In order to elucidate the still poorly understood pathogenetic pathways of acute tinea, the inflammatory cellular infiltrates in this infection were analyzed. Lesional punch biopsies were cryostat-sectioned and stained with monoclonal antibodies for immunophenotypization of T cells, B cells, macrophages and activation markers. For each antibody the positively stained inflammatory cells in the dermis and in the epidermis were quantified separately. Most of the dermal mononuclear cells in acute tinea were identified as T helper lymphocytes of the memory type. Furthermore, considerable amounts of Langerhans' cells and macrophages were found, but virtually no B cells. A high proportion of cells expressed markers of activation. Within the epidermis, accumulations of Langerhans' cells and LeuM5+ dendritic macrophages were detected near fungal elements. In view of the otherwise rather similar cellular infiltrates in acute tinea and different non-infectious dermatoses, acute tinea may be particularly suitable to study the functional relationship of Langerhans cells and LeuM5+ macrophages. Key words: Langerhans' cells; Macrophages; T cells; Monoclonal antibodies; Dermatophytoisis.

(Received January 27, 1992)


J. Brasch, Universitäts-Hautklinik, Schittenhelmstr. 7, D-2300 Kiel, Germany.

The understanding of the pathogenesins of skin diseases is facilitated by a precise knowledge of the different types of cells involved in cutaneous inflammation. A discrimination of cellular subsets can be achieved by immunostaining of appropriate cellular surface markers with monoclonal antibodies. This method has given insight into the pathogenesis of a broad spectrum of different dermatoses (1-3) within the last years. Only a few studies, however, were performed to investigate the cellular response in tinea (4-6). Since those studies had focused on a limited number of different cellular antigens with special attention to CD1a (4) or HLA-DR (6), we felt that a complementary analysis including T cells, B cells and macrophages as well as markers of activation might be useful for a more comprehensive pathophysiologic understanding of dermatophytoisis.

**MATERIAL AND METHODS**

Informed consent was obtained from 4 patients suffering from acute untreated inflammatory non-pustulose tinea. Their lesions were located on neck, scalp, thigh and arm respectively, and the pathogenic organisms were identified by culture on Sabouraud's glucose agar as Trichophyton verrucosum, Microsporum canis (2*), and Trichophyton mentagrophytes. Punch biopsies were taken from the progressive border of the lesions, shock-frozen in liquid nitrogen, cryostat-sectioned and stained with a panel of monoclonal antibodies (Table 1) by use of a three-step immunoperoxidase technique (7). The percentage of positively stained infiltrating cells in the upper dermis was calculated based on the evaluation of at least 200 cells in each case. The density of positively stained infiltrating cells in the epidermis was measured lightmicroscopically per 0.1 mm² by use of an ocular grid. Arithmetic means were calculated and are presented together with the

<table>
<thead>
<tr>
<th>moab</th>
<th>CD</th>
<th>Specificity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu6</td>
<td>1a</td>
<td>Thymocytes; Langerhans' cells</td>
<td>Becton Dickinson (BD)</td>
</tr>
<tr>
<td>Leu4</td>
<td>3</td>
<td>T cells</td>
<td>BD</td>
</tr>
<tr>
<td>Leu3a</td>
<td>4</td>
<td>Helper T cells; activated macrophages and Langerhans' cells</td>
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</tr>
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<td>Leu2a</td>
<td>8</td>
<td>Suppressor T cells</td>
<td>BD</td>
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<td>Leu9</td>
<td>7</td>
<td>T cells; killer cells</td>
<td>BD</td>
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<td>CD45R0</td>
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<td>Dakopatts</td>
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<td>11c</td>
<td>Macrophages</td>
<td>M. R. Parwaresch, Kiel</td>
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<tr>
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<td>Macrophages; activated CD8+ T cells</td>
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<td>KM8</td>
<td>-</td>
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<td>Leu17</td>
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<td>B cells; killer cells; monocytes</td>
<td>Dakopatts</td>
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<tr>
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<td>RR 1/1</td>
<td>54</td>
<td>Intercellular Adhesion Molecule 1</td>
<td>Orbi</td>
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<tr>
<td>OKT9</td>
<td>71</td>
<td>Transferrin-receptor</td>
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</table>
Table II. Staining of infiltrating cells in the dermis
For each antibody the mean percentage (n=4) of positively stained infiltrating cells in the dermis is shown, and additionally the minimal and maximal value.

