will another group of genes be affected?

Dr Kragballe: I am not aware that this has been studied, but other members of the Panel might have more experience on this matter.

Dr R. Brattsand (Sweden): I think a clear answer cannot be given. Empirically, I do not know of any glucocorticosteroid which has such a differentiating effect on the receptor but, theoretically, I want to stress the fact that glucocorticosteroid receptors are composed of three different parts, of which the glucocorticosteroid site is one. When that part is triggered, the DNA binding site is opened, so that the DNA binding part of the receptor can fuse with DNA. It is complicated because the receptor has to be potentiated further by some other factor, and that could be on the part of the molecule where the steroid is situated. It is therefore this enhancing factor which induces the true promotion of the gene.

In this very complicated process there could be some minor differences because of the differently structured glucocorticosteroids, but much more time is needed to determine that explicitly.

Dr H.L. Muston (UK): If the effects of steroids are mediated only by means of this receptor, does it not follow that it would be impossible to differentiate between anti-mitotic, anti-synthetic and anti-inflammatory effects? If that is the case, if we cannot distinguish between different corticosteroids, does it not also follow that the only advance possible would be through manipulation with pharmacokinetics, so that the advances will be in terms of how the compound behaves in the body generally?

Dr Brattsand: I think I can agree with that. We can indeed not only change potency but also pharmacokinetics.

Dr Muston: Would it not follow that if it is impossible to distinguish between the wanted anti-inflammatory effects and the anti-mitotic and anti-synthetic effects, there is no point in worrying about the potency of new steroid molecules, and that we should really be looking at steroids in terms of their behaviour once they get into the body, which means their distribution and systemic side-effects?

Dr G.E. Piérard (Belgium): I do not think this will be the case. All different tissue and cell types do not respond at the same speed. In the epidermis, Langerhans cells are the first cells to respond, followed by keratinocytes, and subsequently there is the interrelation between keratinocytes and melanocytes. In the dermis, the dendrocytes respond first, and then other elements are affected which become manifest by characteristic side-effects. By manipulating the pharmacokinetics it would perhaps be possible to modify the metabolic activity of some cells and not that of others.

It seems, at least to me, that indeed all the cells that are involved in the immune response are more sensitive than other cells. Perhaps one day we may be able to find a good corticosteroid which would have an anti-inflammatory effect without any significant consequences for the structure of the skin.

Dr J. White (UK): Could the Panel explain the mechanism of tachyphylaxis? Secondly, are there differences between the various steroids, so that we could

possibly use them in a different intermittent way, and thereby get greater effect from them?

Prof. Havu: The phenomenon of tachyphylaxis means that the steroid first appears to cause vasoconstriction and decreased DNA synthesis but, as the days go by and treatment continues, vasoconstriction disappear or decrease while DNA synthesis starts increasing again even when steroid is present. The question is what happens, and why?

Dr Brattsand: We know that there is a downregulation. When studied actively in cells, we can see that one of the protein's messenger RNAs, which is downregulated within a few hours, is the glucocorticosteroid receptor itself.

That has been studied in vitro by Oikarinen and co-workers in Finland in cultured tissue fibroblasts. They cultured for 7 or 9 days, and reported a very strong downregulation of the receptors. There also seems to be an escape from the downregulation. At least, J.-A. Gustafson's group at the Karolinska Institute reported that there is an escape when the receptor is downregulated to about 40%. After that it is impossible to get any more downregulation. This may be one of the mechanisms of tachyphylaxis.

A further possibility — but this is more in the way of speculation — is that if an inflammation is present, there may be changes in the receptors by proteolytic enzymes. I have, however, no sound proof of that.

The tachyphylaxis problem does not occur in asthma inhalation, which seems strange, but it could have been due to the pulse exposure used. Inhalation is carried out twice a day, with a dose-free period between the inhalations. In rheumatoid arthritis it is a well-known fact that glucocorticosteroids help for some months, but subsequently the effect will decrease.

Dr Murray: It is an interesting question from the practical point of view because of course sometimes we GPs make use of tachyphylaxis in an odd sort of way. If I refer a patient to you because he is no longer responding to my therapy, and you change it to something wonderful, I do not tell the patient that you are a better doctor. I explain about tachyphylaxis and that it is the reason why treatment has been changed and become effective.

Dr A. Walker (UK): Are there any good studies to show that *when* we apply steroids to the skin, for example, when the skin has just been made warm by having a bath, or when the skin is cold, this will significantly affect the amount of absorption or indeed the efficacy of treatment?

Dr P.J. August (UK): I can answer that only indirectly. We measured some blood levels of Locoid® under various conditions, one of which was after people had been exercised and their skin made hot and sweaty, and of course the dermal blood flow had also been increased. The hydrocortisone butyrate level in the blood went up three- or fourfold. I suspect, therefore, that if the homeostasis of the skin is disturbed more steroid goes in.

I do not know whether this fact can be used therapeutically. On the whole, we prefer our patients with skin diseases not to exercise too much. Of course, one of the main purposes of admission to hospital is to avoid just that.

Dr Walker: It might explain, however, why sometimes steroid treatment does not appear to be effective. If it is put on when the skin is very cold, and therefore presumably vasoconstricted, it is not absorbed so well.

Dr August: Perhaps it has some relevance, say, to the hands, which are usually colder. I do not think we have ever considered it seriously before.

Prof. Havu: Dr Thalén, how would you explain the fact that de-acetylation or cleaving of the acetyl group from budesonide is more effective than, say, the same processes in corresponding prednisolone 16,17-acetonides?

Dr A. Thalén (Sweden): I think it is a matter of steric hindrance in the molecule, so that cytochrome P450 enzymes cannot reach the acetonide side chains of the 16,17-carbon atoms.

Question from the audience: Dr Ashworth, I very much enjoyed your paper. Could you give some idea about the dynamics of the processes? I am not quite clear about how long the steroid has to be on before the Langerhans cells all disappear, and also about the disaggregation and stimulation experiments.

Dr J. Ashworth (UK): The first experiment I did with different strength steroids was to look for an alternative way of classifying them simply. We initially as-

sessed the effect on six different strength steroids after one application. These preliminary experiments showed that a single application of most corticosteroids reduced Langerhans cell numbers. The assessment took place 24 hours after application.

Although five of the six different strength steroids reduced Langerhans cell numbers, there was no statistically significant difference between these five and hydrocortisone. That is why in the following assessments twice a day for 1 week was arbitrarily chosen. In other words, 24 hours after a single application there is some effect on Langerhans cell numbers and, one would assume, therefore some effect on immune function. Langerhans cell numbers seem to return to normal within about 14–21 days after stopping the application of topical corticosteroids.

With regard to function, we can observe that psoralen and ultraviolet-A (PUVA) therapy has similar effects on skin immunity because it decreases the allo-antigen presenting capacity of skin. If immune function is measured by allergic contact dermatitis reactions there is a return to normal about 3 weeks after stopping PUVA therapy. I guess that PUVA treatment and steroids have the same type of immunosuppression, but I have no data to prove it.

I think the effect starts almost immediately, and recovery takes about 3 weeks.

Prof. Havu: You suggested that the immune function tests which you presented could be used as an alternative to the vasoconstrictor test in screening steroids. However, your tests are very complicated and time-consuming. Are they a realistic alternative?

Dr Ashworth: The vasoconstrictor assay should be accepted for what it is, which is a very crude way of measuring an effect of a topically applied corticosteroid. It is also a reasonable screening procedure if you are considering very preliminary modes of screening of topical corticosteroids. I have great difficulty in believing that the anti-inflammatory effect of a topical corticosteroid is primarily upon blood vessel smooth muscle, which is what causes vasoconstriction.

As has been mentioned before, it is the GP who presumably prescribes the vast majority of topical steroid preparations that patients use. If we want to give them the best possible information in order to allow them to decide what they want to prescribe, it makes more sense to measure a function of steroids which is presumably much more directly relevant to their anti-inflammatory mechanisms. In general, most of the functional assays are expensive and time-consuming. I would not recommend using them as a primary screening procedure.

However, the measurement of their effect upon Langerhans cell markers is very simple, and lends itself easily to statistical analysis. Many different steroids may be compared together in the same individual, if desired, by using the skin of the back, for example. I do not think that is an unreasonable alternative.

The other point, of course, is that if we continue to use a vasoconstrictor assay, which is now 27 years old, and just accept it as a measurement of steroid efficacy, it does not help our understanding of what steroids do. It was asked earlier whether it is really true that the anti-inflammatory actions of steroids on the skin are not understood. I agree with that. We know that they do various things to IL-1 and so on, but what steroids do to the skin is not understood. I am simply trying to encourage people to look at the effects of steroids much more scientifically.

Dr Murray: From the point of view of the pharmaceutical industry, obviously the vasoconstrictor assay is extremely useful as a sort of blunderbuss type of screen. One of the biggest problems with which we are faced — and, as a clinician, I sometimes find it rather confusing — is the problem between formulations, where there is the same glucocorticoid but different vasoconstrictor assay results with different vehicles. Perhaps having something more precise or standardized would help us to choose the most appropriate vehicle for that particular steroid.

Prof. Havu: Dr Piérard, you showed us some very interesting pictures of dendritic cells in the dermis. Can you explain what those cells are and what they are doing?

Dr Piérard: We do not know what dendritic cells are doing. They have been identified by the fact that they have several features in common with the monocyte macrophage system. Dendrocytes are known to be capable of phagocytosing many things. They are present in the superficial dermis and are known to participate in several disorders. Specifically, dermatofibromas have now been described as dendrocytomas because they consist of cells which are probably dendrocytes. They are also found in reasonable numbers in other skin disorders, mainly those related to inflammatory and immune disorders.

It is now thought that these cells play a certain role in the immune surveillance within the skin, particularly within the dermis. They are affected by corticosteroids.

Dr E. Sahan (UK): Do you think that the different experimental tests available at present for the estimation of potency of local steroids give any idea of their long-term side-effects, for example, skin atrophy?

Dr Piérard: That is difficult to answer. There are many methods by which this type of effect can be measured. For example, ultrasound is now used to measure the thickness of the skin, but when differences in skin thickness occur exactly which layer of the skin is thinned, or what part of the manipulation has induced that skin thinning, is not known. Each device which is placed on the skin will exert a certain pressure, and it is therefore perhaps not the real thickness of the skin that will be measured at that time.

There are other methods of assessing the long-term effects of steroids. It is possible, for instance, to measure the suppleness of the skin. We did some of this work, and have tried to make some comparison between the early and the late changes. Usually, but not always, there is a correlation between the speed of appearance of the side-effects and the intensity of the late effects — but what seems to be the general rule will not always represent the true happenings.

Question from the audience: Dr August, future restrictions which will be in force for UK practice in an attempt to control the cost of drugs will mean that the introduction of new steroids will be more difficult. Any new steroid will have to supplant an existing steroid already in the drug formulary. It will have to have some major advantage in use or in cost. Do you not think that even the incidental use of these steroids will be questioned?

Dr August: If you fail to be interested in the hospital formulary. It is up to you and your hospital to ask for something if you are convinced of its value. I think it is an appalling impertinence by a general physician to tell us what we may or may not use. We have had this once or twice in hospitals I serve, but in general I think they understand that if you feel you want something you should have it. Just as they would not like to be told what to use, say, on the lung. I think you have to go out and fight for what you want.

Dr P.C. van Voorst (The Netherlands): I found Dr August's presentation interesting because such a personal view may generate a lively discussion. He stated that Locoid® is often prescribed in The Netherlands because it is manufactured there. On the other hand, I suspect that Dr August mentioned those steroids in his presentation because they are manufactured in the UK. Perhaps, however, a supranational approach should be taken, especially with regard to paediatric dermatology which Dr August mentioned. This is an important field in terms of problems with topical steroids, in which preparations both of the Dutch and of the UK firms could be of value.

Could you give any more recently available information about the clinical effectiveness of Preferid® as compared to Dermovate® (clobetasol propionate)? Is anything known about their clinical efficacy?

Dr Murray: Dermovate® is clearly in a class of its own. It is a very potent steroid, more potent than Preferid®. Preferid® is much closer to Diprosone® (or Diproderm®) (betamethasone dipropionate) in potency, and it is certainly more potent than betamethasone-17-valerate in the clinical studies that we have done. That is fortunately quite a clear position.

Question from the audience: Dr Brattsand, I was fascinated by the idea how we could look at steroids and how they worked on the lung and on the skin and then think backwards. You are suggesting that if there was a steroid that could be hydrolyzed or in some way inactivated more quickly, there would be less unwanted effects. What I could not understand is that you did not take into account the depot effect in the epidermis and the fact that the steroid filters through from this depot for another 2 weeks after it has arrived there. Is not the penetration through the epidermis and the fact that the steroid continues for a long while in skin and not in lung presumably one of the major factors in those unwanted effects?

Dr Brattsand: There are two ways by which such effects might be regulated. First, perhaps by using a different contact time that may be required to induce some but not all the effects. That is the case, for instance, in the lung where pulse exposure can be applied, and where some anti-inflammatory effects can be induced without getting a prolonged concentration.

Theoretically, that would be of value in skin if it

was possible to get a very rapid penetration which stopped after a time. However, we are not close to doing that; it is not at this moment a realistic possibility.

The second, and only realistic possibility for differentiation arises because, as we have heard, many important therapeutic effects may happen in the stratum Malpighii in the epidermis. If steroids could be found that are naturally broken down continuously during the absorption process, but still reach sufficient concentration at that layer, and which could further be reduced in the dermis where there is perhaps not the same need for the same high concentration from the therapeutic point of view, then we could have exactly what we need.

However, we do not yet have such steroids. For example, I mentioned that prednicarbate causes atrophy of the skin on sites with a high permeability, which in itself is a condition for even more rapidly biotransformed compounds. This is of course only a theoretical assumption. I have no valid evidence for it, but I think the principle can be used to develop moderately potent steroids that are poorly absorbed or where the small portion that is absorbed on intact skin is biotransformed during the absorption procedure so that there are neither therapeutic effects nor side-effects. This may offer some possibilities for improvement.

Dr Ashworth: With regard to the question about the depot effect and one or two earlier questions about the reason for developing more steroid molecules, in my opinion the vital question is where the steroid will eventually exert its action, rather than whether or not it will be further absorbed systemically. Is this not a question of the transport ability of the vehicle rather than of the intrinsic properties of the steroid molecule itself?

Dr Brattsand: I am not familiar with the pharmaceutical aspects of steroid preparations. I think that the vehicle is important only for the transfer of the steroid through the stratum corneum, after which it does not do much. The further effects of the steroid therefore depend much more upon the inherent properties of blood supply and metabolic turnover at the site of application.

Prof. Havu: We can establish from this session that corticosteroids remain the most potent topical anti-inflammatory agents that we have, and will probably remain so for a long time to come. Constant progress is taking place in research, and we can only guess what we will have in our hands after some years.

I would like to thank all the speakers, discussants and the audience who took part in this part of the symposium. Thank you very much.

The Immunology of Skin Diseases

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Immunology of Skin Diseases: Chairman's Introduction

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The skin has always been recognized as an organ of defense. With the development of the scientific discipline of immunology, a new area of research, immunodermatology, became established. In immunodermatology, both the immunophysiology and immune homeostasis as well as the immunopathology of skin diseases are being investigated.

The program of this afternoon will focus on immunological aspects of the most important dermatoses having immunological factors or even being entirely immune-mediated. After an introduction emphasizing new areas of interest for immunodermatologists, we will hear an overview of one of the most exciting new developments in immunodermatology, that of the recognition of keratinocytes as a source for a wide variety of cytokines. Following this, we will have an overview of the most important dermatoses in which immune mechanisms are operative. These include the acquired blistering diseases, cutaneous lupus erythematosus, psoriasis, contact dermatitis, and atopic dermatitis. Progress in the understanding of these diseases will be summarized by a faculty of specialists in these skin diseases.

Progress in immunology has been impressive, especially during the last decade. For dermatologists, it will be increasingly difficult to follow the developments in the understanding of the immune system, not in the least because immunology is shifting towards molecular biology. It is expected that the lectures of this afternoon session will help us to better understand some of the most prevalent dermatoses with which we are confronted in daily practice.

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Concepts in Immunodermatology

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This review describes the various processes which take place in the immunological network of the skin under physiologic or pathological circumstances. A full understanding of the mechanisms in action is important as it could lead to the development of an entirely new class of therapeutics.

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The skin forms the outer surface of the body. The high impermeability of the stratum corneum, its physical strength and its relatively fast renewal rate protect the body against external influences. Besides this physico-mechanical barrier function, it has recently become clear that the skin also functions as an immune organ (1). This is evidenced by the presence of resident and recirculating bone marrow-derived cells and the interconnection of the skin with other immune organs.

Immunodermatology is concerned with the study of the immunological events in healthy and diseased skin. This article describes the immune system of the skin and also focuses on three areas in which recent immunological developments have contributed to our understanding of the pathophysiology of the skin. These areas are cellular adhesion, antigen presentation and the cytokine network (Fig. 1).

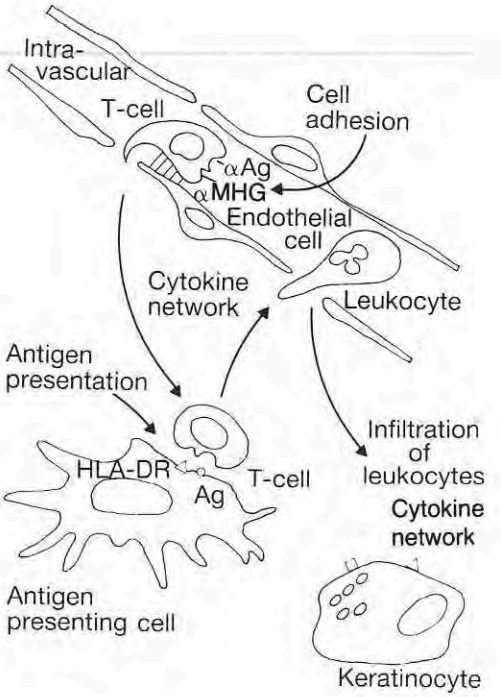


Fig. 1. The three topics of this paper in one figure: 1) cell adhesion involved in extravasation of immune cells; 2) antigen presentation by the Langerhans cell to a T-helper lymphocyte and 3) the immunoregulatory cytokine network, with a participating keratinocyte.

THE IMMUNE SYSTEM OF THE SKIN

As an immune organ the skin is capable of sequestering, processing and presenting antigens and of eliciting a local immune response. To that end, skin contains immunocompetent bone marrow-derived cells such as resident histiocytes and mast cells, recirculating or recruitable Langerhans cells, lymphocytes and granulocytes (Fig. 2).

In particular the Langerhans cells — as the antigen presenting cells of the skin — play a pivotal role in the initiation of an immune response (2). They form an intricate, almost closed network of dendrites. It is estimated that the epidermis of human skin contains more than 10^9 Langerhans cells (3). In addition to their distinctive morphology and the typical Birbeck granules, Langerhans cells can easily be recognized microscopically by staining their membrane markers such as human leukocyte antigen (HLA)-DR and CD1.

It has been shown in mice experiments that Langerhans cells are circulating cells with a limited stay of approximately 3 weeks in the epidermis (4). They probably leave the skin by means of lymphatic vessels and migrate as veiled cells to skin draining lymph nodes.

Lymphocytes are also present in the skin. They can

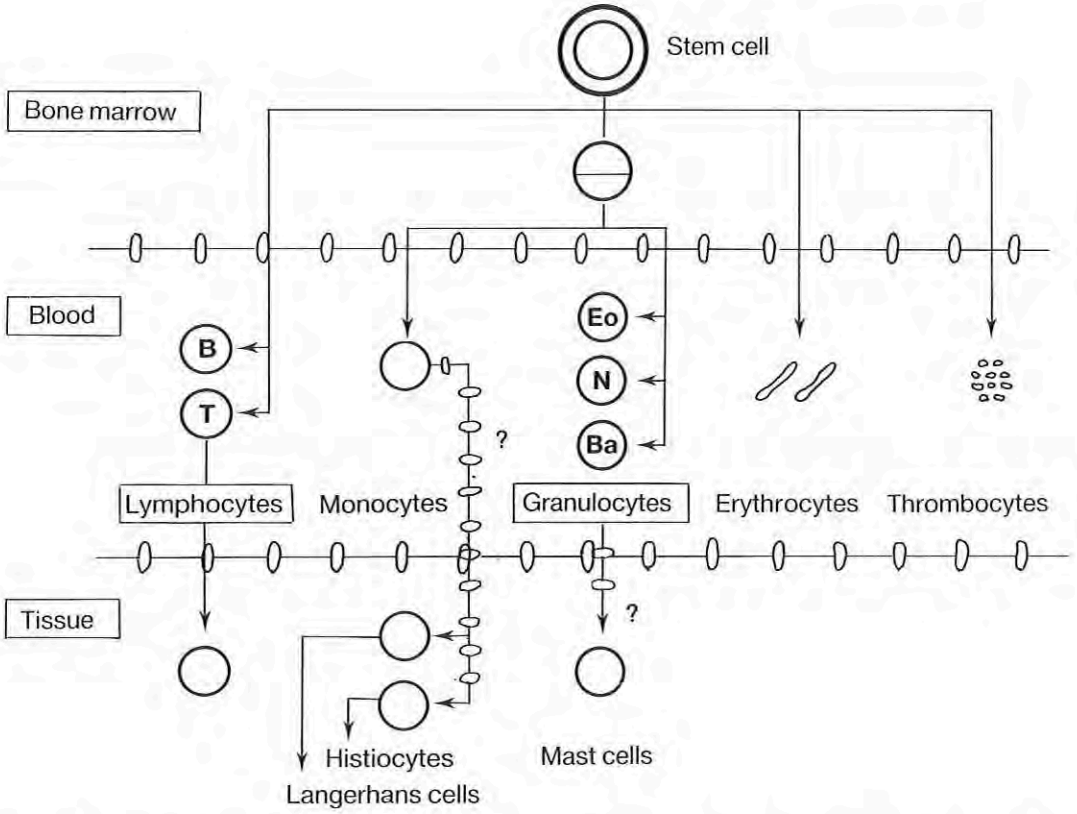


Fig. 2. Cells of the immune system are derived from bone marrow stem cells. They either circulate in the blood or migrate to tissues, from where they recirculate via the lymphatic system or differentiate into tissue-specific resident cells.

be divided into B-lymphocytes, responsible for the humoral immune response and T-lymphocytes, responsible for the cellular immune response. Only T-cells appear to be dermatotrophic. In this respect it is of interest that although the majority of leukemias is of B-cell origin, virtually all lymphocytic malignancies with infiltration of the skin are of T-cell origin (5).

At the moment there is some debate about the existence of a specific skin T-cell population, bearing a particular type of T-cell receptor.

Other well known bone marrow-derived cells are the histiocytes or tissue macrophages, which act as a first-line defence system of scavenger cells, and mast cells, which are of importance in allergic reactions and the release of vasoactive amines.

Keratinocytes and endothelial cells are also involved in the immune response of the skin. Although it is still unclear whether they play a role in the immunological homeostasis of the skin, they certainly do so during an inflammatory response. Keratinocytes are then induced to express HLA-DR antigens and to produce cytokines such as epidermal cell-derived thymocyte activating factor which later turned out to be Interleukin-1. Endothelial cells also become HLA-DR positive and express increased numbers of adhesion molecules, thus allowing rapid extravasation of inflammatory cells.

Two important concepts have been proposed to

Table I. *The cellular and humoral components of the skin immune system.*

Cellular components	Humoral components
Keratinocytes	Anti-microbial peptides
Endothelial cells	Fibrinolysins
	Eicosanoids
Langerhans cells	Neuropeptides
Histiocytes	
Mast cells	Complement components
Granulocytes	Immunoglobulins
T-lymphocytes	Cytokines

collectively describe factors which are involved in the immunological homeostasis of the skin. These are the skin-associated lymphoid tissue (6) and the skin immune system (7). The first comprises Langerhans cells, epidermotrophic T-lymphocytes, keratinocytes and draining lymph nodes. The components of the latter, which also include humoral factors, but exclude lymph nodes, are listed in Table I.

ADHESION MOLECULES

Direct cell to cell contact is an important prerequisite for many biological functions. A helper T-lymphocyte, for example, must bind physically to antigen-presenting cells to become activated. Extravasation of circulating lymphocytes in lymph nodes or granulocytes in inflammatory lesions also involve a specific adhesion step between these cells and the vascular endothelium (8). Extravasation is not a random process but is directed by the distribution of 'homing receptors' regulating the homing and trafficking of immune cells. The highest rate of extravasation takes place in the lymph nodes which therefore have a specialized endothelium, the high endothelial venule. High endothelial venule cells have, in contrast to other vascular endothelial cells, a typical morphology of cuboidal cells (9). Structures like high endothelial venule have also been found in non-lymphoid organs, but only in conditions of chronic inflammation, including autoimmune lesions. This suggests that this typical morphology favors extravasation of cells.

Different types of adhesion molecules have been identified on circulating lymphocytes, monocytes and macrophages and on endothelial cells. Some of these molecules are descriptively called 'homing receptors' and 'vascular addressins', as their distribution is organ specific. This is illustrated by the preferential extravasation of T-lymphocytes in peripheral lymph nodes and dermis and of B-lymphocytes in mucosa or gut-associated lymphoid tissue.

The lymphocyte function-associated antigen on lymphocytes and its receptor, the intercellular adhesion molecule on endothelial cells, are amongst the best studied adhesion molecules (10, 11). The intercellular adhesion molecules are present at low density on vascular endothelial cells, but their expression is greatly increased in inflammatory conditions to allow increased extravasation of immune cells. Several cytokines such as interleukin-1, tumor necrosis factor and gamma-interferon have been shown to be potent inducers of endothelial adhesion molecules.

The indispensability of adhesion molecules is shown by patients with a leukocyte adhesion deficiency. These patients have a genetic defect in the production of lymphocyte function-associated molecules resulting in clinical symptoms such as impaired wound healing, recurrent and progressive soft tissue infections and persistent leukocytosis (12).

ANTIGEN PRESENTATION

The Langerhans cell is the antigen-presenting cell of the skin (13). In healthy epidermis it is the only cell type which is positive for major histocompatibility complex class II molecules (HLA-DR in man). T-cell receptors on antigen-specific T-lymphocytes recognize antigens on Langerhans cells only in association with HLA-DR. This phenomenon is called major histocompatibility complex restriction of antigen recognition. Classical experiments on mice have shown that the T-cell response elicited in allogeneic skin grafts, whereby donor and recipient differed in their class II antigens, was restricted to the class II of donor origin, thus proving the role of Langerhans cells in antigen presentation.

Any immune response must be balanced and therefore both T-helper and T-suppressor cell circuits must be activated. In general the net result will be a helper response which must be suppressed by the time an infection is under control. Langerhans cells preferentially activate T-helper cells (5). Small doses of ultraviolet B radiation selectively deplete the skin of functional Langerhans cells and the skin becomes refractory to the induction of contact hypersensitivity. A particular cell, the Granstein cell, which is more resistent to ultraviolet radiation than Langerhans cells, has been implicated in the induction of T-cell suppression. T-helper and T-suppressor cells can generally be distinguished by their membrane markers. T-suppressor cells carry the CD8-antigen and T-helper cells the CD4-antigen (which is also the receptor for the human immunodeficiency virus).

The binding of the T-helper cell to the Langerhans cell is the first step in the initiation of the skin immune response. The following steps have only partly been elucidated. Activated T-helper cells produce gamma-interferon, which induces keratinocytes to express HLA-DR and to produce interleukin-1, which in turn activates new T-lymphocytes to enhance the immune reaction. T-helper cells migrate to the draining lymph

nodes where they proliferate by locally produced interleukin-2. Antigen-specific effector cells leave the lymph node and 'home' to the original lesion. This is facilitated by local production of chemotaxins and the induced expression of cell adhesion molecules and HLA-DR on the endothelial cells.

This is, of course, only part of the story, since many different mediators with immunoregulatory functions have been identified in inflammatory lesions. These interact in a complex cascade of events. Before they will be discussed, some background information on the major histocompatibility complex system and the CD-antigens will be presented first.

Major histocompatibility complex system

The MHC system (HLA in man and H2 in mouse) is a polymorphic, codominant system of loci coding for a series of membrane glycoproteins which are involved in the immune response. HLA-antigens can be subdivided into class I and class II antigens. Class I antigens (HLA-A, B and Cw) are present on all nucleated cells and serve as restriction elements for cytotoxic T-lymphocytes of the CD8 phenotype. Class II antigens (HLA-DR, DQ, DP and DZ) are expressed on antigen-presenting cells such as the specialized Langerhans cell, but also on B-lymphocytes, macrophages and activated T-lymphocytes. They are inducible on several non-immune cells, such as keratinocytes and endothelial cells, in inflammatory conditions. Class II antigens serve as restriction elements for cytotoxic T-lymphocytes of the CD4 phenotype and, most importantly, for antigen-presenting cells.

There is a long list of diseases with an immunological component associated with particular HLA-antigens. The strongest association is of HLA-B27 with ankylosing spondylitis with a relative risk of 87.4. Dermatological diseases with an immunological component, such as dermatitis herpetiformis (HLA-DR3, with a relative risk of 15.4) and psoriasis (HLA-Cw6, with a relative risk of 13.3) also show significant associations. The reason for these disease-HLA associations is still unclear (14).

Cluster determinants of leukocyte differentiation antigens

CD-antigens are a large group of membrane markers (glycoproteins) which were originally identified by different monoclonal antibodies on bone marrow-derived immune cells. Many of the more than 70 CD-antigens are now biochemically and functionally well characterized. A few examples follow below.

CD1 (originally T6) is an early thymocyte differentiation marker, which is also present on Langerhans cells. CD3 is part of the T-cell receptor which consists of five subunits. CD4 (T-helper) and CD8 (T-suppressor) are markers for functional T-cell subsets. CD11 and CD18 make up the lymphocyte function-associated adhesion molecule family.

THE CYTOKINE NETWORK

The skin contains many immunoregulatory mediators (15). In particular, the arachidonic acid metabolites, the prostaglandins and leukotrienes have been studied most intensively. Steroidal inhibitors of phospholipase A_2, one of the key enzymes in the biosynthesis of these metabolites, proved to be a valuable class of anti-inflammatory drugs. More recently attention has shifted to the study and clinical application of cytokines.

Cytokines is a collective term for a heterogeneous group of proteins with an immunoregulatory function. They include:

— interleukins (interleukin-1 to interleukin-8);
— hematopoietic growth factors or colony stimulating factors;
— interferons alpha (20 subtypes), beta and gamma;
— tumor necrosis factors alpha and beta;
— growth factors, like platelet-derived, epidermal, transforming, fibroblast and nerve growth factors;
— suppressor factors.

An overview of dermal cytokines and their function is given elsewhere in this issue by Dr T.A. Luger.

Cytokines play a role in the differentiation, proliferation and activation of cells. Not so long ago, it was thought that these factors were produced by and acted on immune cells only, but now there is ample evidence that non-immune cells can also be induced to produce one or more cytokines.

The elucidation of the role of cytokines in homeostasis and pathophysiology is hampered by the fact that most cytokines have pleiotropic effects and that they can act locally as well as systemically, in a hormone-like fashion. Moreover, they often act synergistically and interact with cells and other mediators to finely tune the immune response.

Due to recombinant DNA technology most of the cytokines are now produced in large quantities so that their therapeutic value can be assessed. Interferon

alpha-2 has already been registered for dermatological disorders and trials with epidermal growth factor show some promise in wound healing, although most likely cocktails or consecutive dosages of different cytokines must be used for optimal efficacy in the long run (15, 16).

CONCLUSION

It is beyond doubt that a fundamental understanding of the processes involved in cell adhesion and antigen presentation, and the elucidation of the role of cytokines will create the tools to modulate the immune response. This will lead to the development of a new class of therapeutics in the near future. Dermatology is very likely to profit from such developments.

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Epidermal Cytokines

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The concept of the epidermis being a site for the initiation of immune responses has only been developed over the past decade. There is strong evidence that epidermal cells have immune functions. Accordingly the epidermis harbors dendritic cells having antigen presenting capacity and cells belonging to the T-cell family. Moreover, keratinocytes are capable of secreting various immunomodulating cytokines or secretory regulins. Cytokines are glycoproteins which are synthesized and secreted by various cells, bind to specific receptors and regulate activation, proliferation and differentiation of immune as well as non-immune cells. Keratinocytes upon injury i.e. mechanical irritation, ultraviolet irradiation, tumor promotors, synthesize and release interleukin-1α, -1β, -6, -8, colony stimulating factors, tumor necrosis factors-α as well as growth (transforming growth factor β) and suppressor (epidermal cell-contra-interleukin-1) factors. Since there is strong evidence in support of a network of interacting cytokines maintaining a proper balance which only partially has been discovered, so far no causative role of a single cytokine has been established in any disease. However, excessive or insufficient production of these mediators may contribute to certain disease states, particularly those of infectious and autoimmune origins. Thus, cytokines appear to be promising candidates for the treatment of infectious, autoimmune, immunodeficiency and malignant diseases. Future studies are necessary to clarify the therapeutic efficacy of the combined application of different cytokines and more investigations are also needed to elucidate the lymphokine cascade in vivo.

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The concept of the epidermis not simply being a mechanical barrier to the outside but also a site for the initiation of immune responses has only recently been proposed. This concept is based on findings that at least three types of epidermal cells have immune functions. Dendritic bone marrow derived cells such as Langerhans cells are potent stimulators of many types of T-lymphocyte responses (1). Recently cells belonging to the T-cell family also have been identified within the epidermal cell layers (2). Moreover, keratinocytes are capable of producing a variety of immunomodulating secretory regulins (3). Although keratinocytes appear to be the major source of these mediators there is evidence that other epidermal cells such as Langerhans cells and melanocytes may also release these factors (3).

Secretory products of lymphocytes and monocytes regulating growth and differentiation of immunocompetent and hematopoietic stem cells were originally defined as lymphokines. Since it became evident that neither production nor effect of these factors is restricted to the immune system, names such as cytokine or secretory regulin appear to be more appropriate (4). Cytokines are proteins or glycoproteins which are produced by various cells and by means of binding to specific receptors on target cells regulate cell growth and differentiation. Moreover, there is increasing evidence for a complex network of interacting cytokines which are not only involved in the activation of immune and non-immune cells, but also mediate non-specific inflammatory reactions. This is further complicated by the finding of other soluble factors such as hormones and eicosanoids interfering with cytokine activities (5).

Murine keratinocytes have been proven to synthesize and release interleukin-1α,-1β, -3, -6, colony stimulating factors (granulocyte/macrophage colony stimulating factors) and transforming growth factor β. Human keratinocytes and keratinocyte cell lines are known to produce interleukin-1α,-1β, -6, -8, both granulocyte, macrophage, and granulocyte/macrophage colony stimulating factors, tumor necrosis factor-α, basic fibroblast growth factor, and transforming growth factor-α and -β. Moreover, several suppressor factors such as epidermal cell suppressor factor and epidermal cell-contra-interleukin-1 have been identified in supernatants of murine and human keratinocyte cultures (Table I) (6).

In the following secretory regulins produced by

Table I. *Epidermal cell derived cytokines.*

A.	Non-specific multifunctional mediators
	Interleukin-1α and -β
	Interleukin-6
	Tumor necrosis factor-α
B.	Leukocyte stimulating factor
	Interleukin-8
C.	Colony stimulating factors (CSF)
	Interleukin-3 (multi CSF)
	Granulocyte-macrophage CSF
	Granulocyte CSF
	Macrophage CSF
D.	Growth factors
	Transforming growth factor-α and -β
	Basic fibroblast growth factor
	Platelet derived growth factor
E.	Suppressor factors
	Contra-interleukin-1
	Keratinocyte lymphocyte inhibitory factor (transforming growth factor-β)

epidermal cells will be described briefly and their role in the pathogenesis of skin diseases as well as possible therapeutical approaches will be discussed.

INTERLEUKIN-1

A mediator which augmented T-lymphocyte response to mitogens and antigens and co-stimulated the proliferative response of thymocytes, was originally named lymphocyte activating factor. Subsequently it was shown that several previously described cytokines such as endogenous pyrogen or catabolin, osteoclast activating factor and hemopoietin-1 were identical molecules and therefore the term interleukin-1 was introduced (7). Moreover, it became evident that interleukin-1 production is not only confined to the monocyte/macrophage, but that many different cells and tumor cells are capable of producing this mediator (7). Accordingly, the first cytokine proved to be produced by keratinocytes was a product which like interleukin-1 co-stimulated thymocyte proliferation and was thus named epidermal cell derived thymocyte activating factor (8, 9). It soon became evident that this epidermal cell derived thymocyte activating factor is biochemically as well as biologically indistinguishable from interleukin-1. Definite proof that both molecules are identical was finally provided by recent investigations showing that both murine and human keratinocytes express the mRNA specific for interleukin-1 (Fig. 1) (10, 11).

Two different forms of interleukin-1, -1α and -β encoded by distinct genes both located on chromosome 2, have been identified. Both interleukin-1 forms are initially translated as 31 kD precursor polypeptides lacking a signal sequence and subsequently cleaved into the 17 kD extracellular form. Although there is only 27% homology between the interleukin-1 subtypes, they share the same spectrum of biological activities (12). Keratinocytes are capable of synthesizing and secreting both forms of interleukin-1. In contrast to macrophages the predominant biologically active form to be released by keratinocytes is interleukin-1α (10). This may be explained by the observation that biologically inactive 31 kD interleukin-1β has to be cleaved into the active 17 kD moieties by a specific protease which in contrast to macrophages is not present in keratinocytes (13). However, since interleukin-1α and -1β bind to the same receptor with the same affinity and have identical biological activities, the different capacities of distinct cells to produce either one of the interleukin-1 forms appears to be biologically irrelevant. Interleukin-1 receptor expression has been demonstrated on many different cells emphasizing the important regulatory role of interleukin-1 (14). The expression of interleukin-1 receptors by keratinocytes and the observation of interleukin-1 inducing keratinocyte proliferation and cytokine production indicates an autocrine function of interleukin-1 within the skin (15).

Interleukin-1 is a multifunctional secretory regulin and its production is induced by damaging stimuli such as ultraviolet irradiation, tumor promotors and bacterial products, but also by cytokines including colony stimulating factors, tumor necrosis factors α, interleukin-6 and interleukin-1 itself (Fig. 1) (3, 16). The lymphocyte activating capacities of interleukin-1 include the co-stimulation of T- and B-lymphocytes as well as natural killer cells (Fig. 2). Accordingly, interleukin-1 stimulates T-cells to produce interleukin-2 and -4, interferon and colony stimulating factors, increases T-cell interleukin-2 receptor expression and is chemotactic for T-cells (7, 17). Moreover, interleukin-1 co-stimulates B-lymphocytes by enhancing the effects of B-cell stimulatory factors (interleukin-4, -5 and -6) (18). Monocytes are stimulated by interleukin-1 to produce more cytokines such as interleukin-1, -6 and tumor necrosis factors-α as well as eicosanoides (16). Among immunocompetent cells in the epidermis, interleukin-1 has recently been shown to be one of the mediators responsible for

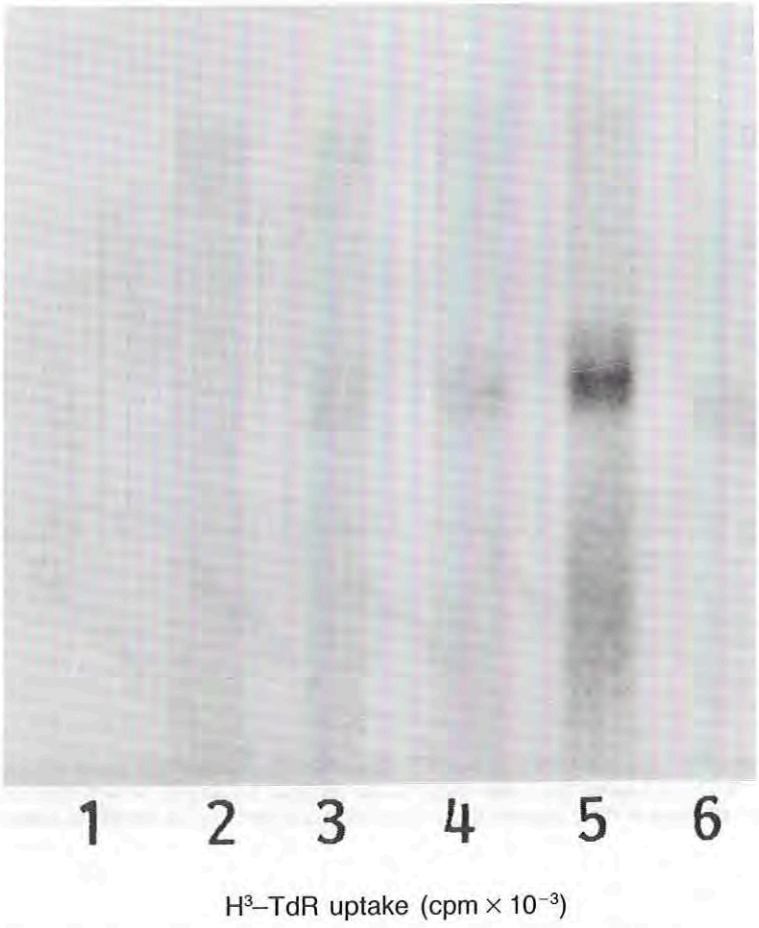


Fig. 1. Northern blot analysis for the expression of interleukin-1α. Human epidermoid carcinoma cells (A431) were incubated for 0 hours (lane 1), 3 hours (lane 2), 6 hours (lane 3), 12 hours (lane 4), 24 hours (lane 5) or 36 hours (lane 6) in the presence of rh-interleukin-1α (1 U/ml). For Northern blot analysis of total cellular RNA (20 μg/ml) a ^{32}P-labelled cDNA encoding for interleukin-1α was used.

Langerhans cell differentiation (19).

In addition to its effect on cells of the immune system, interleukin-1 also induces hepatocytes to produce acute phase proteins, activates both fibroblasts and osteoclasts and causes proteolysis as well as fever (7, 16). The effects of interleukin-1 on vascular endothelial cells include stimulation of growth factor production such as plasminogen activator or platelet activating factor as well as induction of intercellular adhesion molecule 1 expression, which causes adherence of neutrophils, monocytes and lymphocytes (3, 20). Among epidermal cells interleukin-1 in an autocrine manner stimulates the proliferation of keratinocytes and induces keratinocyte intercellular adhesion molecule 1 expression as well as cytokine (interleukin-1, -6 and granulocyte/macrophage colony stimulating factor) production (3). Recently interleukin-1 has also been found to up-regulate melanocyte stimulating hormone receptor expression on melanocytes and melanoma cells as well as melanin production in the presence of melanocyte stimulating hormone, indicating a possible role of interleukin-1 during pigmentation. In addition, interleukin-1 stimulates the release of hypothalamic and pituitary gland hormones (20). Moreover, interleukin-1 has been identified as hemopoietin-1 which in the presence of colony stimulating factors may function as regulator of hematopoiesis (20). These multiple functions of one cytokine support the notion of its central role in immunity and inflammation.