<table>
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<th>Antibody</th>
<th>Arith. mean</th>
<th>Min.</th>
<th>Max.</th>
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<tr>
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<td>18</td>
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<tr>
<td>Leu4</td>
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<td>75</td>
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<td>68</td>
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<td>Leu2a</td>
<td>21</td>
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<td>Leu9</td>
<td>15</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>UCHL1</td>
<td>75</td>
<td>63</td>
<td>81</td>
</tr>
<tr>
<td>KiM1</td>
<td>24</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>LeuM5</td>
<td>43</td>
<td>33</td>
<td>60</td>
</tr>
<tr>
<td>KiM6</td>
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<td>35</td>
</tr>
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<td>KIM8</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
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<td>Leu17</td>
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<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Ki67</td>
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<td>5</td>
<td>13</td>
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<td>HLA-DR</td>
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</tr>
<tr>
<td>OKT9</td>
<td>31</td>
<td>15</td>
<td>55</td>
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</tbody>
</table>

lowest and highest value for each antibody in the tables. In addition, double staining with a modified PAS-method not interfering with immunostaining was performed with selected sections. Normal skin for control purposes had been processed by us previously with the same antibodies (3), showing no positively stained infiltrates of relevance.

RESULTS

A similar composition of the cellular infiltrates was found in all biopsies (Tables II, III).

In the dermis (Table II), a considerable proportion (17%) of the infiltrate consisted of Langerhans' cells (LC). Approximately 70% of infiltrating cells were Leu4+ T cells. 68% of dermal cells were stained with Leu3a (helper T cells or activated macrophages), and 21% expressed CD8 (Leu2a+, suppressor cells). Most of the infiltrating cells were of memory-phenotype (UCHL1+, 75%), whereas Leu9+ cells made up only 15%. Leu18+ naive T cells and Leu7+ cells were virtually absent; furthermore, there was a nearly complete lack of B cells in the dermis.

A considerable amount of macrophages was detected in the dermis, a major part (43%) of which was LeuM5+, and a minor portion of them was positive for KiM1 (24%), KiM6 (18%), KIM8 (20%) or Leu17 (9%). The majority of infiltrating cells were activated as revealed by expression of HLA-DR (66%), and a considerable degree of expression was found for transferrin receptor (31%), ICAM-1 (27%), interleukin 2 receptor (16%), HLA-DO (8%) and the proliferation-associated Ki67-antigen (7%).

A highly distinctive feature of the epidermal infiltrates (Table III) was the presence of LC (230.1 mm²), which tended to aggregate near PAS-positive fungal elements located in the stratum corneum or in hair follicles. In addition, some Leu4+ cells (30.1 mm²) and Leu3a+ cells (70.1 mm²) as well as UCHL1+ cells (80.1 mm²) were regularly found. In contrast to dermal infiltrates, Leu2a+ suppressor cells and Leu9+ cells were not detected in the dermis. Similar to dermal infiltrates, no B cells were identified intraepidermally. The presence of epidermal LeuM5+ dendritic macrophages was found in each case (60.1 mm²; Fig. 1). Additional PAS-staining revealed a similar tropism of LeuM5+ cells for fungal elements as was observed with LC. Staining of epidermal cells with KiM1, KiM6 and KIM8 was negative in all cases.

Table III. Staining of infiltrating cells in the epidermis
For each antibody the mean number (n=4) of positively stained infiltrating cells/0.1 mm² epidermis is shown, and additionally the minimal and maximal value.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Arith. mean</th>
<th>Min.</th>
<th>Max.</th>
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<tr>
<td>Leu6</td>
<td>23</td>
<td>17</td>
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</tr>
<tr>
<td>Leu4</td>
<td>3</td>
<td>0</td>
<td>7</td>
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<td>Leu3a</td>
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<td>UCHL1</td>
<td>8</td>
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<td>LeuM5</td>
<td>6</td>
<td>3</td>
<td>8</td>
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<tr>
<td>HLA-DR</td>
<td>16</td>
<td>12</td>
<td>22</td>
</tr>
</tbody>
</table>

Fig. 1: LeuM5+ macrophages in acute tinea, stained by use of an immunoperoxidase technique. The cells have well developed dendrites and appear to accumulate and invade the epidermis especially below fungal material.

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From the activation markers studied, only HLA-DR was detected on dendritic cells (160.1mm²), while a minimal expression of HLA-DO and interleukin 2 receptors was seen in only one case each. Expression of ICAM-1, transferrin-receptor and Ki67-antigen by keratinocytes was a common feature.

DISCUSSION

The pattern of inflammatory cells described here for acute tinea is to a large extent identical with corresponding observations in various benign inflammatory dermatoses of quite different origins (1, 2, 6, 8–10). Common characteristics are a dominance of memory T cells, a lack of B cells, a participation of macrophages (11), and a certain cellular activation. Compatible with this resemblance, similarities between dermatophytosis and contact dermatitis had previously been pointed out (12).

However, the rather high number of LC is noteworthy and in accordance with the previously reported accumulation of epidermal (4) and dermal (6) LC in tinea. We now could demonstrate that this aggregation of epidermal and dermal LC does occur in the same lesions. Being capable to induce a cellular response to trichophyton in vitro (13), one possible function of the epidermal LC may be to take up fungal antigens. Furthermore, since a cutaneous enrichment of LC can also be induced by skin inflammation unrelated to exogenous antigens (14), these LC may also have other functions.

A new observation of particular interest is the prominent accumulation of LeuM5+ dendritic cells, which were detected in considerable amounts in the dermis and, similar to LC, within the epidermis, too. A weak reactivity of some LC has been reported with LeuM5, but not with KiM1, KiM6 and KiM8 (15). The similar dendritic morphology and epidermal location of LC and LeuM5+ cells in acute tinea (Fig. 1) is therefore intriguing and raises the question of a possible functional relationship between the two types of cells. Although KiM1 antibodies as well as LeuM5 antibodies are directed against CD11c, no epidermal KiM1+ cells were seen in tinea. A possible explanation may be the binding of both antibodies to different epitopes of CD11c. A differential expression of various CD11c epitopes was seen in dermal macrophages (15). Acute tinea may be a suitable model to study more closely the role of epidermal dendritic macrophages in the initiation of a defense reaction against exogenous noxae.

REFERENCES

Quantitative Analysis of Langerhans’ Cells in Epidermis at Irritant Contact Reactions Using Confocal Laser Scanning Microscopy

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1Department of Clinical Immunology, Karolinska Hospital, Stockholm, 2Department of Physics IV, The Royal Institute of Technology, Stockholm, 3Sarastro AB, Stockholm, and 4Department of Dermatology, University Hospital, Uppsala, Sweden