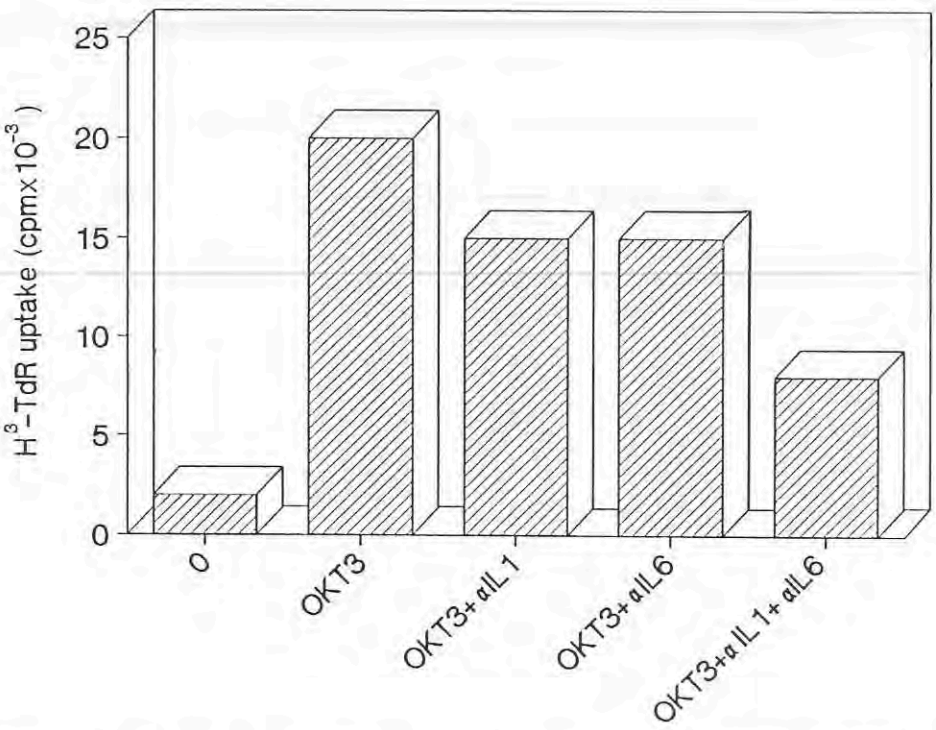


Fig. 2. Effect of interleukin-1 and -6 on OKT3 induced T-cell proliferation. Peripheral blood mononuclear cells (1×10^5) were cultured with OKT3 (12.5 ng/ml) for 3 days either in the presence or absence of anti-interleukin-6 (1:400) and/or anti-interleukin-1 (1:200). Data of three representative experiments are given as ^{3}H-thymidine incorporation (cpm).

INTERLEUKIN-6

Previously described soluble factors such as B-cell stimulatory factor 2, interferon-β2, hybridoma growth factor, hepatocyte stimulating factor, macrophage granulocyte inducer-2 and 26 kD protein were found to be derived from the same gene and are now named interleukin-6 (21–23). Although macrophages and fibroblasts primarily were initially thought to be the predominant source of interleukin-6, it is now clear that many cells, including T-cell lines, endothelial cells, osteocytes, epidermal cells and melanoma as well as carcinoma cell lines, are able to produce this cytokine (21, 24). The synthesis and release of interleukin-6 is up-regulated by cytokines such as tumor necrosis factors, interleukin-1, platelet derived growth factors and interferons as well as bacterial toxins and different viruses including human immunodeficiency virus I, abnormal nucleic acids and tumor promotors such as phorbol myristate acetate (21, 24, 25). Moreover, as recently has been shown ultraviolet irradiation is a potent stimulator of interleukin-6 synthesis and release (26). Therefore, interleukin-6 like interleukin-1 appears to be an ubiquitous cytokine which is immediately released after injury and stimulates inflammatory reactions.

Recently, normal human keratinocytes and epidermoid carcinoma (A431, KB) cell lines have been shown to produce a mediator, which induces the proliferation of an interleukin-6 dependent murine hybridoma cell line (B9). Moreover, as detected by immunoblotting experiments the different forms of interleukin-6 secreted by keratinocytes co-migrated with those obtained from monocytes and fibroblasts and exhibit molecular weight heterogeneity between 21 and 28 kD indicating that epidermal cell-interleukin-6 appears to be closely related to monocyte or fibroblast interleukin-6. This was further confirmed by Northern blot analysis and in-situ hybridization using a cDNA probe encoding for interleukin-6. Accordingly, low amounts of interleukin-6 specific mRNA were detected in unstimulated keratinocytes, which was highly induced by the addition of interleukin-1α, -1β, lipopolysaccharides, phorbol myristate

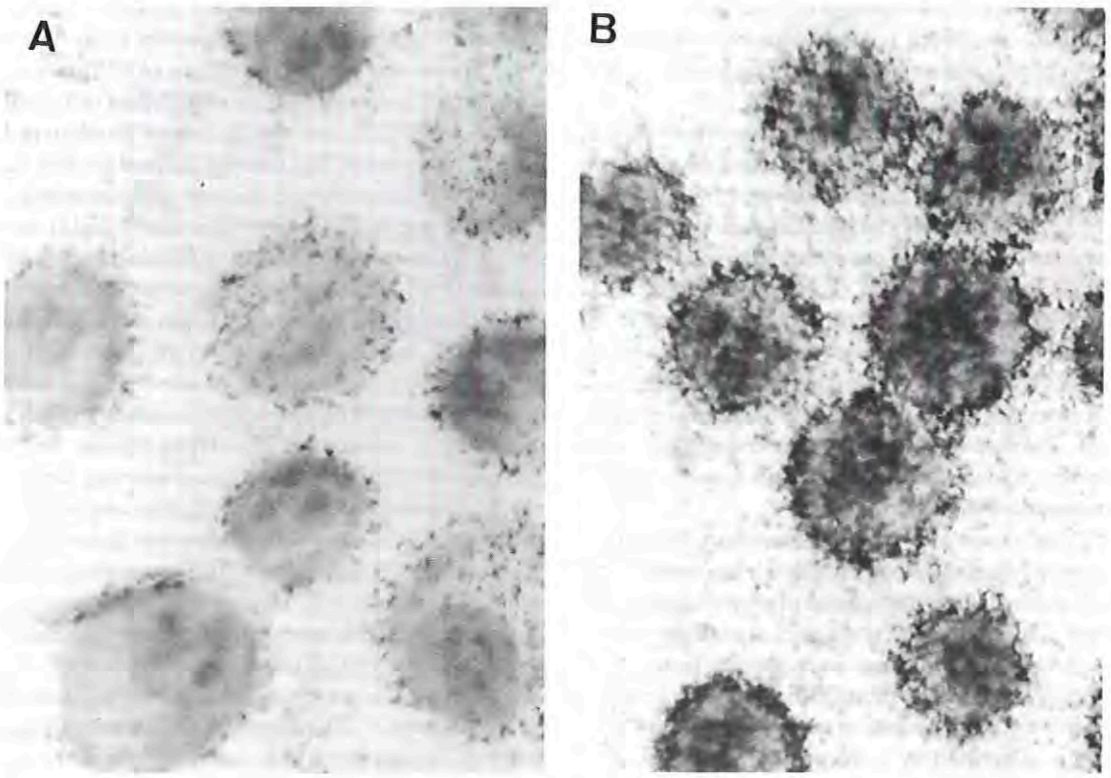


Fig. 3. In-situ hybridization of human A431 cells using an interleukin-6 cDNA probe. Human epidermoid carcinoma cells (A431) were incubated 12 hours without stimulus (A) or following treatment with phorbol myristate acetate (50 ng/ml) (B). Subsequently cells were cytocentrifuged and hybridized with ^{35}S-labelled cDNA probe for human interleukin-6.

acetate and following ultraviolet B irradiation (Fig. 3) (26–28). Although these findings indicate that keratinocytes definitely can be regarded as a potent source for interleukin-6, it is not yet clear whether other cells within the epidermis, including melanocytes and dendritic cells, are also capable of producing this cytokine.

The biological activities of interleukin-6 on different target cells are pleiotropic. Interleukin-6 is a major mediator of the acute phase response, since it enhances the production of acute phase reactants and inhibits secretion of albumin (29). Moreover, interleukin-6 has been identified as one of the endogenous pyrogens (30). Another important biological function of interleukin-6 includes its B-cell growth and differentiating capacity (31). Accordingly, interleukin-6 enhances the proliferation of Epstein-Barr Virus transformed B-cell lines (32) and murine hybridoma plasmacytoma cell lines (33). It also plays a crucial role in B-cell differentiation by enhancing immunoglobulin production in B-lymphoblastoid cell lines and activated normal B-cells (34). The capacity of interleukin-6 to activate T-cells has been assessed in several studies, indicating that interleukin-6 like interleukin-1 is able to function as a second signal required for thymocyte and T-cell proliferation (24, 27). This is further supported by the finding that the addition of anti-interleukin-6 to anti-CD3 stimulated peripheral blood mononuclear cells leads to the suppression of blastogenesis. Moreover, in the presence of anti-interleukin-1 an additional inhibitory effect was observed, indicating that interleukin-6 similar to interleukin-1 reacts as an accessory cell derived signal by augmenting early events in T-cell activation (Fig. 2) (24).

Interleukin-6 has also been shown to stimulate cytotoxic T-lymphocytes and natural killer cells. The enhancement of natural killer cells by interleukin-6, however, is due to the induction of interleukin-2 since it stimulates T-lymphocytes to produce more interleukin-2 and an antiserum against interleukin-2 blocks interleukin-6 mediated natural killer cell acti-

vation (35). Recent experiments also indicate that a previously described natural killer cell augmenting factor produced by epidermal cell is apparently identical to interleukin-6 (36).

In addition, to its effects on lymphocytes interleukin-6 in synergy with stimulating factors may enhance the proliferation and differentiation of multipotential stem cells. Moreover, interleukin-6 stimulates early human hematopoietic progenitors derived from patients with leukemia (37). There is an obvious similarity between the effect of interleukin-6 and -1 on hematopoietic cells, as many biological functions of interleukin-1 and -6 are overlapping and interleukin-1 is a potent inducer of interleukin-6 in many cell types (38). Further studies will be necessary to clarify whether some effects of interleukin-1 may be due to interleukin-6 induction.

There is some evidence that interleukin-6 may play a role in tumor development since it has been shown that several tumors are capable of producing interleukin-6 (28, 39) and that some such as multiple myeloma require interleukin-6 as a growth factor (39). Therefore, autocrine stimulation may be one mechanism in the pathogenesis of human myeloma. This is further supported by a recent finding that interleukin-6 stimulates pheochromocytoma cells to express proto-oncogenes and to differentiate into neurite extending cells (40). In contrast, interleukin-6 also exhibits antiproliferative activity since it suppresses the growth of certain fibroblasts and tumor cell lines (41).

TUMOR NECROSIS FACTOR

Mediators causing tumor necrosis and cachexia were recently identified as the same macrophage derived cytokine named cachectin/tumor necrosis factor-α (42). Another factor exhibiting similar biological activities and found to be produced by lymphocytes is lymphotoxin/tumor necrosis factor-β (43). Although both tumor necrosis factors are structurally distinct proteins, they bind to a common receptor (44). Human tumor necrosis factor-α is an important mediator of endotoxic shock and cachexia, causes hemorrhagic necrosis of tumors in animals and exerts cytostatic and cytolytic effects on many tumor cell lines in vitro (45). It also induces fever by stimulating prostaglandin E_2 release in hypothalamic neurons and by enhancing the production of pyrogenic cytokines such as interleukin-1 and -6 (46). Moreover, tumor necrosis factor-α activates neutrophils, eosinophils, macrophages as well as osteoclasts and stimulates the release of collagenase and prostaglandin E_2 by human synovial cells and dermal fibroblasts (45). Tumor necrosis factor-α also stimulates endothelial cell proliferation, migration, and production of interleukin-1, procoagulant factor and platelet derived growth factor (45). Moreover, tumor necrosis factor-α increases major histocompatibility complex class I and II antigen and intercellular adhesion molecule-1 expression on many cells, including endothelial cells, fibroblasts and keratinocytes (47, 48). The effect of tumor necrosis factor-α on T-lymphocytes include co-stimulation of proliferation and induction of interleukin-2 receptor expression as well as cytokine production such as interferon γ and colony stimulating factors. In the presence of other cytokines tumor necrosis factor-α also co-stimulates B-cell differentiation and antibody production (49, 50). Tumor necrosis factor-α also affects the cytotoxic activity of macrophages and stimulates several cells to produce interleukin-1, -6 and -8, granulocyte/macrophage colony stimulating factor and platelet derived growth factor (3, 51). Moreover, tumor necrosis factor-α has been demonstrated to induce differentiation of myeloid cell lines (52) and to block the proliferation of leukemic cells as well as hematopoietic progenitors (53). Finally tumor necrosis factor-α also belongs to the group of cytokines exhibiting antiviral activity (54).

Recently it has been demonstrated that long-term cultured human keratinocytes and epidermoid carcinoma cell lines were able to produce a tumor necrosis factor-α-like mediator. Moreover, upon Western blot experiments epidermal cell derived tumor necrosis factor co-migrated with recombinant human tumor necrosis factor-α and using a cDNA probe encoding for tumor necrosis factor-α keratinocytes contained tumor necrosis factor-α specific mRNA. Similar to interleukin-1 and -6, tumor necrosis factor-α synthesis and release were significantly increased by ultraviolet light, phorbol myristate acetate and by tumor necrosis factor-α itself (55). Although the role of tumor necrosis factor-α in the epidermis is not yet fully elucidated, there is some evidence that tumor necrosis factor-α released by keratinocytes in concert with other epidermal cell cytokines such as interleukin-1 and granulocyte/macrophage colony stimulating factor may serve as a crucial signal for Langerhans cell viability in the epidermis (56).

NEUTROPHIL ACTIVATING FACTORS (INTERLEUKIN-8)

A new group of cytokines being chemotactic for neutrophils was recently described by several investigators and named monocyte chemotaxin, neutrophil activating factor, neutrophil activating peptide, and monocyte derived neutrophil chemotactic factor (57–60). According to the present cytokine nomenclature it has been proposed to call this mediator interleukin-8. The mature functional protein has a molecular weight of 6.5 kD and was found to be similar to previously characterized mediators such as human platelet factors: β-thromboglobulin, platelet factor 4, interferon γ inducible protein, Rous sarcoma virus (v-src) inducible protein and is completely identical with a previously described 3–10C cDNA (3). Moreover, interleukin-8 appears to be similar to recently described murine macrophage derived inflammatory proteins which have been shown to be chemotactic and to activate polymorphonuclear neutrophils (61).

Interleukin-8 only affects neutrophils via interaction with a specific surface receptor (62). It has been shown to be strongly chemotactic for polymorphonuclear neutrophils, to increase cytosolic free Ca^{2+}, to activate respiratory burst and to induce granule exocytosis (58–60). In addition of being able to activate neutrophils interleukin-8 recently has been shown under certain conditions to function as a chemoattractant for T-lymphocytes (63). In vivo, upon i.v. injection interleukin-8 induces granulocytosis rapidly and when injected into the skin it causes an early local inflammatory reaction (57). The production of interleukin-8 was primarily thought to be restricted to monocytes, which only upon stimulation with lipopolysaccharide, mitogens, interleukin-1 or tumor necrosis factor-α produced this cytokine (57–60, 64). Recent findings, however, indicate that several similar polymorphonuclear neutrophil activating factors are also synthesized and released by various different cells such as endothelial cells (65), dermal fibroblasts as well as keratinocytes (66, 67). Since both interleukin-1 and tumor necrosis factor-α are potent inducers of interleukin-8, they are indirectly causing polymorphonuclear neutrophil infiltration via induction of chemotactic interleukin-8 as well as intercellular adhesion molecule-1 (48, 59, 60). Moreover, T-cell derived cytokines such as interferon γ have been demonstrated to be potent inducers of keratinocyte interleukin-8 expression (68). The relationship of interleukin-8 and previously characterized monocyte or epidermal cell derived granulocyte activation mediators responsible for the stimulation of oxidative metabolism of neutrophils is not yet completely clear. However, they partly appear to be related to granulocyte/macrophage colony stimulating factor which also has neutrophil activating capacities (69). The production of neutrophil attracting and activating cytokines including granulocyte/macrophage colony stimulating factor, interleukin-1, tumor necrosis factor-α and interleukin-8 by epidermal cells suggest a regulatory role of keratinocytes in the pathogenesis of skin diseases characterized by an infiltration of neutrophils.

COLONY STIMULATING FACTORS

The family of the colony stimulating factors consists of four major species: interleukin-3, granulocyte/macrophage colony stimulating factor, granulocyte colony stimulating factor and macrophage colony stimulating factor. Interleukin-3 (also termed multicolony stimulating factor) and granulocyte/macrophage colony stimulating factor stimulate the proliferation of multilineage colonies containing granulocytes, monocytes, megakaryocytes and erythrocytes. Granulocyte colony stimulating factor mainly induces colonies of neutrophils and macrophage colony stimulating factor primarily causes monocyte colony formation (70). For the generation of mature erythrocytes in addition to granulocyte/macrophage colony stimulating factor or interleukin-3 erythropoietin is required (71).

The cellular source of colony stimulating factors is not restricted to immunocompetent cells since it became clear that many different cell types are able to produce mainly the non-lineage specific colony stimulating factors: interleukin-3 and granulocyte/macrophage colony stimulating factor. Normal cells constitutively do not produce high amounts of colony stimulating factor, however, upon stimulation with tumor promotors endotoxin, or cytokines such as interferon, interleukin-1, or tumor necrosis factor-α, monocytes, endothelial cells or fibroblasts express high levels of granulocyte and macrophage colony stimulating factor mRNA, but no message for granulocyte/macrophage colony stimulating factor. In contrast, normal T-cells produce high levels of granulocyte/macrophage colony stimulating factor mRNA and low levels of interleukin-3 mRNA, but no mRNA encoding for granulocyte or macrophage colony stimulating factor (70, 72).

Recently, it has been shown that normal murine keratinocytes and transformed keratinocyte cell lines release a mediator which stimulates the proliferation of murine interleukin-3 dependent cell lines. Moreover, the usually low constitutive interleukin-3 mRNA expression in keratinocytes is markedly augmented by the addition of the tumor promotor phorbol myristate acetate and following ultraviolet irradiation (73). Murine keratinocytes also synthesize and release granulocyte/macrophage and macrophage colony stimulating factor. Granulocyte/macrophage colony stimulating factor production by murine keratinocytes is significantly upregulated by ultraviolet irradiation, phorbol myristate acetate and cytokines such as interleukin-1, tumor necrosis factor-α and granulocyte/macrophage colony stimulating factor itself (3, 74). In contrast to murine keratinocytes, it was not yet possible to detect interleukin-3 mRNA expression in human epidermal cells. Human keratinocytes, however, constitutively release low amounts of granulocyte/macrophage and granulocyte colony stimulating factor, and addition of interleukin-4 significantly increases both granulocyte/macrophage and granulocyte colony stimulating factor mRNA expression in human keratinocytes (3, 75).

Colony stimulating factors interact via distinct cell receptors which are displayed on many different cells. Receptors for macrophage colony stimulating factors have recently been identified as the gene product of a protooncogene (cfms) suggesting a possible role of macrophage colony stimulating factor in tumor development (76, 77). Granulocyte/macrophage colony stimulating factor has multiple biological activities. It supports the proliferation of macrophage, eosinophilic and neutrophilic colonies. Moreover, in the presence of erythropoietin granulocyte/macrophage colony stimulating factor induces the proliferation of erythrocyte and megakaryocyte progenitors. Granulocyte/macrophage colony stimulating factor also enhances the biological activities of mature neutrophils, eosinophils and macrophages and has been shown to act as a growth factor for myeloid leukemic cells (78). Furthermore, granulocyte/macrophage colony stimulating factor enhances tumoricidal activity, oxydative burst activity as well as receptor expression for the Fc portion of an antibody on macrophages and promotes growth and activation of monocytes by inducing macrophage colony stimulating factor gene expression (78). Interleukin-3 is a growth factor for primitive multilineage progenitors and enhances neutrophilic, eosinophilic, basophilic, macrophage, megakaryocytic and erythroid colonies. Whether human interleukin-3 shares the growth factor activities described for murine interleukin-3, such as activation of mast cells and killer cells, is still unknown (79). Granulocyte colony stimulating factor supports the proliferation of neutrophilic colonies, and activates mature neutrophils by inducing superoxide production, stimulates them to kill tumor targets through antibody dependent cellular cytotoxicity and enhances their ability to phagocyte particles. Moreover, granulocyte colony stimulating factor may also promote the differentiation of some myeloleukemic cells (80). The biological activities of human macrophage colony stimulating factor include primarily the stimulation of macrophage colony formation and activation of mature macrophage colony stimulating factor receptor expressing macrophages (70).

SUPPRESSOR FACTORS

Secretory regulins of cell growth and differentiation such as transforming growth factors were also recently identified as mediators of inflammation and immunity (81). Transforming growth factor-α is substantially homologous to epidermal growth factor and to vaccinia virus growth factor, and binds to the epidermal growth factor receptor (82). Transforming growth factor-β is a homodimer exhibiting a molecular weight of 25 kD. There are three subtypes called transforming growth factor-β1, -β2 and -β3 (83). Transforming growth factors are produced by various tumour cells and have also been detected in normal tissues such as platelets, placenta, kidney, bone and keratinocytes (81). Using in-situ hybridization transforming growth factor-α mRNA was found expressed in the stratified epidermis. In psoriatic plaques as well as both basal and squamous cell carcinomas, significantly increased amounts of transforming growth factor-α were observed. Similarly, transforming growth factor-β was demonstrated in the epidermis and transforming growth factor-β mRNA expression was enhanced after topical application of tumor promotors (81, 84, 85). Transforming growth factor-α induces angiogenesis, increases calcium resorption, promotes wound healing and influences estrogen metabolism (86). Transforming growth factor-β inhibits the proliferation of normal cells and certain tumor cell lines, whereas other tumor cells and mesenchymal cells proliferate in response to transforming growth factor-β (83). Moreover, transforming growth

factor-β stimulates the expression of fibronectin and collagens in fibroblasts, it promotes chondrogenesis and epithelial cell differentiation in vitro, and blocks adipogenesis, myogenesis and hematopoiesis (83).

Transforming growth factor-β has also been detected in activated T-cells and macrophages, and recently it became evident that transforming growth factor-β significantly blocks interleukin-1 as well as interleukin-2 activity (87, 88). Transforming growth factor-β2 also inhibits selectively early hematopoietic progenitor cell growth and differentiation (89). Moreover, a keratinocyte derived mediator blocking mixed leukocyte reaction called keratinocyte lymphocyte inhibitory factor recently appeared to be closely related to transforming growth factor-β (90). These findings indicate that transforming growth factor-β exhibits potent immuno-inhibiting capacities and thereby may participate in the downregulation of the epidermal immune response.

In addition to transforming growth factors it was demonstrated that keratinocytes and epidermoid carcinoma cell lines when ultraviolet irradiated or treated with phorbol myristate acetate produce a cytokine which upon i.v. injection into mice blocked the induction of contact hypersensitivity to potent sensitizing agents in the recipient animals (91). This factor exhibits a molecular weight between 20–40 kD and appears to be distinct from other immunomodulators such as prostaglandins, leukotrienes and urocanic acid, a soluble compound located in the stratum corneum which has been suggested to be a photoreceptor for immunosuppression (92). There may be some similarity to a recently observed transferable serum suppressor factor found in ultraviolet-light-exposed mice, which induces suppression of contact hypersensitivity via generation of T-suppressor cells (93). Epidermal cell-contra-interleukin-1 is another recently observed immunosuppressive cytokine released by murine keratinocytes upon ultraviolet exposure or stimulation with phorbol myristate acetate (94). Partially purified epidermal cell-contra-interleukin-1 blocks both natural and recombinant interleukin-1 specifically, exhibit a molecular weight of 40 kD and an isoelectric point of 8.8. Epidermal cell-contra-interleukin-1 does not inhibit interleukin-2 or -3 activity and does not block the spontaneous proliferation of different cells and cell lines, thus excluding a toxic effect (94).

The production of epidermal cell-contra-interleukin-1 does not seem to be an in-vitro phenomenon

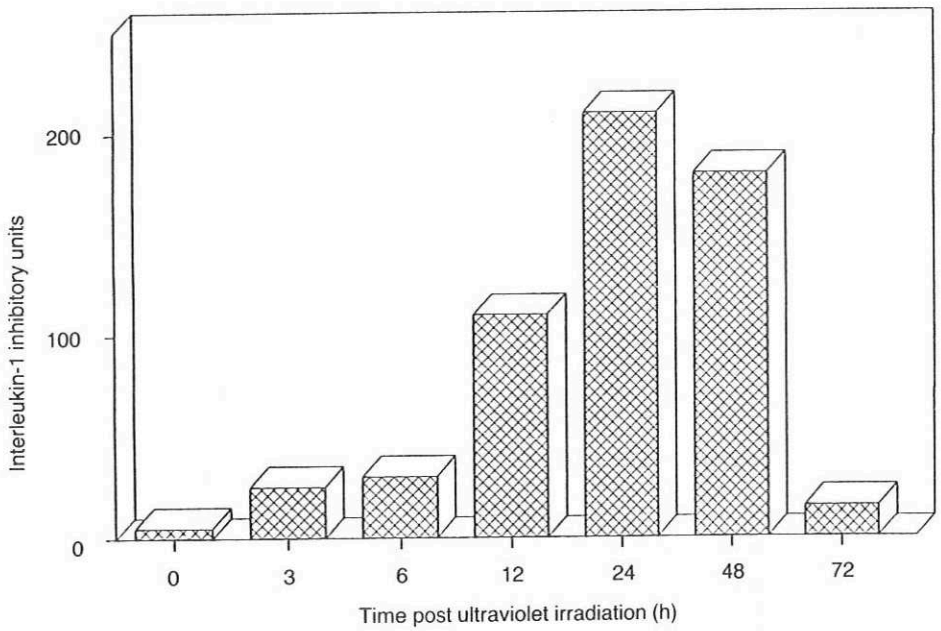


Fig. 4. Effect of ultraviolet-B irradiation on serum levels of contra-interleukin-1. Shaved balb/c mice were exposed to ultraviolet-B irradiation (1×10^4 J/cm^2). HPLC-gel filtration fractions (40 kD) of serum obtained prior to ultraviolet irradiation and 3, 6, 12, 24, 48 and 72 hours post ultraviolet irradiation were tested for interleukin-1 inhibitory activity in the thymocyte co-stimulator assay after addition of 1 U/ml rh-interleukin-1. Results were expressed as inhibitory units.

only as recently an interleukin-1 inhibitor with similar biochemical characteristics could be isolated from the serum of ultraviolet exposed mice (Fig. 4) (95). These findings support the notion that epidermal cells release epidermal cell-contra-interleukin-1 upon ultraviolet irradiation which penetrates the basal lamina, enters the circulation and probably causes systemic immunosuppression.

There seems to be an obvious similarity between epidermal cell-contra-interleukin-1 and the contact hypersensitivity inhibitor. Both factors are released by the same cells and cell lines under identical conditions, they are both induced by ultraviolet light and they share a similar molecular weight (6). However, only further biochemical characterization, sequencing and gene cloning of both mediators will clarify whether these factors are identical or whether they are two distinct cytokines released by keratinocytes. Purification will also indicate whether epidermal cell-contra-interleukin-1 is a new mediator or a well known immunomodulator with an as yet undescribed activity.

SECRETORY REGULINS AND PATHOGENESIS OF SKIN DISEASES

The capacity of epidermal cells upon stimulation to release secretory regulins may have important implications for the pathogenesis of many skin diseases. These cytokines are released following injury and may regulate local as well as systemic inflammatory reactions. Accordingly, exposure to ultraviolet light leads to both local and systemic inflammatory reactions as well as alterations of the immune response (92). Ultraviolet light has also been shown to be a very potent inducer of epidermal cytokine production. It has been demonstrated that following ultraviolet-B irradiation, keratinocytes synthesize and release increased amounts of immunostimulating cytokines such as interleukin-1, -3 and -6, tumor necrosis factor-α and granulocyte/macrophage colony stimulating factor, but also immunosuppressive factors such as transforming growth factor-β, epidermal cell-contra-interleukin-1 and epidermal cell stimulating factor (6). These ultraviolet light induced mediators may be involved in the activation of the cytokine cascade inducing both the expression of specific receptors on various cells and the production of other mediators such as interleukin-8 causing the attraction of inflammatory cells which by themselves may contribute different cytokines not released by keratinocytes such as interleukin-2 and -4 or interferon γ (6). Therefore, through the release of cytokines epidermal cells apparently play an important role in the regulation of the local inflammatory reaction following exposure to ultraviolet irradiation.

There is also strong evidence that increased levels

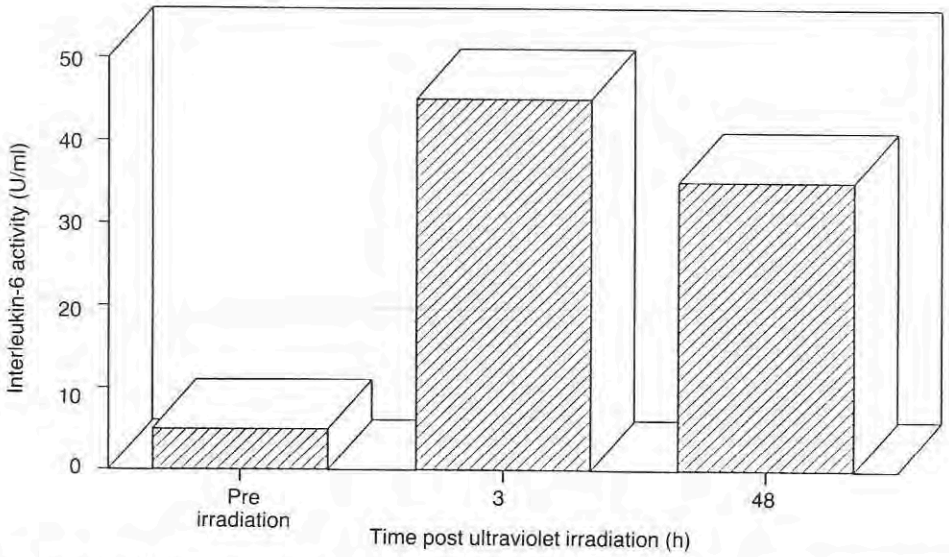


Fig. 5. Effect of ultraviolet-B irradiation on serum interleukin-6 activity. Shaved balb/c mice were exposed to ultraviolet-B irradiation (1×10^4 J/cm^2). Subsequently, serum samples prior to ultraviolet-B irradiation or 3 and 48 hours post irradiation were tested for interleukin-6 activity using an interleukin-6-dependent plasmacytoma cell line (B9). Results are expressed as units of interleukin-6 activity/ml.

of circulating cytokines including interleukin-1 and -6 may be detected in the serum following ultraviolet irradiation (Fig. 5) (6, 24). Although it has not been formally proven, one may speculate that ultraviolet only partly penetrating the epidermal cell layers may induce cytokine production in epidermal cells which may gain access to the circulation and subsequently cause systemic inflammatory effects. Since both interleukin-1 and -6 are highly pyrogenic and since they are mediators of the acute phase response, increased levels of these cytokines following ultraviolet irradiation may be involved in the pathogenesis of severe sunburn reactions accompanied by fever, malaise and chills (20, 22). Because of its B-cell stimulatory capacity and elevated serum interleukin-6 levels in patients with autoimmune-like conditions (96), increased interleukin-6 production following ultraviolet irradiation may also be of importance in the pathogenesis of autoimmune diseases such as lupus erythematosus. Therefore, through the induction of autoantibodies interleukin-6 may be responsible for ultraviolet induced exacerbation of lupus erythematosus. This is further supported by an observation demonstrating that transgenic mice infected with constitutively interleukin-6 expressing cells developed plasmacytomas in association with permanently elevated serum interleukin-6 levels (96).

The concept of interleukin-6 being an important mediator of systemic inflammation is further confirmed by recent investigations demonstrating significantly elevated levels of interleukin-6 activity in the serum of patients with severe burns (30), sepsis (97) and following administration of endotoxin to volunteers (98). Moreover, increased interleukin-6 activity was found in synovial fluid from rheumatics and in the spinal fluid of patients with meningitis (99, 100). In patients with psoriasis an increased interleukin-6 mRNA expression by keratinocytes was found to be associated with increased circulating interleukin-6 levels (101, 102). Moreover, it has been shown that colony stimulating factors, in particular granulocyte/macrophage colony stimulating factor present in normal skin, are induced by ultraviolet light suggesting that ultraviolet irradiation induces the cutaneous release of colony stimulating factor in amounts sufficient to reverse acute myelosuppression (74). These findings may suggest that ultraviolet induced release of epidermal cell derived cytokines may play a role in the recovery from bone marrow suppression.

The observation that epidermal cells and in particular keratinocytes are capable of releasing immunosuppressive cytokines supports the crucial role which keratinocytes may play in the regulation of immunological reactions. Thus, epidermal cells may not only be involved in the initiation of an immune response by providing immuno-enhancing mediators, but they may also turn down inflammation by secreting suppressive cytokines. This capacity to release agonistic as well as antagonistic regulins such as interleukin-1 and contra-interleukin-1, however, might not be unique for the keratinocyte but might be a common feature of all immunosecretory cells. Another important implication of these findings is that immunosuppression can originate from the keratinocyte. This seems to be valid for ultraviolet induced immunosuppression, as ultraviolet mediated release of immunosuppressive factors by keratinocytes may at least partly explain local and in particular systemic immunosuppression following ultraviolet exposure. This is supported by the finding of circulating immunoinhibitory cytokines such as epidermal cell-contra-interleukin-1 following ultraviolet exposure which may contribute to the immunosuppressive state following ultraviolet exposure by blocking the immunologic function of interleukin-1 (Fig. 4) (6). These observations may have new implications for the pathogenesis of skin cancer and autoimmune disorders.

Epidermal cell derived cytokines may also affect T-cell activation via modulating growth and differentiation of antigen presenting cells within the epidermis. The capacity of freshly isolated Langerhans cells to stimulate T-cells was found to be weak. However, during culture immunological immature Langerhans cells require many features of dendritic cells and become more potent concerning their T-cell stimulatory capacity. The signals required for this maturation include epidermal cytokines. Accordingly, granulocyte/macrophage colony stimulating factor in the presence of interleukin-1 enhances the maturation of Langerhans cells whereas granulocyte/macrophage colony stimulating factor alone maintains Langerhans cell viability and increases Langerhans cell functions (19). In addition to granulocyte/macrophage colony stimulating factor and interleukin-1, tumor necrosis factor-α only recently was recognized as an epidermal cell derived cytokine affecting Langerhans cells (55). Accordingly, Langerhans cells cultured in the presence of tumor necrosis factor-α had not matured as measured by their T-cell stimulatory capacity but had survived (56). These findings support the importance of cytokines released by epidermal cell which by maintaining the viability of Langer-

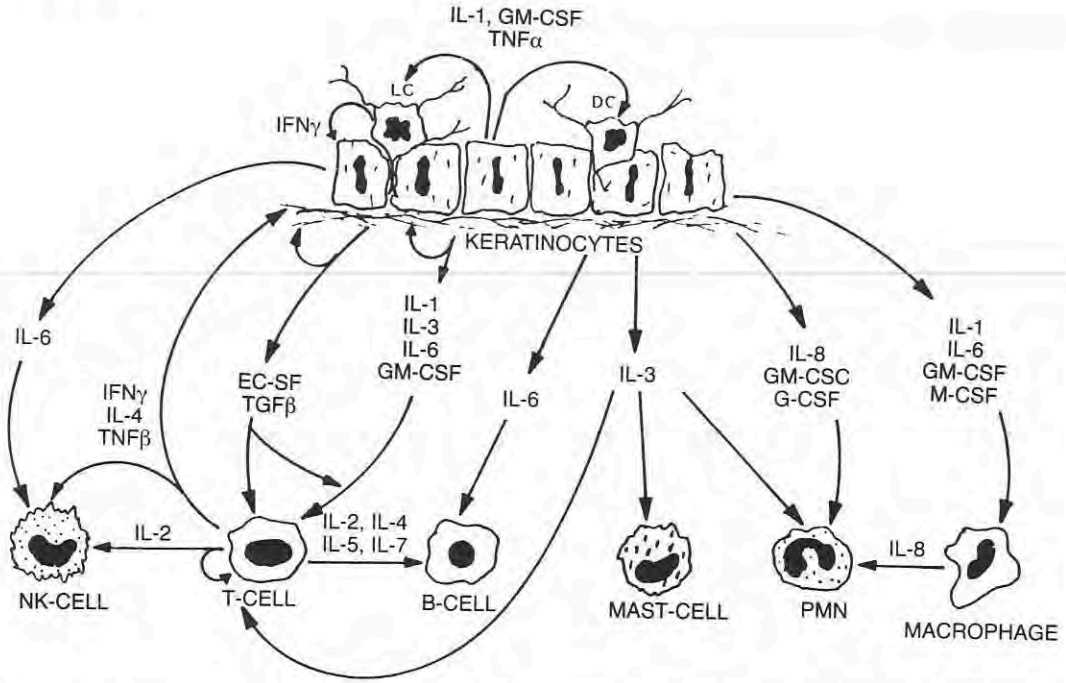


Fig. 6. The skin immune system. Cytokines produced by keratinocytes such as interleukin-1, granulocyte/macrophage colony stimulating factor and tumor necrosis factor-α regulate Langerhans cell viability and differentiation. The differentiation, activation and growth of inflammatory cells in the dermis is modulated by keratinocyte derived secretory regulins such as interleukin-1, -6 and -8, tumor necrosis factor-α and colony stimulating factors.

hans cell and modulating Langerhans cell function may serve as important mediators during the initiation of immune responses in the skin (Fig. 6).

Cutaneous T-cell lymphoma is characterized by the immigration of transformed T-cells of the helper phenotype into the dermis and epidermis. There is evidence that epidermal cytokines may be responsible for this attraction of T-cells. Accordingly, interleukin-1 appears to be chemotactic for T-cells and granulocyte/macrophage colony stimulating factor functions as a growth factor for some T-lymphocytes (10, 20). Moreover, interleukin-3 is a growth factor for certain T-cells and cytokines released by epidermal cells such as interleukin-1 and -6, and thymopoietin may participate in the activation and differentiation of T-lymphocytes (20, 22). In addition, tumor necrosis factor and interleukin-1 up-regulate keratinocytes intercellular adhesion molecule-1 expression which functions as ligand to which T-lymphocytes bind via their leukocyte function antigen-1 molecules. This interaction may also contribute to T-cell epidermotropism (48). The capacity of keratinocytes to release mediators of T-cell activation and differentiation may provide an explanation for the affinity of T-lymphocytes to the epidermis.

Mechanical irritation of epidermal cells has been shown to result in the release of different cytokines (3). These epidermal cell derived secretory products may function as regulators of the different events occurring in wound healing including inflammatory reaction, vessel formation, matrix protein formation and re-epithelisation. Accordingly, the invasion of inflammatory cells during the initial phase of wound healing may be regulated by epidermal cell derived chemotactic cytokines such as transforming growth factors and interleukin-8, a recently discovered neutrophil activating as well as attracting cytokine (60, 83). The proliferation and differentiation of keratinocytes appears to be under the regulation of epidermal growth factor, platelet derived growth factor, interleukin-1 and -6 (82, 102). Vessel formation is known to be regulated by cytokines such as transforming growth factor, fibroblast growth factor and platelet derived growth factor (81). Fibroplasia and formation of new matrix proteins again is regulated by epidermal cytokines such as platelet derived, transforming,

epidermal and fibroblast growth factors, and interleukin-1 (81). These findings indicate the important function of epidermal cytokines during wound healing and scar formation. There is a striking similarity between tissue repair and carcinogenesis, both characterized by matrix destruction and synthesis as well as angiogenesis. Moreover, the same secretory regulins participate in both tissue repair and malignant differentiation. Thus, it may be speculated that the difference between these two phenomena appears to be based on the appropriate control of cytokine expression rather than solely on their presence or absence.

CONCLUSION

The production of secretory products regulating growth and differentiation of inflammatory as well as non-inflammatory cells by epidermal cells supports the important function of the epidermis in protecting the body against the environment. Therefore, the skin represents not only a simple mechanical barrier, but upon injury may be activated to release cytokines which actively participate in repair mechanisms and the elimination of the injurious agents (Fig. 6). These findings further implicate that secretory regulins may be promising candidates for treatment of infectious autoimmune, immunodeficient and malignant diseases. However, one has to consider that a balanced network of different secretory regulins interacting with other mediators of inflammation such as prostaglandins or hormones appears to be important for the regulation of inflammatory as well as immunological events, and dysfunction may play a role in the pathogenesis of inflammation, autoimmunity and tumor development.

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Immunology of Atopic Dermatitis

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Atopic dermatitis is a chronic eczematous skin disease which characteristically starts early in life and later tends to disappear. Genetic predisposition seems important for the development of the disease. The immune system is involved through a lymphocyte-mediated inflammation in the skin creating the eczema, and an increased incidence of type I and possibly type IV allergies induced by environmental allergens. Recent findings of a changed concentration of some interleukins in atopic patients support the evidence for an increased T-lymphocyte activation and may explain the increased amount of polyclonal IgE in many of the patients. Hypothetically, atopic dermatitis can be considered to be due to an inborn error of the maturation of epithelial tissue. Maturation of epithelial tissue is essential for both the appearance of normal skin and for correct maturation of the cell-mediated immune system. The immune deviation is later more or less corrected through maturation which explains why the disease in most patients disappears in childhood. However, it still leaves a certain increased capacity for inflammation of the atopic persons.

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Atopic dermatitis is a chronic skin disease of which the main symptoms are severe itching, dry skin and eczema localized mainly in the face, neck and on the flexor sides of the extremities. The skin changes are erythema, dryness, scaling and lichenification. The disease starts typically within the first two years of life (1). In up to 90% of the patients it disappears during childhood (2). Symptoms may, however, continue into adulthood or the disease may relapse following several years of a disease-free period (2).

The immune system participates in atopic dermatitis. The previous two decades have produced an enormous amount of information about immune changes in atopic patients. However, it is still not known whether these changes are of primary importance for the development of atopic dermatitis or whether they influence the symptomatology of the disease.

This review intends to summarize the present main immunological findings in atopic dermatitis, to discuss their significance for the disease and to suggest a hypothesis for the development of the disease, which will include our present immunological knowledge.

HISTOLOGY

The histological changes in the skin of patients with atopic dermatitis are comparable to those seen in contact eczema, seborrhoic eczema or nummular eczema. Spongiosis and microvesicles are found intraepidermally in the acute phase with increased cell proliferation and slight acanthosis or sometimes parakeratosis. There is hypertrophy of endothelial cells in the dermal vessels with increased thickness of the basal membrane and an infiltrate of lymphocytes. The number of mast cells is doubled in comparison to normal skin (3).

The cellular infiltrate in the skin consists of T-lymphocytes of the T-helper phenotype (4). The ratio of Leu 3a+:Leu 2a+ (helper:suppressor) is double the ratio in peripheral blood (5). Of the T-cells 85% express class II antigens, which are also found on the interdigitating cells in the dermis implying immune activation. Very few natural killer cells and B-lymphocytes are found (5, 6).