Confocal laser scanning microscopy (CLSM) was used for quantitative analysis of CD1a+ cells in epidermis at irritant reactions. Sodium lauryl sulphate (2% and 4%) or non-anoic acid (20% and 80%) were applied to the skin of healthy volunteers under occlusion for 24 h. Skin biopsy specimens were taken after additional 24 h and were snap frozen. Freeze-sections, 25 µm thick, were stained with anti-CD1a antibodies (Leu-6) followed by FITC-labelled rabbit anti-mouse IgG. The sections were viewed and optically sectioned in the CLSM at 10 depth levels. The data was analysed using a threshold value for the fluorescence. The obtained result is presented as the proportion of specimen area having a fluorescence intensity above the threshold. The result demonstrates that the CLSM is a useful tool for obtaining not only structural information but also quantitative information from a defined tissue volume. In the present investigation it was possible to demonstrate variations in CD1a+ reactivity in epidermis at detergent-induced irritant reactions with a marked decrease in CD1a+ after 80% non-anoic acid exposure and only minor differences in the CD1a+ after 2% and 4% sodium lauryl sulphate exposure. Key words: Contact dermatitis; Immunology; Patch test.

(Accepted April 14, 1992.)


M. Lindberg, Department of Dermatology, University Hospital, S-751 85 Uppsala, Sweden.

Today it is well established that the skin is a heterogenous organ that provides a microclimate for immunological and inflammatory events (1, 2). Langerhans’ cells, keratinocytes and lymphocytes interact in a dose and time dependent manner during the induction and elicitation of contact allergic reactions. The keratinocytes also act as a key cell in non-specific inflammation in the skin (3, 4, 5). It has been shown that mild irritant stimuli such as occlusion with water and 0.5% sodium lauryl sulphate (SLS) induces morphological alterations in the epidermal Langerhans’ cell population (6, 7). It is thus possible that minor single, or repetitive, exposures of the skin to mild irritants might alter the micromilieu of the epidermis, affecting subsequent immunological events. It has also been shown in immunohistochemical and transmission electron microscopic studies (8, 9, 10) that different detergents induce different responses in the epidermis regarding Langerhans’ cell morphology and number and in the expression of the intercellular adhesion molecule-1 (ICAM-1) on keratinocytes. To obtain a better understanding of the temporal, spatial and quantitative alterations in the different epidermal cell populations and their expression of surface markers, there is a need for a technique that allows both a morphological and quantitative analysis of specific structures in a given tissue volume.

The ability of a confocal laser scanning microscope (CLSM) to detect fluorescent signal from a uniformly thin "optical section" eliminates ambiguities arising from variable thickness of specimen tissue (11). The technique can also be applied for the three-dimensional reconstruction of cellular structures and has recently been applied for the reconstruction of epidermal Langerhans’ cells stained for HLA-DR antigens (12). In the present study we have used CLSM to quantitatively determine alterations in the Langerhans’ cell population at contact reactions induced by two different detergents, SLS and non-anoic acid (NAA).

MATERIALS AND METHODS

Subjects

Irritant reactions were induced in 4 healthy volunteers without any signs of skin diseases. All had given their informed consent. The investigation was approved by the Ethics Committee at Karolinska Hospital.

Patch testing

On each of two persons (2 males, age 26 and 52 years) 2% and 4% SLS in distilled water and distilled water alone were applied. These persons are referred to as subjects 1 and 2. On each of two persons (2 males, age 23 and 37 years) 20% and 80% NAA in isopropanol and isopropo- nol alone were applied. These persons are referred to as subjects 3 and 4. The test substances (thirteen microliters) were applied under occlusion for 24 h in small Finn-Chambers®, diameter 8 mm (13). On the gluteal area to ascertain non-sun-exposed skin. In both groups unexposed skin served as control.

Skin biopsy specimens

Skin biopsies, diameter 3 mm, were taken at 48 h after dermal injection of local anaesthesia (Lidocaine®, Astra, Södertälje, Swe- den). The biopsies were immediately placed in transport medium (Histofon®, Histolab, Gothenburg Sweden) on ice and subsequently snap-frozen in chilled isopentane and stored at −70°C. The strength of the test reactions were graded visually at the time of biopsy.