Within the last few years IgE antibodies have been observed on the surface of Langerhans cells in epidermis of atopic skin (7, 8). This may be important for lymphocyte activation through binding of allergen to IgE on the antigen-presenting Langerhans cells.

The histological changes indicate an activation of T-lymphocytes in the skin. The question is whether a specific cell-mediated immune reaction is taking place and, if so, towards which antigen(s), or whether the changes are an immune inflammation induced by more non-specific factors.

DELAYED HYPERSENSITIVITY SKIN REACTIONS IN ATOPIC DERMATITIS

Skin testing of patients with atopic dermatitis give indications of a deficient cutaneous cell-mediated immunity (9–11). Sensitization with dinitrochlorobenzene succeeded in 77% of patients with atopic dermatitis compared with over 90% in non-atopics (12). Thus, the general capacity to react with a type IV-like skin reaction is reduced. This could in part be due to a continuing immune reaction in the skin (13).

A revived interest for skin reactions in atopic dermatitis has come with the observation that antigens inducing type I reactions can also elicit type IV-like reactions in the skin. Histological examinations of patch test reactions with these allergens have shown 5–10% basophils (14, 15) or eosinophils in the infiltrate (16), which for basophils is comparable to the number seen in contact sensitivity reactions — at least in animal studies (17). Several studies have now confirmed these observations (18, 19). In the case of atopic dermatitis, for instance, the amount of house dust mite antigen had to be increased 500-fold (18). We have not been able to repeat these results using 48-hour patch tests with house dust mite antigen in lower concentrations, but a twice daily skin application of mite antigen solution for 5 days can induce an eczematous reaction in one third of patients with atopic dermatitis who are type I allergic to house dust mite (19). Even though only a fraction of patients reacted in these tests, the real-life situation of continued exposure to mite antigen may be sufficient to elicit symptoms in those allergic to the antigen. However, many patients with atopic dermatitis do not have IgE antibodies towards house dust mites and still have severe atopic dermatitis. It is likely that any 'damaging' effect of atopic epidermis may induce the release of biological potent mediators during epidermal regenerative proliferation (discussed later).

T-LYMPHOCYTES AND ATOPIC DERMATITIS

T-cell number and subsets

Many investigators have documented that the total number of T-lymphocytes in peripheral blood is decreased in atopic dermatitis (20). The reduction seems related to the extent and severity of the disease (21, 22). Several studies have shown that it is the subpopulation of T-lymphocytes with receptors for IgG (23–26) or the OKT8+ cells (27–30) that are reduced with approximately 25%. These T-lymphocytes show suppressor activity in certain in-vitro systems and their decrease in blood has been considered a sign of deficient suppressor cell activity thereby creating the basis for the hyper IgE responses in atopic patients. The CD4+:CD8+ ratio is increased in patients with atopic dermatitis, even in children from atopic parents before the development of atopic dermatitis (31, 32).

T-cell in-vitro function

In-vitro proliferation studies of T-cells from patients with atopic dermatitis have given varying results (20), which seems due to the disease activity at the time of investigation and several technical details such as the use of fetal calf serum, which improves the responsibility of atopic lymphocytes (33) compared with human serum (21). In general, T-cells from patients with severe atopic eczema show decreased responsiveness, but this can be overcome when culturing the cells for longer periods of time (34).

The reactivity in the concanavalin-A suppressor assay is decreased (26, 29, 35) or normal (36). This may again be due to the severity of the disease. The reactivity of T-cells in a mixed leukocyte reaction was normal except in the autologous mixed leukocyte reaction, but the biological significance of this finding is not evident (37). Other in-vitro investigations such as excretion of leukocyte migration factor was decreased after concanavalin-A stimulation (38), but not after tuberculin stimulation (21). We have observed that the natural killer cell cytotoxicity towards K562 was decreased (39), but this has not been confirmed by others (40). Increased cytotoxicity has also been observed towards autologous fibroblasts (41), but cytotoxic lymphocytes are only rarely seen in the infiltrate in atopic eczema (5). Thus, in general, minor deviations have been observed of in-vitro functions, probably secondary to disease activity.

Stimulation of T-lymphocytes in-vitro using house dust mite antigen have demonstrated an increased reactivity among 11 of 16 atopic children with eczema compared with two of 14 non-atopics (42). There was no correlation with type I reactivity towards the same antigen and the true specificity may be discussed.

B-LYMPHOCYTES AND ATOPIC DERMATITIS

The total number of B-cells is normal in peripheral blood of patients with atopic dermatitis (20). The

total amount of immunoglobulin is within normal limits, although some have found a slight increase of IgG and a slight decrease of IgA (43, 44). The total amount of IgE is increased in most, but certainly not all patients with atopic eczema. The IgE is polyclonal and the amount is correlated to the extent and severity of the disease (45–47). No correlation has been found between the amount of IgE and the number of positive prick tests, which may be present in 25%–70% of patients, especially in those with concomitant respiratory allergy (48). Specific IgE responses are normally seen in parasitic diseases (49). Non-specific IgE production is seen in animal experiments following whole-body X-ray irradiation, anti-lymphocyte globulin injections, thymectomy or cyclophosphamide (50). It is also seen in patients with lymphomas.

In-vitro studies of IgE production have recently shown that T-lymphocytes of the CD4+ subpopulation can release factors following mitogen stimulation, which can both enhance or suppress a polyclonal IgE response (51–53). Even more recently the interest has focused on interleukin-4. Interleukin-4 is a T-cell product which affects the expression of various genes and consequently the activation, proliferation and differentiation of lymphoid cells and hematopoietic precursor cells. It has been found to induce an increased release of IgE from normal human B-cells from some persons in vitro (54). The increased IgE production could be inhibited with small amounts of alfa- or gamma-interferon, prostaglandin E_2, or anti-CD23 antibodies, which primarily react with B-lymphocytes (55, 56). An augmenting effect has been observed of interleukin-5 (eosinophil chemotactic factor), if soluble CD23 was released (57). It has also been observed that interleukin-4 could enhance a continuing IgE production by B-cells from atopic patients, which again could be blocked by interferons (58). However, others have looked at the mRNA level for various lymphokines in non-atopic and atopic patients and did not see any difference concerning mRNA expression of interleukin-4 or -2. Pokeweed mitogen stimulation of lymphocytes revealed, however, a significantly reduced mRNA for interleukin-1β in cells from atopic patients. Interleukin-6 mRNA levels were also lower in cells from atopics for all stimulations used (59).

These findings may open a new era for the study of IgE release in atopic patients. A recent investigation has demonstrated that IgE-committed B-lymphocytes belong to unique B-cell sublineages which carry IgM and IgD on their surface and which can secrete IgE after Epstein-Barr virus transformation in vitro. Their differentiation pathway leading to IgE release seems highly restricted and regulated (60). Many patients with atopic dermatitis may have defects in this regulation, leaving their IgE-committed B-lymphocytes open to the influence from altered concentrations of interleukins and/or virus infections. But, it will not explain atopic dermatitis in patients with normal levels of IgE.

MONOCYTES, GRANULOCYTES AND COMPLEMENT

Approximately 25% of monocytes have crystallizable fragment receptors for IgE. The affinity is 10 times higher than the few crystallizable fragment IgE receptors on T- and B-lymphocytes (61, 62). Atopic patients have an increased IgE-mediated cytotoxicity (62). Patients with extensive eczema have a decreased IgG-mediated monocyte cytoxicity also during a quiscent period of their disease. The cytotoxicity is normal in patients with slight eczema (63). Neutrophil chemotaxis is decreased in atopic dermatitis, especially if impetigo exists (64, 65). The granulocyte release of enzymes is also decreased and may in part explain the decreased resistance towards bacterial infection in atopic patients (66).

Complement C3, C4 and C1 are increased in atopic dermatitis. Therefore, these changes may indicate complement activation, but the changes are similar to what is found in psoriasis and thus seem of no primary importance for the disease (67).

INTERLEUKINS

The evolution of new sensitive techniques enables the study of interleukins in patients. The significance of some interleukins is indicated in the paragraph on B-lymphocytes. Interleukin-1 is known to be released from a large variety of cells including monocytes, fibroblasts and keratinocytes. It augments the T-lymphocyte reactivity through an increased release of interleukin-2 and an increased expression of interleukin-2 receptors on the T-cells. Two recent reports have found a decreased release of interleukin-1 from lipopolysaccharide-stimulated monocytes in patients with atopic dermatitis (68, 69). However, we have consistently found increased interleukin-1 activity using the C3H assay, when studying patients with se-

vere atopic eczema (unpublished observations). Increased levels of interleukin-2 receptors have been observed in serum from patients with atopic eczema (Söderberg et al., unpublished), but a decrease of interleukin-2 release following phytohemagglutinin-stimulation in vitro was found (70). Increased levels of interleukin-2 receptors is seen following mitogen stimulation of lymphocytes and does not necessarily imply allergen-specific stimulation (71).

The significance of interleukins for the activity of inflammation including atopic dermatitis seems very important. They need not necessarily originate from lymphocytes, but could arise from monocytes, endothelial cells, keratinocytes and fibroblasts. Whether changes in interleukins may be of primary importance for the development of atopic dermatitis is not as yet known.

CYCLIC NUCLEOTIDES, LEUKOTRIENES AND CATECHOLAMINES

A detailed discussion of these topics falls outside the limits of this review. However, readers can obtain in-depth information concerning cyclic nucleotides in atopic dermatitis from a recent review by Archer (72), who also discusses the partial β-blockade theory of Szentivanyi (73). Leukotriene B_4, prostaglandin E_2, 12- and 15-hydroxy-eicosa-tetra-enoic acid are increased in lesional, but not normal, atopic skin indicating their possible significance during inflammation (74, 75).

A recent study on catecholamines in 41 patients with atopic eczema demonstrated a significant increase (two-fold) of norepinephrine, whereas epinephrine and dopamine were within normal levels (76).

IMMUNE REACTIVITY BEFORE THE DEVELOPMENT OF ATOPIC DERMATITIS

Kjellmann has studied the IgE levels in the serum of 207 children and observed a significantly increased incidence of eczema or rhinitis in those children who at their first investigation had an IgE level which was above 1 standard deviation of the normal average level (77). This observation has been confirmed by others (78). Thirty children of atopic parents were followed for 2–3 years. It was found that the number CD8+ lymphocytes in blood was decreased in those developing clinical symptoms of atopy later in life (31). The ratio of CD4+:CD8+ T-cells was found to be increased at 4 months of age in those children who later developed atopy within an observation period of 2,5 years (32). The breastfed children had a higher number of CD8+ lymphocytes than children on cow's milk, whereas the latter group tended to have more B-lymphocytes (32).

These studies indicate that the increased amount of IgE and the drop in CD8+ T-lymphocytes is present before clinical disease.

IMMUNE REACTIONS AND ATOPIC DERMATITIS

It is difficult to assess the exact importance of the immune deviations in atopic dermatitis for the development of the disease, or how significantly they contribute to the symptomatology of the disorder. A recent study of atopic eczema in twins showed that the monozygotic twin runs a risk of 0.86 of having atopic eczema if the twin partner has the disease, whereas the disease risk is 0.21 in dizygotic partners, which is equal to the risk among children in families where one child has atopic eczema (79). These results strongly support the concept that atopic dermatitis can only develop in genetically susceptible individuals and that environmental factors are of secondary importance.

All studies indicate that there are more or less profound immunological changes in atopic dermatitis compatible with an inherited immune dysregulation. It is possible that this 'dysregulation' is a lack of proper maturation. A maturation defect would likely be present in epithelial tissue and it might influence T-cell maturation with ensuing consequences. It has recently been demonstrated that thymic epithelium can release a peptide chemotactic for T-cell precursors, called thymotaxin (81). This 11 kD thymotaxin seems responsible for the attraction of hemopoietic precursor cells into the thymus and during an ensuing interaction matures T-cells to CD4+ cells. If this process is impaired in children of atopic parents, then a dysregulation develops in the immune system leading to an imbalance between the release of interleukins. This imbalance could be aggravated by stress factors such as viral infections, which are common in the early years of life. This then creates the basis for a dysregulation of B-lymphocytes with an IgE polyclonal activation (58, 60). The IgE including IgE reacting towards the common environmental aller-

gens is bound to the monocytes and mast cells of the skin and also to the mucosa in the intestine (81), turning the patient into an armored immunological 'bomb'.

When a child grows, it matures. This is probably also true for epithelial tissues. It can be *hypothesized* that an epithelial defect(s) is (are) overcome during aging and that, eventually, the immune deviation is more or less controlled. This then leads to the regulation of the immune system with more or less appropriate control of T-lymphocyte functions and ensuing the synthesis of IgE. The immunological imbalance early in life due to a lack of maturation of certain epithelial tissues may be disturbed by infections leading in some patients to the heightened allergic reactivity with concomitant symptoms.

It has been firmly established that patients with previously severe atopic dermatitis have an increased risk of developing irritant contact dermatitis later when they are exposed to skin irritation (82). This demonstrates that the skin of an atopic person, even when eczema is not present, has an increased capacity to initiate a lymphocyte-mediated skin inflammation. Again, epidermal derived interleukins may be of primary importance if transcribed and released from epithelial tissue, which proliferate following a damaging, non-immune influence.

The immediate, excellent effect of systemic steroids in atopic dermatitis supports the view that the condition is based on a continued mediator-sustained immune inflammation. The not so dramatic effect of cyclosporin A and the ensuing break-through of symptoms in some patients even during continued cyclosporin A therapy would tend to support the hypothesis that a specific blocking of interleukin-2 activity alone is not sufficient to stop the disease (83).

CONCLUSION

Atopic dermatitis is a multifaceted disease in which the altered immune reactivity dominates the symptomatology. The heightened inflammatory reactivity in the patients seems only partly due to specific allergies. Other factors such as tissue maturation, tissue expression and secretion of interleukins may be equally important in explaining the sum of effects which is clinically recognized as atopic dermatitis.

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Immunology of Contact Dermatitis

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Allergic contact dermatitis is a classical type IV delayed hypersensitivity immune response. This cell-mediated response is also known as hapten-type delayed hypersensitivity. Allergic contact dermatitis may be viewed as hyperreactivity of the skin immune system. In the present view of allergic contact dermatitis, individuals are born in a state of tolerance to environmental haptenic allergens. During life, sensitization to any hapten(s) may occur. Subsequent elicitation of a sensitized individual then leads to dermatitis, often accompanied by severe pruritus.

Human epidermal Langerhans cells play a central role during the sensitization stage. These antigen presenting dendritic cells, loaded with environmental haptens, continuously leave the epidermis through the lymph vessels and, upon arrival in the paracortical T-cell areas of the skin draining lymph nodes, they differentiate into interdigitating cells. There is now in-vitro evidence for such a maturation of human Langerhans cells into interdigitating cells. Any given individual may be sensitized to any particular hapten by this route. Allergic contact dermatitis is probably a skin-specific disease because of the capacity of Langerhans/interdigitating cells to induce relatively naive T-cells to become memory T-cells. Factors determining the ultimate outcome in this continuous hapten presenting process, i.e. whether or not the original state of tolerance will persist, are still enigmatic.

During elicitation, when the allergenic hapten is applied epicutaneously to a sensitized individual, a focal accumulation of immune response associated cells producing a wide variety of cytokines and inflammatory mediators ultimately results in the clinical condition of allergic contact dermatitis. Langerhans cells do not seem to play a major role during this stage. Although sensitization is systemic in nature, local mechanisms that divert hapten-specific cells into the elicitation site must exist. Mast cells and endothelial cells are probably involved.

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Allergic contact dermatitis is a classical type IV delayed hypersensitivity immune response. Subtypes of delayed hypersensitivity responses, which are cell-mediated, include Jones-Mote, tuberculin, granulomatous and hapten-type reactions. Allergic contact dermatitis is a typical example of the latter and can be defined as an abnormal response of the skin immune system (1) to a hapten-carrier complex, in which the hapten has a molecular weight generally less than 500 Dalton.

In the present view of allergic contact dermatitis, individuals are born in a state of tolerance to environmental haptenic allergens. During life, sensitization to any hapten(s) may occur. Subsequent elicitation of a sensitized individual then leads to dermatitis, often accompanied by severe pruritus. Clinically, the affected skin may show symptoms varying from simple erythema to the characteristic erythematous papulovesicular eczema. When chronic and repeated contact with the provoking hapten(s) is or cannot be avoided, lichenified hyperkeratotic lesions may ensue.

ALLERGIC CONTACT DERMATITIS AT THE CELLULAR LEVEL

Most data obtained in studies directed at the elucidation of the cellular mechanisms involved in allergic contact dermatitis have been performed in rodents. From these studies, which have been well summarized by Polak (2), it has become apparent that presentation of a hapten through the epicutaneous route may induce the development of at least two distinct (suppressor and effector) T-cell lines from their immature precursors. Suppressor precursors will develop into hapten-specific suppressor effector T-cells which are able to suppress the simultaneously activated effector (delayed hypersensitivity) T-cells from their respective immature precursors. In allergic contact dermatitis the most widely supported hypothesis is that this suppressor circuit fails, resulting in a lack of inhibition of hapten-specific effector T-cell activ-

tivity effector cells, memory T-cells specific for the hapten involved will be induced. Re-stimulation then will bring these memory T-cells into immunoblastoid stages, resulting in the production of even more delayed hypersensitivity effector T-cells.

Unfortunately, the results obtained in rodents cannot easily be applied to the human model of delayed hypersensitivity. There are two major and obvious differences between the skin immune systems of rodents and man. First, the elicitation of allergic contact dermatitis in sensitized rodents, more specifically in guinea pigs and mice, results in the establishment of a mixed cutaneous infiltrate consisting of T-cells, monocytes, basophils and neutrophils. Although it is difficult to find literature on this subject, the presence of especially neutrophils within the inflammatory infiltrate of elicitation reactions in rodents may be very prominent (3). This acute inflammatory response fades away within a few days. In man, however, the elicitation of allergic contact dermatitis results in the establishment of an infiltrate mainly consisting of T-cells with small numbers of dendritic cells, eosinophils and monocytes/macrophages, but virtually without neutrophils (4). This response of the human skin immune system to elicitation has a natural history of approximately 6 weeks, which is the time needed for the dermatitis to resolve.

In addition to this major difference in the immunohistopathology and course of allergic contact dermatitis, the skin immune system of mice and man differ in the actual presence of an intra-epidermal subset of T-cells. In normal mouse skin, dendritic epidermal T-cells expressing the gamma/delta type of T-cell receptor form a major cellular constituent (5, 6). In man, however, this T-cell subpopulation is extremely rare and its actual frequency is so low that these cells are only rarely present in an unselected epidermal specimen (7–9). The function of the gamma/delta T-cell receptor bearing T-cell subpopulation is at present still a mystery. However, data obtained in studies of allergic contact dermatitis in mice, where epidermal cell preparations have been used, need re-appraisement.

LANGERHANS CELLS AND THE INDUCTION OF SENSITIVITY

In man as well as in rodents, epidermal Langerhans cells play a central role during the sensitization stage. These antigen presenting dendritic cells, loaded with environmental haptens, continuously leave the epidermis through the lymph vessels and, upon arrival in the paracortical T-cell areas of the skin draining lymph nodes, they differentiate into interdigitating cells (Fig. 1).

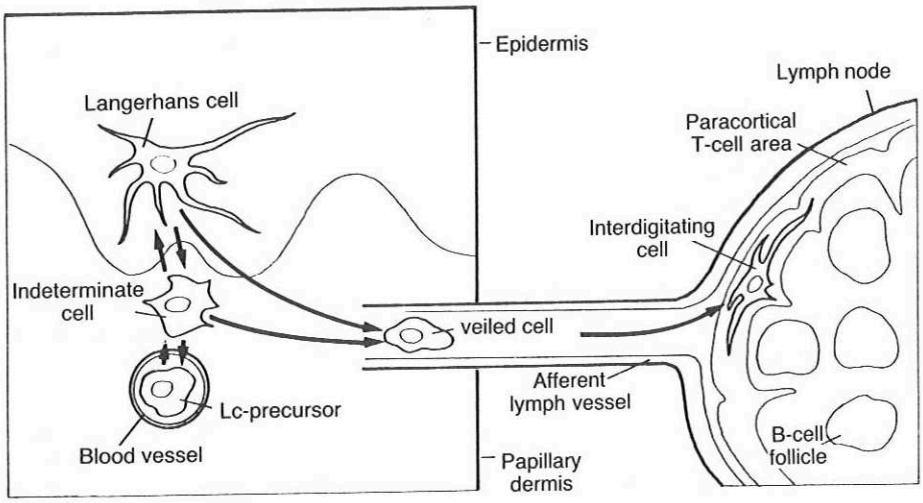


Fig. 1. Differentiation stages of dendritic cells of the skin immune system. In the present view, bone marrow derived blood borne cells enter the papillary dermis as indeterminate cells, they then differentiate within the epidermal micro-environment into Langerhans cells and continuously leave the epidermis as veiled cells, reaching the paracortical T-cell areas of the lymph nodes through afferent lymph vessels. Within the skin-draining lymphoid tissues these dendritic cells then differentiate into interdigitating cells. With permission from Elseviers Science Publishers, Cambridge, U.K. (1).

Evidence for such a differentiation pathway of dendritic cells within rodents has been supplied by a large number of studies. For example, most recently it has been shown that in rats lymph-derived dendritic cells do not recirculate, but enter the lymph nodes and differentiate into interdigitating cells (10). There is now in-vitro evidence for such a maturation of human Langerhans cells into interdigitating cells. For these in-vitro studies a method was developed by which millions of unlabelled human Langerhans cells could be obtained (11). In a subsequent investigation using a variety of monoclonal antibodies as well as electron microscopy and functional studies, it became apparent that human Langerhans cells in-vitro upon 3 days of culture lose their characteristic Birbeck granules as well as the expression of the CD1 glycoproteins. In addition, Langerhans cells cellular membrane expression of class II molecules of the major histocompatibility complex increased. Further results from our laboratory indicate that human Langerhans cells cultured for 3 days are more effective in antigen presentation when compared to freshly isolated Langerhans cells (12). Thus, in-vitro human Langerhans cells obtain the immunophenotype morphology and function of interdigitating cells within a relatively short period of 3 days.