Immunofluorescence staining

Vertical frozen sections were cut, 25 µm thick, in a cryostate at −20°C. The sections were acetone-fixed and incubated with mouse monoclonal anti-CD1a antibodies (Leu-6, working dilution 1/32, Beckton Dickinson, Sunnyvale, CA, USA) for 30 min at room temperature, followed by washes in phosphate-buffered saline (PBS). Fluorescein-isothiocyanate (FITC)-labelled rabbit-anti-mouse IgG (working dilution 1/20, Dakopatts, Copenhagen, Denmark) was used as a secondary antibody. The sections were mounted with glycerol in PBS containing p-phenylenediamine (PDD) to reduce the fading of immunofluorescence (14). The coverslip was sealed with nailpolish to avoid oxidation of PPD leading to redbrown discouraling of the specimens. The dilutions of the antibodies were determined using sections from normal skin. Staining was not observed in controls without the primary antibodies.

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Table I. The mean fluorescence intensities (arbitrary scale; min = 0; max = 255) for the two subjects exposed to SLS for 24 h. Biopsies were taken at 48 h. Data obtained from 4 levels in the sections. Mean and standard deviation (SD) are given.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Water</th>
<th>2% SLS</th>
<th>4% SLS</th>
</tr>
</thead>
<tbody>
<tr>
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<td>23.6</td>
<td>24.8</td>
<td>21.2</td>
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<td></td>
<td>(5.7)</td>
<td>(3.2)</td>
<td>(3.1)</td>
<td>(3.0)</td>
</tr>
<tr>
<td>Person 2</td>
<td>39.9</td>
<td>23.7</td>
<td>27.5</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>(4.4)</td>
<td>(0.7)</td>
<td>(4.8)</td>
<td>(2.2)</td>
</tr>
</tbody>
</table>

The total number of measured optically sectioned areas in each case is 16.

Microscopy

In the CLSM the recorded image represents a thin optical section of the specimen with almost all out-of-focus information eliminated. It is thus possible to get a three-dimensional (3D) recording of the specimen by scanning the object several times at different levels. A set of such images scanned at different depth in a specimen is referred to as a "stack". In this investigation each image consisted of 512 x 512 8-bit pixels.

The CLSM setup

In the present study two different microscopes were used, a PHOIBOS 1000 (a predecessor of the Sarastro 2000 instrument from Molecular Dynamics, Sunnyvale, California, USA) with photomultiplier (PM)-voltage 860 V (subject 1), barrier filter 515 nm longpass, and beam splitter 510 nm longpass, and the CLSM at the Royal Institute of Technology with PM-voltage 660 V (subject 2) and 720 V (subjects 3 and 4), barrier filter 515 nm (subject 2) and 560 nm (subjects 3 and 4) longpass, and beam splitter 510 nm longpass. To excite the FITC, the 488 nm line from an argon ion laser was used.

The two microscope setups have been described in detail elsewhere (15, 16). The CLSMs were built around light microscopes (Nikon Optiphot 1 or Zeiss Universal microscopes) with plano-apochromate 40X (N.A. 1.30) oil objectives.

Analysis of the biopsy specimens

Two freeze-sections were analyzed from each biopsy specimen. All material from one subject was stained and scanned at the same time under coded conditions. With both microscopes the stained histological sections were scanned to obtain 4 images (optical sections) of 512 x 512 pixels of each specimen, with three µm in between each section and with at least 2 such "stacks" per stained section yielding a minimum of 16 optical sections per biopsy specimen. For each analysis session the operating sensitivity of the CLSM was configured firstly by minimising laser output to a level where maximal fluorescence output from the specimen was still obtainable, whereafter the photomultiplier voltage was set to give maximum image brightness without saturating the 256-level display scale. Three different measuring methods were used:

Method 1: Practiced on person 1 and 2. Two areas chosen in the central parts on each of the 2 sections were scanned with the CLSM as described above. The average fluorescence intensity was measured in each digitized picture (optical section) on subject 1 with a built-in function in the PHOIBOS4D program or with a software package, ISIS (Information System for Image Studies) on subject 2. For both persons the FITC-labelled CD1a+ cells in the epidermal area (excluding the stratum corneum) was measured on each optical section. Instead of a grayscale we used pseudo colours to be able to clearly see the basal membrane and the stratum corneum. A value for each optical section was thus obtained.