Any given individual may be sensitized to any particular hapten by this Langerhans/interdigitating cell route. Allergic contact dermatitis is probably a skin-specific disease because of the capacity of Langerhans/interdigitating cells to activate relatively naive T-cells into effector or memory T-cells (13). We have preliminary evidence in man that the development of expanded clones of 'memory' T-cells from their immature 'virgin' or 'naive' precursors depends on the presentation of antigens by dendritic antigen presenting cells. Such a maturation cannot be induced by other important antigen presenting cell populations, i.e. cells of the monocyte/macrophage lineage. Factors determining the ultimate outcome of this continuous hapten presenting process, i.e. whether or not the original state of tolerance will persist, are still enigmatic.

ELICITATION OF ALLERGIC CONTACT DERMATITIS

During elicitation, when the allergenic hapten is applied epicutaneously to a sensitized individual, a focal accumulation of immune response associated cells producing a wide variety of cytokines and inflammatory mediators ultimately results in the clinical condition of allergic contact dermatitis. Epidermal Langerhans cells do not seem to play a major role during this stage, because their depletion (by ultraviolet-B or psoralens ultraviolet-A) cannot prevent elicitation in a sensitized individual. Although sensitization is systemic in nature, local mechanisms that divert hapten-specific cells into the elicitation site must exist. Mast cells and endothelial cells are probably involved.

In the mouse model, there is evidence that cutaneous mast cells bear antigen-specific T-cell derived factors (14, 15). Upon elicitation, haptens penetrate the skin, possibly by binding to major histocompatibility complex II bearing cells. Interaction of antigen and the T-cell derived antigen-specific membrane molecules on the mast cells result in the release of serotonin. The release of this compound leads to vasodilatation, facilitating extravasation of especially lymphocytes from the circulation into the challenged site. A small minority of these T-cells is antigen-specific and will proliferate and actively secrete cytokines upon antigen presentation.

In experimentally induced elicitation sites, i.e. in nickel-positive patch tests in humans, we have determined the percentage of hapten-specific T-cells within such an allergic contact dermatitis lesion by an antigen-independent T-cell cloning system. Lesional T-cells were cloned by limiting dilution and subsequently challenged with nickel in the presence of varying populations of antigen presenting cells. Up to 10% of the CD4+ T-cell clones were found to be nickel-specific (16). In addition, by studying major histocompatibility complex II restriction in this system, it was found that these different nickel-specific T-cell clones recognized different epitopes of the nickel-carrier complex (17).

FUTURE DEVELOPMENTS IN THE IMMUNOLOGY OF ALLERGIC CONTACT DERMATITIS

There is, as yet, no effective approach by which a state of sensitization, which may have to be endured for decennia, might be reversed into a state of tolerance. Therefore, continuous surveillance for new environmental highly allergenic haptens must be performed, preferably by national committees. In addition, new and reliable bio-assays by which the intrinsic allergenicity of a hapten can be predicted,

need to be developed. Finally, more detailed understanding of the molecular biology of sensitization might perhaps lead to the introduction of methods that can re-introduce a state of tolerance for the hapten(s) in a patient with allergic contact dermatitis.

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Patient Subgroups and the Inflammatory Pattern in Psoriasis

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Despite great numbers of recent studies on immunological parameters in psoriasis, the question whether psoriasis is an immunological disease is still open. Also, it is not clear how the three main abnormalities of this disease, i.e. the association with the human leukocyte antigen system, excessive epidermal new cell production and a unique neutrophilic infiltrate within the diseased epidermis, are linked together. Analysis of large patient cohorts has now shown that two types of non-pustular psoriasis exist: one showing early onset and linkage disequilibrium for human leukocyte antigen Cw6, B13, Bw57, the other type showing late onset associated with Cw2 and B27. The pathological features of increased cell proliferation and neutrophilic inflammation are likely to be regulated by potent peptide mediators some of which are mitogenic and/or proinflammatory. There are two powerful regulatory peptides ($C5a_{des\ arg}$ and neutrophil activating peptide-1), large amounts of which have now been isolated from psoriatic scale material. Both are able to stimulate other cells to migrate and to produce further signals. The initiating agent still remains an enigma.

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Psoriasis is a chronic hyperproliferative inflammatory disease without known cause. Histologically, aside from epidermal hyperplasia, inflammatory cells are numerous in the dermis and epidermis. These include neutrophils (as the predominant and characteristic cell type), lymphocytes, macrophages and mast cells (1, 2). There are no eosinophils among these cells. The chronic character of psoriasis with frequent relapses as well as the presence of the inflammatory infiltrate may suggest an immune pathogenesis. In fact, a number of observations has provided some evidence for immunologic alterations. These were related to changes seen in T-cells, B-cells, macrophages, anti-stratum corneum antibodies as well as cross-reactivities with microbial antigens. Recently Bos (3) has carefully discussed the possibilities of immune pathomechanisms in psoriasis and has pointed out that 'at present, psoriasis still has not been proved definitively to be an immunological disease'.

It is noticeable that even with whole body involvement psoriasis patients generally are in good health, and apart from concomitant joint disease (psoriatic arthritis) in ca. 15% of the patients (Henseler, in preparation) no other organ appears to be involved. Also, despite the fact the psoriatic skin is heavily colonized by pathogenic organisms (4), infectious skin diseases (e.g. pyoderma, herpes simplex, etc.) are rare in psoriasis patients (5). This indicates that the defence mechanisms including immune reactivity against microbial infection are not impaired, which is in contrast to atopic dermatitis (5).

GENETIC CONSIDERATIONS

When data concerned with immune defects in psoriasis are carefully assessed, no clear picture evolves and, like some decades ago, uncertainty remains as to the pathogenesis of this disease. In 1962 Burch and Rowell (6) in a paper discussing 'the mystery of psoriasis' referred to this dilemma with the statement that 'the only firmly established aetiological feature is its genetic basis...'.

Years later when studying human leukocyte antigen haplotypes associated with psoriasis, it became clear that a number of human leukocyte antigens, e.g. B13, B17 and Cw6, are significantly associated with psoriasis. In addition, several authors noted that early onset of psoriasis is frequently associated with human leukocyte antigen Cw6 (7–9). By following these observations we were able to provide evidence for the existence of two types of non-pustular psoriasis (10, 11). These types could be distinguished according to the age of onset, family inheritance and human leukocyte antigen-typing as well as disease patterns. Some of the data from that study as well as more recent results will be briefly reported here.

With the help of computerized data files from the Department of Dermatology in Kiel we were able to analyse data about the age of onset obtained from

2147 patients (10). It was found that the age at which psoriasis first begins varies considerably. Interestingly two peaks for age of onset could be identified in both men and women. One group of patients, consisting of nearly 75% of the total patient cohort, showed an age of onset of 16 (females) and 22 (males) while a second group showed an age of onset with a peak at 56 years. Both patient groups demonstrated a Gaussian distribution and proved to be distinct with an overlap of approximately 15% (between 46 and 51 years of age).

In the same study class I antigens were determined in over 100 patients of both groups and it was found that the Cw6 haplotype was predominantly present in the patient group with an early onset. On the other hand, such Cw6 linkage was nearly absent in patients with a late onset of the disease. It was noted also that the disease was following a more severe course in patients with early onset plus Cw6 as compared to patients with a late onset. These findings led to the conclusion that there exist two distinct types of non-pustular psoriasis, namely type I demonstrating early onset together with human leukocyte antigen Cw6 and type II without such associated findings (10).

It is interesting to note that a bimodal age of onset curve as seen in our patient group has been previously observed by several authors (6, 11, 12). Also, the presence of human leukocyte antigen Cw6 in patients with an early onset of psoriasis was suggested (13, 14). Thus our study revealed that there are indeed two patient cohorts which can be distinguished by several parameters.

Recently we have been able to add further data on human leukocyte antigen typing in early and late onset psoriasis (Henseler and Christophers, in preparation). In this study 256 patients were investigated for a total of 46 class I antigens. By plotting age of onset again bimodality was seen and as before the majority of our patients demonstrated early onset around the second decade of life (Fig. 1). Patients under 40 years of age again were significantly more often Cw6 positive (73.8%) when compared to patients with a late onset (22.8%) (15).

Further, a high frequency of additional antigens was present in type I psoriasis; B13, Bw57 and to a lesser extent A2. In type II the latter two were completely absent so that linkage disequilibrium for these haplotypes relates to type I. The antigens seen in type II (late onset, Cw6 negative) were B27 (25.8%) and Cw2 (27.3%) (Table I).

It is noteworthy that because of the human leuko-

Table I. *Human leukocyte antigen typing and relative risk (RR) in patients with early and late onset psoriasis (n = 256).*

	≤40 years of age		>40 years of age		Normal
	%	RR	%	RR	
Cw6	73.8	11.0	31.5	1.8	20.4
B13	34.3	7.7	9.1	1.5	12.3
Bw57	29.7	6.4	12.1	2.1	6.2
Cw2	11.6	2.3	27.3	6.4	5.5
B27	7.6	0.7	25.8	3.1	10.1

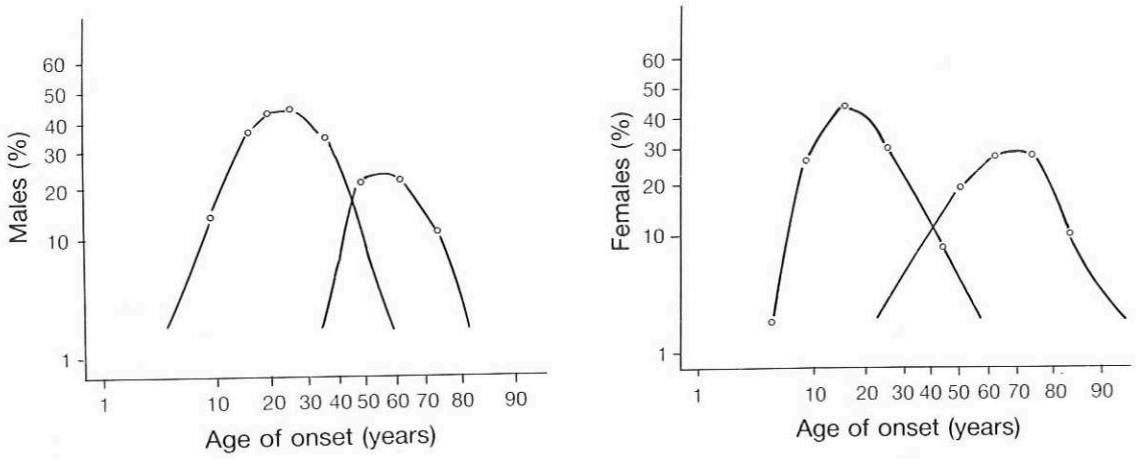


Fig. 1. Age of onset (per cent distribution) in 256 patients with psoriasis. Two patient cohorts can clearly be distinguished with age of onset peak at 25 and 60 years (males) and 17 and 63 years of age (females). Data from (15).

cyte antigen B27 (relative risk of 3.1) type II psoriasis appears to be more related to the group of human leukocyte antigen B27 positive diseases (Bechterew's disease, Reiter's disease) which often occur after infectious episodes predominantly of the intestinal tract. In these syndromes an immune mechanism is suspected.

In recent years a large number of studies has demonstrated significant associations between human leukocyte antigen genes and autoimmune diseases. In a review on the role of genetics in autoimmunity published by Batchelor (16) it was stated that from diseases in which there is strong evidence that autoimmunity is responsible for the pathological tissue damage, there is also evidence that the human leukocyte antigen region genes influence immune responsiveness. In fact 'all chronic autoimmune diseases studied have been shown to be associated with one or the other human leukocyte antigen' (14). In addition it has been observed that in some autoimmune diseases (e.g. diabetes type I, rheumatoid arthritis) specific alleles of different loci tend to occur together more often than expected. This linkage disequilibrium is relevant to the genetic susceptibility of the disease. As shown above, linkage disequilibrium appears to be present in type I psoriasis (Cw6, B13, Bw57) and to a lesser extent in type II (Cw2, B27).

In multiple sclerosis, which represents another chronic inflammatory disease, Van Lambalgen was able to identify two distinct subgroups showing different human leukocyte antigen profiles. These two types demonstrated different sets of resistance and susceptibility markers (17). As pointed out by Batchelor (14), in autoimmune diseases microbial infections may act as possible triggers for persisting autoimmune responses. Recurrent respiratory tract infections are known to be associated with human leukocyte antigen DR3 in multiple sclerosis patients (17). This observation bears similarities to the situation in psoriasis where streptococcal throat infections are known to trigger eruptive (type I) psoriasis. Thus it may be that a distinct set of human leukocyte antigen genes is associated either with susceptibility to infection or with abnormal responses to microbial antigens. There is evidence, as has been stated recently (16), that environmental agents such as infective agents, drugs and toxins are important initiating factors of autoimmune responses. The problem might lie in the fact that at present there are too many to be identified.

THE INFLAMMATORY ACTIVITY

Aside from the peculiar association to certain class I antigens described above we consider two additional features highly characteristic for psoriasis so that research concerned with these abnormalities could be rewarding. One of them is a highly increased proliferative activity of psoriatic epidermis which results in the highest degree of benign epidermal hyperplasia seen in human skin. In fact, psoriatic epidermis produces more keratinocytes per unit time and unit surface area than any other skin disease (2, 18).

The second most characteristic abnormality of psoriasis is the presence of an unusual infiltrate within the diseased epidermis. This consists predominantly of neutrophils and some mononuclears such as T-helper inducer cells and macrophages (3). In patients with extensive psoriasis the withdrawal of systemically applied steroids or recurrent streptococcal infections may provoke pustule formation (*psoriasis cum pustulatione*) where nearly all epidermal inflammatory cells consist of neutrophils. This is peculiar since no other skin disease except seborrhoic dermatitis shows persistent epidermal infiltration with neutrophils (19).

Work in our laboratory conducted during the last years was focussed on the contents of psoriatic scales because it was felt that by analysing the remains of the diseased epidermis one might be able to detect factors related to cell proliferation as well as neutrophilic inflammation. The results of these studies, which were successfully elaborated by Jens M. Schröder in our laboratory, shall briefly and superficially be reviewed here.

The first strongly chemotactic component present in psoriatic scales was isolated by column and ion exchange chromatography, radioimmunoassay and chromatofocussing. It proved to be the desarginated form of the C5 split product, $C5a_{des\ arg}$ (20). Large quantities of this strong proinflammatory chemotaxin could be isolated and purified. In addition, $C5a_{des\ arg}$ was not detectable in scales from other scaling disorders suggesting that it could play a role in psoriasis.

A second compound isolated from psoriatic scales was found to be a potent neutrophil activating peptide (called anionic neutrophil activating peptide) (20). This peptide proved to be distinct from interleukin-1/epidermal cell derived thymocyte activating factor for several reasons. Furthermore, the peptide was shown to be strongly chemotactic for polymorphonuclear neutrophils whereas its potency in elic-

iting the release of β-glucuronidase as well as the generation of $Oxigen_2$ by neutrophils was approximately 50 times lower (20).

Since anionic neutrophil activating peptide was isolated from scales it became important to know the cellular sources of this proinflammatory factor. Therefore, large amounts of supernatants from cultured human monocytes were analysed. A factor (molecular weight ~ 8 kD) was purified to homogeneity (21) showing high chemotactic activity in neutrophils but no chemotactic activity in eosinophils or monocytes (21). A factor, which was purified from stimulated human T-cells (human lymphocyte derived neutrophil activating peptide) (22) proved to be biochemically identical. This was subsequently analysed for amino acid sequence (23) and showed partial homology to a group of growth parameters (platelet factor 4, connective tissue activating peptide). Nearly identical material (by SDS-PAGE and terminal amino acid sequence) was subsequently isolated from the supernatants of human endothelial cells (24). This molecule which has in the meantime been isolated and characterized by various groups (25–28) is called neutrophil activating peptide 1 (29). Whereas this peptide was demonstrated to be a strong in-vitro chemotaxin for neutrophils in our laboratory, recent work by Larsen et al. revealed that neutrophil activating peptide 1 also 'chemo'-attracts T-cells (29). This appears important because apart from neutrophils T-cells are numerous within the psoriatic epidermis for reasons so far unknown. It is conceivable that in psoriasis neutrophil activating peptide 1 chemoattracts both neutrophils and lymphocytes.

CONCLUSION

Recent work has shown that psoriatic epidermis contains highly potent peptide mediators, which unlike platelet activating factor or leucotrine B_4 are not produced by neutrophils. Two of these, $C5a_{des\ arg}$ and neutrophil activating peptide 1 are able to activate several cell systems to secrete a second set of mediators. It is obvious that we are not dealing with a simple cause–effect relationship but with a spectrum of proinflammatory and mitogenic mediators from different sources. Their activities and targets act in concert producing a disease with a limited extent of tissue destruction and a lack of systemic involvement. It may be postulated that these mediator-dependent tissue reactions represent the persisting phase of psoriasis, which continues for years to produce the characteristic lesion. As mentioned before, the more specific events could be related to the initiation phase in which human leukocyte antigen associated susceptibility to (infectious) diseases may be important.

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The Immunology of Cutaneous Lupus Erythematosus

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The immunologic mechanisms involved in cutaneous lupus erythematosus are closely tied to the histologic and ultrastructural changes seen at the dermo-epidermal junction. These alterations are reviewed and an attempt is made to interrelate them with the current ideas on pathogenesis and therapy.

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HISTOLOGY AND ULTRASTRUCTURE OF THE DERMO-EPIDERMAL JUNCTION

The basement membrane region of skin lesions in lupus erythematosus patients has been the focus of considerable interest since the discovery that it appears thickened in histological sections, especially when stained with the periodic-acid-Schiff stain (1). With the advent of immunofluorescence it became apparent that immunoglobulins and complement are deposited in the same area, not only in the lesional skin of many lupus erythematosus patients, but also on the non-lesional skin of patients with systemic lupus erythematosus (lupus band). The deposition is granular or fibrillar at the basement membrane zone when it is examined with the immunofluorescence microscope after staining with the appropriate fluorescein isothiocyanate conjugated anti-human immunoglobulin (usually IgG or IgM, less commonly IgA) and complement (2, 3).

Electron microscopy later revealed that the deposition of the immunoglobulins was located on and below the lamina densa of the basement membrane in the skin of lupus erythematosus patients (3). The components of the lamina densa are type IV collagen, heparan sulfate proteoglycan, chondroitin-6-sulfate proteoglycan, nidogen and two antigens designated by the abbreviations KF-1 and LDA-1 for their respective antibodies (4). It has now become apparent that the anchoring fibrils are composed of type VII collagen, and the carboxyl terminus of the molecule, which is located in the lamina densa, is recognized by the LH7:2 and epidermolysis bullosa acquisita antibodies (5, 6) (Table I). Through the use of electron microscopy it also became evident that the basement membrane thickening seen on periodic-acid-Schiff staining with light microscopy corresponded to reduplication of the lamina densa of the basement membrane. Moreover, disruption of the basal cells of the epidermis was observed (Table II) (7).

Table I. *Antigens in the basement membrane zone*

Lamina lucida
Laminin
Bullous pemphigoid antigen
Cicatricial pemphigoid antigen
GB3
Lamina densa
Type IV collagen
Type VII collagen
Heparan sulfate proteoglycan
Chondroitin-6-sulfate proteoglycan
LDA-1
KF-1
Nidogen
(Fibronectin)

Table II. *Histologic and ultrastructural changes at the basement membrane zone*

Histology	Immunofluorescence	Ultrastructure
Thickening (especially on periodic-acid-Schiff staining)	IgG IgM C3 (IgA) granular or fibrillar	Reduplication of lamina densa with immunoglobulins on and below the lamina densa

CHANGES IN MATRIX MOLECULES

Our work examining the status of six matrix molecules at the basement membrane zone in lupus erythematosus patients using monoclonal antibodies and immunofluorescent microscopy in biopsies from 37 patients and immunoelectron microscopy of four patients revealed that type IV and type VII collagen, which are both components of the lamina densa, showed considerable alteration in the lesional skin of lupus patients. There was also a statistically significant correlation between the presence of immunoglobulin deposition and alteration of collagen types IV and VII in the lesional skin when examined by immunofluorescence. The changes in type IV and type VII collagen consisted of non-linearity of staining in the form of fragmentation of both antigenic components (Table III). This fragmentation was also seen when the biopsies were examined with immunoelectron microscopy and was more pronounced in type IV collagen. Additionally, there was reduplication of type VII collagen and, in some instances, stained fragments were noted below the lamina densa (8).

Table III. *Changes in matrix molecules*

Indirect immunofluorescence	
	All specimens
Linear vs non-linear staining in lesional and non-lesional skin	Probability of a greater value on χ^2 with 1 DF
Type IV collagen	$0.02 > P > 0.01$
Type VII collagen	$P < 0.001$
Fibronectin	$0.005 > P > 0.001$
	Lesional skin
Linear vs non-linear staining and immunoglobulin deposition	Probability of a greater value on χ^2 with 1 DF
Type IV collagen	$P < 0.001$
Type VII collagen	$P < 0.001$

PATHOGENESIS

The initiating event leading to epidermal cell damage and subsequent antibody formation to antigens made available at the cell surface by this damage may be the immune complex deposition at the basement membrane zone, which can be experimentally induced by ultraviolet light (8). The immune complexes can generate assembly of the so-called membrane attack complex of complement, C5b-C9, which mediates membrane injury (9) such as that seen in the epidermal cells of lupus erythematosus skin lesions. During the course of the skin disease, an infiltrate occurs at the basement membrane zone, adding credence to the theory that antibody-dependent cellular cytotoxicity may play a part in the pathogenesis (10). The inflammatory infiltrate and its constituent cytokines such as interleukin-1 cause increased production of collagenases capable of causing disruption of the collagen components of the lamina densa such as type IV and VII collagen. Levels of interleukin-1 are also increased during ultraviolet exposure, potentially causing increased damage (11) and there is recent evidence supporting mediation by cutaneous interleukin-1 of ultraviolet induced exacerbation in lupus erythematosus (13).

THERAPY

From the afore-mentioned, the use of sunscreens, anti-inflammatory agents such as steroids and other immune suppressants appears quite rational although the effects of the latter two are not specifically directed and have numerous side-effects. Currently the retinoids are receiving some interest since they appear helpful in cutaneous lupus, although it is still unknown how they aid. Possibilities include their anti-inflammatory effects (14), inhibition of polyclonal B-cell stimulation (15) or stimulation of macrophages aiding in elimination of inciting factors (16). Future efforts in treating cutaneous lupus might be directed against the various components instrumental in causing damage at the dermo-epidermal junction and continued research will give further insight into how this occurs.

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Immunopathological Techniques in the Diagnosis of Bullous Disorders

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Immunopathological techniques have become essential to the investigation and management of autoimmune vesiculobullous diseases. In this article the role and practical application of direct immunofluorescence, indirect immunofluorescence and complement C3 binding indirect immunofluorescence techniques, and their relevance to the diagnosis and management of the individual blistering diseases will be reviewed. The diagnostic value and limitations of the new split skin indirect method will also be described. Furthermore, the contribution of immunoelectron microscopy and immunoblotting studies to our knowledge of the pathogenesis of these diseases will be discussed.

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Immunofluorescence techniques are used extensively in the diagnosis of bullous disorders. Utilization of these techniques enables earlier diagnosis and investigation of patients with bullous diseases, thus leading to prompt treatment. Improvements in the optical equipment, commercially available conjugates and standardization of methodology have made immunofluorescence techniques simple to perform, more reliable and reproducible.

SPECIMEN COLLECTION

Blood specimens

Serum samples are needed for the detection of circulating autoantibodies. About 10 mls of blood without anticoagulant should be collected, serum separated and stored at −20°C until it is analysed. Serum and unclotted blood samples should be frozen in order to avoid massive haemolysis. Serum samples can be mailed without freezing.

Skin biopsy

The site of biopsy for immunofluorescence studies is of crucial importance and depends on the skin condition. In bullous diseases, ideally two biopsies should be taken: the first and most important one should be taken from the clinically uninvolved skin; the second from the edge of the blister which should include adjacent cutaneous tissue. A single biopsy of a blister alone is not suitable for immunofluorescence studies since immunoreactants and tissue structures are destroyed thus making microscopic interpretation difficult, if not impossible.

Snap freezing is still the preferred method for preparing biopsies for subsequent staining. In our experience the method originally described by Chayen has proved the most satisfactory (1). The biopsy is mounted in OCT compound and then snap frozen in a cold hexane bath.

If the skin biopsy needs to be transported and liquid nitrogen or carbon dioxide snow are not available, the biopsy can be transported in Michel's liquid medium at ambient temperature (2). This medium prevents the degradation of tissue-bound immunoreactants by inhibiting tissue enzymes. Earlier problems with the use of this medium seem to have been resolved by careful maintenance of neutral pH. This is best achieved by prior washing of the biopsy in normal saline or phosphate buffered saline to remove blood and serum proteins. The biopsies received in Michel's medium should be washed in buffer, snap frozen and stored at −25°C until they are stained.

IMMUNOFLUORESCENCE TECHNIQUES

In clinical immunodermatology there are three basic types of immunofluorescence tests: direct immunofluorescence, indirect immunofluorescence and complement binding indirect immunofluorescence (C3-method). The procedures of these methods are outlined in detail in several major text books on the subject (3).

Direct immunofluorescence

This is a one-step procedure for identifying tissue-bound antibodies, complement components and fi-

brinogen in vivo. Frozen sections of biopsies are incubated with monospecific fluorescein-isothiocyanate labelled antisera. It is recommended that anti-IgG, IgA, IgM, C3 component of complement and fibrinogen should be routinely employed. To obtain maximum tissue-specific fluorescence with minimum background fluorescence, the optimum dilution of the conjugates should be used. This is achieved by a chess-board titration procedure using a known positive tissue specimen (4).

The interpretation of direct immunofluorescence slides should be undertaken by an observer who has a sound knowledge of dermatopathology. Simultaneous examination of haematoxylin and eosin stained cryostat sections can help considerably with the orientation of the tissue and hence avoid potential errors in immunofluorescence interpretation. With a skin biopsy one has to contend with autofluorescence from keratin and elastic tissue and also with non-immunologic staining by fluorescence dye of certain infiltrates, particularly eosinophils (5). These have to be clearly differentiated by the observer from specific immunological staining patterns.

Indirect immunofluorescence

Indirect immunofluorescence is a serological technique for demonstrating circulating autoantibodies in a patient's serum. Frozen sections of normal tissue substrate are overlaid with serially diluted patient's serum followed by fluorescein-isothiocyanate labelled anti-human IgG. Every indirect immunofluorescence run should always include well-known positive and negative controls. It is now generally recognized that normal skin from the flexor surface of the arm, leg and thigh is the most satisfactory and reliable substrate. Neonatal foreskin, skin from the scalp and face or sun-damaged skin should not be used as they yield a high rate of false negative results (6). Substrates such as lip and oesophagus from rabbit and guinea-pig should be avoided because they also produce a high rate of false negative results. However, a recent study suggests the simultaneous use of several different substrates for the optimum result for detecting pemphigus antibodies in various clinical subtypes of pemphigus (7). Also, the use of tris-acetate buffered saline supplemented with calcium chloride is reported to increase the sensitivity of indirect immunofluorescence assay for detecting pemphigus antibodies on human skin (8).

The complement binding indirect method (C3-method)

Complement binding immunofluorescence is a three-stage procedure for demonstrating whether circulating antibodies are capable of fixing complement. This method also amplifies the staining obtained by conventional immunofluorescence when complement fixing antibodies are present. Frozen sections of tissue substrate (normal human skin) are incubated with the patient's serum, followed by complement (normal human serum diluted in complement fixation test diluting buffer) and then with fluorescein-conjugated anti-C3.

Split skin indirect technique

This technique provides a simple and reliable alternative to immunoelectron microscopy for differentiating anti-hemidesmosome antibodies (pemphigoid antibodies) from anti-sublamina densa antibodies (epidermolysis bullosa aquisita antibodies). The method relies on splitting normal skin through the lamina lucida so that pemphigoid antigen adheres to the epidermal side with components of lamina densa, EBA antigen, types IV and VII collagen on the dermal side. The split skin is then used as substrate for the indirect immunofluorescence method (9). This method also increases the detection rate of circulating anti-basement membrane zone antibodies (10).

IMMUNOPATHOLOGICAL FINDINGS IN BULLOUS DISORDERS

Pemphigus

Direct and indirect immunofluorescence are indicated in all forms of pemphigus, i.e. pemphigus vulgaris, vegetans, foliaceus (including the Brazilian type), erythematosus and drug induced pemphigus (11, 12), as well as atypical cases such as pemphigus herpetiformis (13, 14) and eosinophilic spongiosis (15, 16). On direct immunofluorescence intercellular antibodies can be demonstrated in the skin and mucosa of virtually all patients with active pemphigus. These antibodies are generally of the IgG class but IgM and IgA may be present. Although IgG is deposited throughout all layers of the epidermis, some patients with pemphigus foliaceus have IgG deposits in the superficial layers only (17). Interestingly, circulating antibodies from the same patients bind to all layers of normal epidermis. Researchers in this field have begun to examine these paradoxes. Stanley and his co-

workers have isolated a unique antigen to which pemphigus vulgaris antibodies bind and which is distinct from the antigen(s) detected by pemphigus foliaceus antibodies (18, 19). Multiple antigenic determinants may, therefore, be involved in the immunopathogenesis of these diseases. Regional variation in the expression of pemphigus vulgaris antigen(s) has been demonstrated in human skin (20). However, this does not correlate with the distribution of the lesions in the disease.

Frequently components of complement (C3) are bound to the intercellular area together with IgG in the lesional and perilesional skin, especially in untreated cases (21). The finding of intercellular complement deposits in normal skin should not be mistaken as a sign of pemphigus, since such findings may be associated with other conditions (22). However, when present together with IgG this may be a marker for disease activity (23).

Direct immunofluorescence in pemphigus erythematosus can demonstrate a mixed immunofluorescence pattern of intercellular deposits of IgG and C3 in the epidermis associated with granular deposits along the dermo-epidermal junction (24).

Tzanck smears are not satisfactory for immunofluorescence purposes (25). Epidermal intercellular IgG and C3 deposits are widely distributed in pemphigus and not confined to perilesional skin. Therefore, a diagnostic biopsy may be taken from any convenient site. This is of practical importance as perilesional skin is not always a suitable site for biopsy and may be absent in early cases of pemphigus cutaneous lesions (23).

Atypical cases of pemphigus may resemble lupus erythematosus, erythema annulare centrifugum, erythema multiforme, impetigo or apthous ulceration. In such cases immunofluorescence studies of sera and skin biopsy specimens establish the correct diagnosis. Despite extensive acantholysis found in Hailey-Hailey disease and in transient acantholytic dermatosis, both direct and indirect immunofluorescence are consistently negative. However, in lesional areas some secondary changes detectable by direct immunofluorescence have been reported in transient acantholytic dermatosis (24, 26). Such secondary changes occur in many types of skin lesions.

In most active cases of pemphigus, circulating intercellular antibodies are demonstrated in high titres. This serves as an additional, though not definite, diagnostic test. Titres as low as 1 in 10 in the presence of typical clinical and histological findings may be regarded as consistent with pemphigus. However, in early cases (particularly in those with oral involvement only) or when the disease is in remission they may be absent. In these early cases repeated testing is required as the disease evolves. In general a two-fold increase in titre of circulating intercellular antibodies is a sign of impending relapse (27). Similarly, a fall in titre indicates effective control of the disease. Although the titre of intercellular antibodies has been shown to fall following plasmapheresis (28), it is difficult to be sure that a clinical response following such treatment is directly related to the fall in antibody titres as adequately controlled studies have not been performed. It is recommended that indirect immunofluorescence tests should be performed every 2–3 weeks until the disease is in remission and thereafter every 3–6 months.

While it is the experience of most that titres reflect disease activity, it is not possible to predict the clinical status of a given patient on the basis of the antibody titres. Titres vary greatly from patient to patient with similar clinical disease activity. Disease activity can be assessed from titres only by studying them repeatedly over time in an individual patient.

Pemphigus antibodies have been reported to be species-specific in that they will bind to human or monkey epithelium but not to guinea-pig epithelium (6).

Patients with pemphigus erythematosus have antinuclear antibodies in their serum in addition to intercellular antibodies. Pemphigus-like antibodies have been found in the sera of patients with thermal burns, although antibodies in these patients may be directed against the basal cells of the epidermis rather than against the intercellular area. Pemphigus-like antibodies have also been found occasionally in patients with certain drug eruptions (particularly ampicillin and practolol). They are usually of weak intensity, present in low titre and tend to disappear spontaneously. Serum with high titres of antibodies to blood group substances A and B may produce a pemphigus-like staining pattern on indirect immunofluorescence because these antibodies bind to the blood group substances coating the epidermal cells. Absorption of serum with type A and B red blood cells before testing will eliminate this.

PEMPHIGOID

Both direct and indirect immunofluorescence studies

are essential in the diagnosis of bullous pemphigoid. However, in the diagnosis of the atypical forms of pemphigoid (Table I) indirect immunofluorescence studies are less helpful than direct immunofluorescence studies.

Table I. *Types of pemphigoid*

Bullous pemphigoid
Cicatricial pemphigoid
Localized pemphigoid
Nodular pemphigoid
Vesicular pemphigoid
Drug induced pemphigoid, e.g. frusemide induced pemphigoid
Ultraviolet-A induced pemphigoid
Psoralens + ultraviolet-A induced pemphigoid

Bullous pemphigoid

Patients with bullous pemphigoid have antibodies which bind to the basement membrane zone. Therefore, perilesional erythematous skin or normal skin adjacent to a blister are the preferred sites to biopsy rather than the blister itself which may produce false negative results.

Direct immunofluorescence reveals linear deposition of complement (C3) and IgG at the basement membrane zone in virtually all active cases of bullous pemphigoid. However, in one third of patients linear deposits of IgM and IgA may also be present. Occasionally IgD and IgE are also demonstrated (29, 30). Some patients with bullous pemphigoid may have linear deposits of C3 only along the basement membrane zone in the absence of specific immunoglobulins. The linear immunofluorescence pattern of antibody binding is similar to a pencil line and is often described as tubular.

Circulating anti-basement membrane zone antibodies are usually demonstrated in 70% of patients with bullous pemphigoid. However, when chemically split skin (1M NaCl) is used as substrate, the detection rate can be increased to 95% (10). Circulating antibodies in bullous pemphigoid are exclusively of the IgG class and consist mainly of IgG4, a non-complement binding subclass, and IgG1, a complement binding subclass (31). Bullous pemphigoid antibodies have been shown to be species-specific (32). A variety of substrates have been employed to demonstrate them. These include monkey, guinea-pig and rabbit oesophagus, monkey, guinea-pig and rabbit lip and skin from a variety of mammalian species including human. It is now well established that normal human skin is the most sensitive substrate for detecting bullous pemphigoid antibodies (33).

Fluctuation in titres of bullous pemphigoid antibodies does not correlate with disease activity and therefore cannot be used for prognostic purposes. However, with remission of the disease, antibodies usually disappear from the circulation.

Circulating anti-basement membrane zone antibodies may be negative in cases of bullous pemphigoid which are associated with concurrent internal malignancy (34, 35). An interesting observation made by Hodge and Marsden et al. is that 23% of patients with sero-negative pemphigoid developed concurrent malignant disease within 6 months of pemphigoid being diagnosed compared to only 4% of the sero-positive cases.

In bullous pemphigoid, antibodies are known to be produced and directed against the bullous pemphigoid antigen. This antigen is produced by keratinocytes and is a constituent of normal squamous epithelium (36). It is found in the basal keratinocytes where it exists as a large intracellular pool (37). Direct immunoelectron microscopy of bullous pemphigoid skin shows that IgG and C3 are deposited in the lamina lucida of the basement membrane zone and also at the hemidesmosomes of epidermal basal cells. Similarly, indirect immunoelectron microscopy also demonstrates that bullous pemphigoid antibodies bind in the lamina lucida and to hemidesmosomes (38, 39). This supports the pathogenicity of bullous pemphigoid antibody theory since the separation of the bullous pemphigoid blister occurs at the site of antibody binding, i.e. through the lamina lucida and the epidermal basal cell. However, since the serum titres of anti-basement membrane zone antibodies do not vary with disease activity, it is likely that antibody production may be a secondary phenomenon following bullae formation.

Employing biochemical studies, the bullous pemphigoid antigen has been identified as a non-collagenous glycoprotein composed of 220 kD subunits joined by disulphide bonds (40, 41). This glycoprotein is distinct from the other lamina lucida glycoproteins laminin and proteoglycan (42).

Cicatricial pemphigoid

Cicatricial pemphigoid is a rare blistering disorder of the mucous membranes, principally the oral mucosa and conjunctiva. Cutaneous lesions occur in only one third of these patients.

Direct immunofluorescence of both perilesional

skin and mucosal biopsies demonstrates immunoglobulins (IgG) and complement components (C3) at the basement membrane zone in approximately 80% of active cases (43, 44). Biopsies of normal skin are usually negative. An additional immunofluorescence finding that is occasionally seen in cicatricial pemphigoid is the presence of linear deposits of IgG and C3 in the basement membrane of mucous glands (45). The significance of this finding is not known.

Ultrastructural studies demonstrate antibody binding in the lamina lucida similar to bullous pemphigoid (46, 47).

Circulating anti-basement membrane zone antibodies are demonstrated in the serum of only 10% of cases of cicatricial pemphigoid (48). In contrast to bullous pemphigoid these are always of weak intensity and present in low titres. However, in the Brunsting-Perry variant, in which scarring plaque-like lesions appear predominantly on the neck and head, only a minority of cases have circulating antibodies, but when present they may be in high titres (49). The antibodies are often organ- or patient-specific and therefore require human mucous membrane or the patient's own skin as substrate.

Patients with cicatricial pemphigoid who have conjunctival involvement also have an increased incidence of HLA-B12 antigen (47), and about 50% have elevated IgA serum levels (50). The significance of these findings is not known at present.

The cicatricial pemphigoid antigen(s) is yet to be characterized but there is some evidence that it differs from the bullous pemphigoid antigen (45).

Pemphigoid gestationis

Pemphigoid gestationis is a rare blistering disease of pregnancy. The incidence of pemphigoid gestationis is estimated to be 1 in 50,000 deliveries (51). The obvious association between this disease and pregnancy suggests that an immune mediated tissue injury follows exposure to a newly encountered antigen produced or exposed during gestation.

Immunofluorescence studies of both perilesional and normal skin demonstrate C3 at the basement membrane zone in almost all active cases of pemphigoid gestationis (31). In addition, IgG may also be present in 30–50% of cases.

Immunoelectron microscopy studies show that both IgG and C3 are deposited within the lamina lucida and tend to concentrate adjacent to the lamina densa (52).

Usually indirect immunofluorescence fails to demonstrate circulating anti-basement membrane zone antibodies in the serum of pemphigoid gestationis patients. However, complement binding indirect immunofluorescence technique detects an anti-basement membrane zone complement fixing agent in the serum of these patients. This poorly defined molecule is termed 'pemphigoid gestationis factor'. Recent studies in our laboratory by Kelly and Cerio et al. (31) have conclusively shown that this pemphigoid gestationis factor is an IgG1 antibody.

The antigen in pemphigoid gestationis awaits identification and characterization. Kelly and Bhogal et al. (53) have reported that pemphigoid gestationis antigen is present in the basement membrane zone of skin, amnion placenta and amnion cord. They found that it is present in the placenta from 22 weeks gestation until term, but that it is absent from the placenta at 12 weeks gestation.

The primary event in the pathogenesis of pemphigoid gestationis is still unclear. It seems that once the antibodies are synthesized, they are clearly responsible for precipitating the disease, partly due to complement activation and cell lysis.

Linear IgA bullous dermatosis

Recent evidence (54–56) suggests that chronic bullous disease of childhood is the same condition as adult linear IgA bullous dermatosis. Both are characterized by the linear deposition of IgA at the dermoepidermal junction and both appear to share the same target antigen (55). The clinical features, histological and immunopathological findings are also largely identical (56).

All patients with this condition have IgA deposited in a homogeneous linear basement membrane zone pattern in the uninvolved skin. Other immunoreactants may be present in 20–30% of patients but this does not correlate with any difference in the clinical picture or prognosis (56, 57). Direct immunofluorescence becomes negative during clinical remission in the majority of patients (57, 58) although in some cases it may persist for many years after the patient has gone into remission (58–60). Unlike dermatitis herpetiformis it seems that the IgA deposits in linear IgA bullous dermatosis in adults are not of mucosal origin as they lack J-chains (61).

Indirect immunofluorescence demonstrates IgA class anti-basement membrane zone antibodies in the sera of patients with chronic bullous disease of childhood and also in those with linear IgA bullous dermatosis. In a study of 25 adult cases and 25 childhood

cases of linear IgA bullous dermatosis, Wojnarowska and Bhogal et al. (56) found that 72% of the childhood cases had circulating IgA anti-basement membrane zone antibodies while only 20% of the adult cases were positive and tended to be lower in titres. Circulating anti-basement membrane zone antibodies of classes other than IgA may also be detected but these do not relate to any differences in the clinical picture (55, 57, 58, 60).

The site and nature of the target antigen for the circulating IgA anti-basement membrane zone antibodies remains an enigma. Ultrastructural studies in our department on 31 patients with linear IgA bullous dermatosis consistently demonstrated the antibody binding site to be in the sublamina densa region of the basement membrane zone (62). This is in contrast to other reports (63–66) which have shown the binding either in the lamina lucida or in the sublamina densa.

Indirect immunofluorescence studies on split skin have shown variable sites of IgA binding. When skin is split by 1M NaCl, binding occurs to the epidermal side of the split. When the split is performed by the suction blister technique, antibodies bind to the roof and base of the blister (66). These discrepancies may be explained by the fact that different antigenic binding sites are exposed when the skin is split.

Dermatitis herpetiformis

The major criterion for the diagnosis of dermatitis herpetiformis is the presence of granular deposition of IgA on direct immunofluorescence in the uninvolved skin (67–69). These IgA deposits are predominantly in the papillary dermis although they may extend up to the dermo-epidermal junction and down to the deeper dermis. Immunoelectron microscopy has revealed that the IgA deposits are closely associated with fibrillary structures, which have been termed microfibrillary bundles (70, 71).

The biopsy for direct immunofluorescence is best taken from uninvolved skin as the IgA deposits are often absent from vesicular or inflammed lesions (68). If direct immunofluorescence is not positive for IgA in a patient whose clinical features are strongly suggestive of dermatitis herpetiformis, then serial sections of the biopsy should be taken (72). If the result is still negative, a repeat biopsy is indicated. Seah and Fry (73) in a study of 50 patients reported on two in whom the IgA was not found in the first biopsy specimen but was found in the second. It has also been demonstrated that the intensity of IgA fluorescence may be reduced and in some cases disappear completely in patients on a strict gluten-free diet for a long period of time (74–76). The IgA deposits in the skin of dermatitis herpetiformis patients has been found by Unsworth and Payne et al. (77) to be dimeric, thus suggesting that the IgA in this condition originates from the plasma cells in the small intestine.

Occasionally, deposits of IgG and IgM may also be found in the skin of patients with dermatitis herpetiformis (73, 78). In these cases, the IgA staining is of the typical papillary pattern while the other immunoreactants are present in a more linear basement membrane zone pattern. There is no association between the presence of immunoglobulins other than IgA and any clinical or prognostic features. Deposition of the C3 component of complement is also found in the uninvolved skin (67, 79). There is much evidence (80, 81) to suggest that this is deposited as a result of activation of the alternative pathway and is thus relevant to the pathogenesis of the lesions. Circulating immune complexes containing IgA have been detected in 30% of patients with dermatitis herpetiformis. Their presence, however, does not correlate with disease activity (82).

Indirect immunofluorescence is usually negative for circulating anti-basement membrane zone antibodies in dermatitis herpetiformis. However, it should be part of the routine clinical assessment in order to distinguish dermatitis herpetiformis from atypical cases of bullous pemphigoid.

Patients with dermatitis herpetiformis have a high incidence of tissue reactive antibodies in their serum, particularly gastric parietal cell, thyroid and antinuclear antibodies (83). They also have circulating antibodies that react with wheat protein (84, 85). Unsworth and Leonard et al. (86) were able to demonstrate antigliadin antibodies in 46% of patients with dermatitis herpetiformis. The levels of IgA class antigliadin antibodies tend to fall when the patients adhere to a gluten-free diet (87, 88). These antibodies are a non-specific marker of gluten-sensitive enteropathy as they may also be present in up to 15% of gastrointestinal disease control subjects and are not present in all patients with gluten-sensitive enteropathy (89).

Chorzelski and co-workers (90, 91) have described an IgA class antibody to the reticular component of the endomysium of smooth muscle (IgA-EmA). This has been found in 70–75% of patients with dermatitis herpetiformis and is specific for the presence of gluten-sensitive enteropathy, regardless of skin lesions (92, 93). The titres of IgA-EmA correlate strongly

with the severity of gluten-sensitive enteropathy: they fall with the patient's adherence to a gluten-free diet and reappear following gluten rechallenge (94). As the IgA-EmA antibody is a more specific marker, it is of practical use in dermatitis herpetiformis as an indicator of the presence and severity of underlying gluten-sensitive enteropathy and also for monitoring patient compliance with a gluten-free diet.

Epidermolysis bullosa aquisita

The 'classical' clinical picture of epidermolysis bullosa aquisita is that of a mechanobullous disease in which the blisters heal with scarring and milia. However, this condition is notable for the variability of its clinical expression and its capacity to mimic other vesiculo-bullous diseases (95). Thus, determining the correct diagnosis often rests with immunopathological investigations, particularly immunoelectron microscopy.

Direct immunofluorescence studies of lesional and perilesional skin demonstrate linear deposits of IgG at the basement membrane zone. These deposits usually form an intense broad fluorescent band. Components of complement, IgA and IgM may also be seen but with less intense staining than IgG (95). The distribution of IgG deposits in epidermolysis bullosa aquisita appears to be generalized in the skin (96) but may show regional variation. It is thus best to biopsy perilesional skin for direct immunofluorescence studies (95).

Indirect immunofluorescence reveals circulating anti-basement membrane zone antibodies in 25–50% of patients (98–100). These are more likely to be found in the early or inflammatory phases of the disease (95) and there is no evidence suggesting an association of antibody titres with disease severity (97). When chemically split skin (1M NaCl) is used as substrate, the antibodies bind to the dermal side of the split. This is in contrast to the binding demonstrated by bullous pemphigoid antibodies which usually stain the roof of the split and can be used to differentiate epidermolysis bullosa aquisita from bullous pemphigoid. However, Logan and Bhogal et al. (101) have shown that some cases of bullous pemphigoid may show the dermal pattern of staining similar to epidermolysis bullosa aquisita. Therefore, this technique cannot be used in isolation to make the definitive diagnosis of epidermolysis bullosa aquisita.

Immunoelectron microscopy is the most specific means of diagnosing epidermolysis bullosa aquisita. Direct immunoelectron microscopy shows IgG deposits in the upper dermis at the interface of the lamina densa and the sublamina densa. The target antigen has been shown by immunoblotting techniques to be a distinct basement membrane zone component consisting of 290 and 145 kD subunit chains (102). Studies by Yoshiike and Woodley et al. (103) suggest that the 290 kD chain consists of collagen and non-collagen domains of equal size: 145 kD each. The non-collagenous domain is localized in the lamina densa and the collagenous domain is distributed in the sublamina densa region. The antigenic determinants are in the non-collagenous domain.

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Discussion

CHAIRMAN: DR J.D. BOS

Dr J.D. Bos (The Netherlands): Dr Van Lambalgen, like Dr Luger, emphasized that there may perhaps be a very big future for cytokines to be used in therapy. This would also be the case for application of pharmaceutical peptides. Would either of them be able to give an idea of what the future will be for those cytokines, particularly in the therapy of dermatological conditions?

Dr R. Van Lambalgen (The Netherlands): The cytokines are very interesting compounds and possibilities for therapeutical applications develop rapidly. Many different classes of cytokines have been recognized so far, and some of them are currently subjected to assessment in clinical trials. Very few of them, however, are being tested for dermatological disorders, apart perhaps from epidermal growth factor (EGF). Some of the cytokines which increase haematopoiesis — that is, the colony stimulating factors (CSFs) — have been for some time in clinical trials for myelosuppression seen with chemotherapy. These cytokines, in particular, will hold great promise for the future.

One important finding in most of the clinical assessments is that, although these factors are active as single agents, the best clinical efficacy may be obtained by combining different factors either together in a pool of factors or subsequently at different times. With respect to dermatological diseases, as I have said, perhaps EGF may be of interest for the near future.

Dr T.A. Luger (Austria): I agree with Dr Van Lambalgen. There might be some additional aspects which would have to be considered. First, at the moment we do not know enough about all the regulatory mechanisms of this cytokine network. For example, knowledge about the downregulation is rather incomplete because only a couple of studies have been done so far. It is known that the transforming growth factors (TGFs) have immunosuppressive effects in some way, but there is evidence for many other factors which may also downregulate an immune response in a similar way.

On the other hand, with the possibilities for therapy in mind, another promising approach would be the development of cytokine analogues with which there is a specific effect. This might overcome the problem of side-effects. For example, in the interleukin-2 (IL-2) trials and also in the granulocyte macrophage colony-stimulating factors (GMCSFs) trials one of the major side-effects which has been observed is the capillary leak syndrome, which limits the therapeutic efficacy of these mediators.

Secondly, a combination of these mediators of the peptides with drugs might be considered. An example, which might be of interest for dermatology, is the treatment of certain forms of cutaneous T-cell lymphoma with the peptide mediators in combination with low-dose interferon and retinoids. At the moment, this appears to be very promising.

Dr Van Lambalgen: I would like to add that, as we heard earlier, steroids affect the arachidonic acid metabolite formation, such as the production of leukotrienes and prostaglandins. So far, some of the cytokines that have been discussed, such as interferons, but in particular some of the CSFs, also have some very direct and profound effects on the production of these prostaglandins and leukotrienes. As far as we know, some of these cytokines interfere with the pathway in the same manner as steroids.

Dr Bos: Dr Luger said that ultraviolet-B (UV-B) is able to induce or to enhance the production of IL-1 by keratinocytes. On the other hand, it is also known that UV-B is an immunosuppressive therapy, so how can those two findings be reconciled?

Dr Luger: This again points to our lack of knowledge about the regulation of cytokine production. It also focuses our attention on another problem; that is, that the light sources used to investigate the synthesis of cytokines by any cell contain a mixture of different wavelengths. It might well be possible that a particular wavelength could be responsible for the induction of a certain signal, but this is not known at the moment. It is certainly true that UV-B irradiation turns on positive signals such as IL-1, IL-6 and also the CSFs, but it also turns on the downregulating

signals, including the TGFs and what we call contra-IL-1.

Again, not enough is known about the quality of the ultraviolet light which may be responsible for triggering either the positive or the negative pathway.

Dr G.E. Piérard (Belgium): Prof. Thestrup-Pedersen, why is it that all the diseases presented by you manifest themselves so symmetrically in patients? There is generally a symmetry in the distribution of the lesions on the body. We have been informed about immunology, but what about the distribution? Why do lesions present on some sites and not on others? Are there differences in the immunology of skin according to the location on the body? Is anything known about that?

Prof. K. Thestrup-Pedersen (Denmark): That is a very interesting question. It should be realized that skin does not follow the political pattern of left and right. The diseases with which we are concerned are diseases which involve the general organ; skin. By their nature, they necessarily have to be symmetrical. They are not nevoid, or linked to any vascular deformation; they are not linked to any haemangiomas or anything else which is determined by anatomically demonstrable defects. They are spread over the total skin organ, and that is necessarily symmetrical. I think that could be the explanation, if I understand your question correctly.

Dr Bos (The Netherlands): I have a question more specifically related to your presentation on atopic dermatitis. At least according to your hypothesis, you think that atopic dermatitis patients have an immature epidermis. Could you perhaps explain more about this? As far as I know, this is a new aspect of, or a new hypothesis about, this disease.

Prof. Thestrup-Pedersen: I want to stress again that it is my own private hypothesis that the epithelial tissues show some kind of immature defect of the immune system. My reason for saying that is the nature of the disease itself, in which as the individual matures we see how the immune system regulates itself towards a normal state.

Also, as I stressed in my talk, there is the clinical fact that these patients are not healthy even when they are without any eczema. Depending upon the climate, they may still develop xerosis of the skin or a dry skin, without having a clear-cut eczematous state. If their skin is damaged by such normal levels of irritation that occur in washing, contact with detergents or other irritating agents, a threshold can again be passed, inducing a lymphocyte-mediated inflammation.

Some kind of 'maturation defect' may exist. It is very important to look much more carefully, also in atopic dermatitis, at the whole cytokine cascade as discussed today by Dr Luger.

Dr H.W. Rothenborg (Denmark): Sometimes when patients even with severe atopic dermatitis have, for example, a severe virus infection, the eczema disappears completely. As the patient recovers, the eczema returns. Is it possible to link that with any of the phenomena that we have been confronted with today?

Prof. Thestrup-Pedersen: My answer would be speculative. I do not know how many cases you have seen in which the eczema clears up completely during a virus infection. I do not think it is a common event that can be seen in our wards.

Of course, if the immune system is strongly influenced, if the lymphocytes have disappeared, there cannot then be a lymphocyte-mediated inflammation in the skin.

However, it is more complicated than that. There was a recent report, I think in the *British Medical Journal,* in which acquired immune deficiency syndrome (AIDS) patients were asked if they previously had atopic symptoms. When the immune paralysis starts in an AIDS patient, that person develops the atopic symptoms again. Apparently, there is a very complicated and delicate immune balance or imbalance.

Dr R. Darhell (UK): With a view to Prof. Thestrup-Pedersen's lecture about the immaturity of the skin, especially in children who eventually get over atopic eczema, could there be the same immaturity in the gut as in the skin which might explain food intolerance playing a part in eczema?

Prof. Thestrup-Pedersen: Do you mean whether this imbalance in the immune system could explain the food intolerance? In relation to your question, an interesting finding in the gut has recently been published by Finnish workers. They have observed that there is an increased number of mast cells in atopic dermatitis. The higher the amount of IgE in the se-

rum, the more these mast cells were armed with IgE. Many of these 25 patients had had symptoms of intolerance, but not completely correlated to the number of IgE-armoured mast cells in the intestines.

At the moment, the subject of food intolerance has, in my opinion, more question marks than answers.

Dr L. Juhlin (Sweden): Prof. Thestrup-Pedersen mentioned that there was a 100% increase of mast cells. Was this in normal-appearing skin or only in the lesions?

Prof. Thestrup-Pedersen: I am quoting the paper by Nick Sota, who presented his results at the Oslo meeting. I cannot say whether it was eczematous or normal skin, but I think it was the latter.

Dr S.A. George (UK): Is Prof. Thestrup-Pedersen aware of any immunological markers that can be used to monitor the progress of the disease activity in atopic dermatitis clinically?

Prof. Thestrup-Pedersen: I do not know. I presented some results of the IL-2 receptor level in serum. The patients we followed for 1 month did not clear their eczema and had, on average, the same activity in their atopic eczema. It would be interesting to take samples from children with atopic eczema, keep them in the freezer and look at the children after 10 years. That would be a good study to perform. I have not done it, and do not know whether anyone else has.

Dr Bos: There are no biological markers to assess disease activity in atopic dermatitis or in psoriasis, as far as I know. They are needed urgently.

Dr Bos: Prof. Christophers, I was slightly surprised when you said something about Langerhans cells at the end of your presentation, and that you hoped never to have to speak about Langerhans cells and psoriasis. I did not understand that, and perhaps you can explain a little more about what you meant.

Prof. E. Christophers (FRG): I think you understood quite correctly what I said. The situation is that we in dermatology behave at the moment as if we do not understand the world when we do not talk about Langerhans cells. There is a sort of 'Langerhansomania' going on at present. Whenever we go to meetings and talk about immunological aspects in dermatology, the name of Paul Langerhans is mentioned. Unfortunately, in psoriasis this is not applicable. There is no evidence that the Langerhans cell plays any role in this disease. I may be very much mistaken, but I would say that also in the future this cell will not come up — but this is a very careful statement, which I will be ready to withdraw.

Dr Bos: It is a pity that Dr Valdimarsson, from Reykjavik, is not here. He formulated a hypothesis in which Langerhans cells are central in this disease.

Dr H.K. Shamy (UK): I was very interested in your human leukocyte antigen classification of your psoriasis patients into locus CW6-positive and -negative Type I/Type II. Does it include pustular psoriasis, generalized pustular psoriasis and pustulosis of the palms and soles? If so, where can they be classified?

Secondly, did you also assess the leukocyte function in these types of diseases?

Prof. Christophers: We as well as others have looked for an association between pustular psoriasis and histocompatibility locus antigen (HLA) linkage. What I found with the classification Type I/Type II refers only to non-pustular psoriasis. The pustular type of Zumbusch and the palms and sole type pustular psoriasis are different. There is no evidence that they belong in the HLA classification group.

Concerning your second question about the systemic abnormalities in terms of leukocyte function, we had nine patients with a Zumbusch type psoriasis and, depending upon the disease activity, found hyperactive leukocytes within the peripheral blood — that is, neutrophils. Four of those nine patients had a transient receptor downregulation. They had a locus C5a defect for several days when they were in the middle of their eruption, indicating that complement split products like C5a or C5a-des-arg are systemically present and do inactivate the receptor. That is the evidence for the involvement of a priming and a de-activation of circulating leukocytes under these conditions.

Dr D. Court-Roger (UK): Dr Bos, you stated that you did not think Langerhans cells were involved in the elicitation phase of contact dermatitis. However, there is evidence from both in-vivo and in-vitro observations that they are involved. Langerhans cell numbers are increased in the infiltrate in allergic contact dermatitis between 24 and 48 hours. Also, in the

in-vitro model the function of Langerhans or the antigen presenting cells is better than that of peripheral blood mononuclear cells. In view of these findings, why do you not think that Langerhans cells are involved in the elicitation of allergic contact dermatitis?

Dr Bos: Studies both in the mouse model and in man have shown that when there is a state of sensitization and an attempt is made to elicit contact dermatitis, having previously eliminated epidermal Langerhans cells either by psoralens and ultraviolet-A (PUVA) therapy or by UV-B, there is still a normal elicitation response.

I know this matter is still under debate, but I support the idea that even when no Langerhans cells are present it is still possible to elicit allergic contact dermatitis. The fact that Langerhans cells are present in the infiltrate does not mean that they are central in the development of the lesion during elicitation.

Dr Court-Roger: I accept that, but it is not the normal situation not to have Langerhans cells present. Langerhans cells are several times more potent at antigen presentation than macrophages. Macrophages can also present antigen, and if there are no Langerhans cells these macrophages will take over the antigen presentation. However, in allergic contact dermatitis the antigen is presented on the surface of the skin, so the Langerhans cells are the first cells to pick it up. It is therefore only reasonable to think that they may be involved. I am not saying that other cells cannot be involved, but that Langerhans cells would seem to be involved.

Dr Bos: I agree that it is reasonable to accept that idea, but there is no proof that they could indeed be functional in the elicitation of allergic contact dermatitis.

Dr Court-Roger: There is, however, likewise no proof that they are not.

Prof. Thestrup-Pedersen: Prof. Turk, in London, has studied that particular problem by stripping the epidermis. He showed that there was quantitatively the same response in patch testing later. Even though the Langerhans cells are in the first line of defence, we should not forget about the interdigitating cells in the lower layers, of the dermis, as possible mediators of an immune reaction.

Dr Court-Roger: I am not sure that those interdigitating cells always express Class II antigens, though, which would be a prerequisite for antigen presentation.

Prof. V.K. Havu (Finland): Dr Mooney, you mentioned that IL-1 activates collagenase. What was meant by that statement? Do you mean that if you have collagenase and collagen in a test-tube and add IL-1 you would find an increase of collagen reaction products which would indeed mean an activation of collagenase?

Dr E. Mooney (Iceland): These were not my studies to which I was referring. Dr Luger may be able to reply more accurately.

Dr Luger: There are a couple of studies showing that in addition to both types of IL-1, another mediator not mentioned today is also present in the skin. It is tumour necrosis factor-α (TNF-α), which may play a role in lupus erythematosus. These mediators activate fibroblasts to produce more collagenase.

Prof. Havu: That is something different. More collagenase is produced, but it does not activate collagenase. That is a different thing.

Dr Mooney also said that retinoids inhibit collagenase. Again, to be exact, it means that if retinoids are added to collagenase and collagen in a test-tube, there are less reaction products. Is this what you mean?

Dr Mooney: These are studies done by Dr Bower in fibroblast cultures. I believe he measured collagenase *per se,* but he may have been measuring the amount of collagen. There was less collagen degradation when the retinoids were added to his fibroblast cultures.

Prof. Havu: So there was most probably less production of collagenase instead of inhibition of collagenase.

Dr L.J.W. van Ginkel (The Netherlands): Dr Black, you mentioned the central role of plasminogen activator (PA) in the breakdown of the intercellular substances in the blister formation of pemphigus vulgaris. Do you have any experience, or what do you think about the idea of treating patients with pemphigus vulgaris with well-known inhibitors of PA, such as

ε-aminocaproic acid, transhexaminic acid or low molecular weight serum protease inhibitors?

Dr M.M. Black (UK): I have had no experience using any of those suggested types of treatment. The concept of plasminogen activation is very much the work of Gerald Lazarus and his group, in Philadelphia. Although it is a phenomenon that is occurring in pemphigus, I doubt that it would turn out to be the only reason why damage occurs leading to acantholysis. As I demonstrated, a variety of complement factors may well cascade and produce some sort of change in the region of the intercellular cement substance as well as in plasminogen or PA's. It may be rather more complex than that, but I agree that the question is an interesting one.

Question from the audience: You pointed out the close resemblance between bullous pemphigoid and herpes gestationis, in fact calling the latter pemphigoid gestationis. I think there is one histological feature that is different in these two blistering disorders; it concerns the necrosis of individual keratinocytes in the basal layers of the epidermis. Would you comment on that?

Dr Black: You are quite right, but the phenomenon is an early histopathological change of herpes gestationis. I think it is dangerous to make distinctions between one disease and another on one criterion like that alone. The reason for grouping bullous pemphigoid and herpes gestationis together is that there are far more similarities than differences between those conditions.

Of course there are some differences, and we could spend a long time discussing the pros and cons whether or not they are the same disease. Just as I talked about linear IgA disease (on which I could also have spent a long time), there are overlaps occurring between these diseases.

I think what makes bullous disease quite interesting is the fact that there can be a very wide range of both clinical and histopathological expression whether early or late lesions are studied. There are also immunopathological variations differing from the single theme of basement membrane zone deposition. However, the fact that the antigen in herpes gestationis is not yet known and may well differ somewhat from bullous pemphigoid, and the fact that the antibody is deposited in the lamina lucida zone, puts these two diseases much closer together rather than keeping them well apart.

Dr B. Brooks (UK): I was very interested in your remarks about finding and identifying the antibodies in pemphigus foliaceus and pemphigus vulgaris. I thought that over a period of time pemphigus foliaceus gradually changed and became a more pemphigus vulgaris picture. Do you think that the antibodies also vary over a period of time?

Dr Black: Again, we are talking about variability both of clinical and of immunopathological expression. I agree entirely that the clinical picture may overlap, as do the immunofluorescence findings. There may well be findings in pemphigus foliaceus which look identical to those in pemphigus vulgaris, but I think the point is that these techniques are perhaps not sufficiently sensitive. By looking at the molecular level it is possible to detect the obvious differences. This separation of pemphigus foliaceus from pemphigus vulgaris, by Stanley and his group, has been the major contribution of recent years. I would certainly accept their data that there appear to be different antigens, but of course they are probably very closely related and situated in the region of the intercellular zone, perhaps on the desmosomes, and that is why the clinical and the immunofluorescence expressions look very similar.

Dr Bos: Before I close this session I would like to thank all speakers for their excellent contributions this afternoon. I would also like to thank all employees of Gist-brocades who made possible this excellent day.

Closing remarks

Mr A.J. Westerveld (Gist-brocades, The Netherlands): I do not know whether or not it is usual in the international world, but at Gist-brocades we have 10 people who for the last couple of months have been extremely busy, and super-busy the last couple of days, in organizing this symposium. Without their efforts and enthusiasm I think it would not have been possible, even with the best of intentions, to get this meeting organized. If I may be allowed, I would like to thank my collaborators for the work they have done.

I should also like to take this opportunity to thank both Chairmen for their dedication as far as the scientific part of this symposium is concerned.