Method 2: Practiced on persons 1 and 2. The same data as above was used but instead of the average fluorescence intensity the proportion of specimen area having a fluorescence intensity above some selected threshold value was measured. An algorithm was used to obtain a threshold value (17) for each subject. In this algorithm a bend point in the image histogram is used as the threshold value for the staining. The ISIS program was used to make a binary picture out of each of the scanned optical sections. All pixels above the threshold value received the value 1 and all pixels below received the value 0. The binary picture was then used to measure the relative area of the FITC-labelled CD1a+ cells in the epidermal area, excluding stratum corneum.

Method 3: Practiced on persons 3 and 4. As many epidermal fields as possible on the two histological sections, excluding the two fields at the both ends of the sections, were scanned with the CLSM as described above. From each of the optical sections a binary picture was obtained and measured by the ISIS program as in method 2. This was done for two thresholds on each subject, one which was held constant for both and one that was chosen specifically for each of the subjects.

Table II: The relative area of CD1a+ for the two persons exposed to SLS for 24 h. Biopsies were taken at 48 h. Data obtained from four levels in the sections. Mean and SD are given. Threshold value = the value selected on an arbitrary scale for the fluorescence intensities to consider a pixel positive (min = 0; max = 255)

<table>
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<tr>
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<td>Person 2</td>
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<td>(0.01)</td>
<td>(0.03)</td>
<td>(0.01)</td>
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</tr>
</tbody>
</table>

The total number of measured optically sectioned areas in each case is 16.

Table III: The relative area of CD1a+ in epidermis of the two persons exposed to non-anoic acid (NAA) for 24 h. Biopsies were taken at 48 h. Data obtained from four levels in the sections. Mean and standard deviation SD are given. Threshold value = the value selected on an arbitrary scale for the fluorescence intensities to consider a pixel positive (min = 0; max = 255)

<table>
<thead>
<tr>
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<th>80% NAA</th>
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<td>0.35</td>
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<td></td>
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<td>(0.11)</td>
<td>(0.13)</td>
<td>(0.05)</td>
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<tr>
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<td>0.04</td>
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<td>(0.02)</td>
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<tr>
<td>Person 5</td>
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<tr>
<td>Person 6</td>
<td>45</td>
<td>0.14</td>
<td>0.19</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td>(0.11)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td></td>
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<tr>
<td></td>
<td>36</td>
<td>0.36</td>
<td>0.44</td>
<td>0.36</td>
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</tr>
</tbody>
</table>

n = total number of measured optically sectioned areas.
RESULTS

Epikutaneous testing with SLS resulted in a weak erythema after 2% SLS and erythema with oedema after 4% SLS in both persons. The NAA exposure resulted in similar reactions in the skin but the oedema was weaker after 80% NAA exposure compared to 4% SLS. Control skin and vehicle exposed skin were normal looking.

The result of the CLSM analysis is given in Tables I–III. In the four subjects it was possible to describe variations in the amount of CD1a reactivity in epidermis after exposure to the two detergents SLS and NAA. The exposure to 2% and 4% SLS caused minor changes in the CD1a reactivity (Tables I and II). In persons 3 and 4 (Table III) we found a decreased CD1a+ staining after exposure to 20% in one person and after exposure to 80% NAA in both volunteers. In person 3 there was a marked difference in the amount of CD1a+ (the relative area) after exposure to 20% NAA between the two applied threshold values (Table III). The influence of the threshold value on the relative area determination was demonstrated by choosing different values (Tables II and III).

DISCUSSION

In the present paper it is shown that different irritant stimuli do induce variations in the amount of CD1a reactivity in epidermis and that the CLSM can be a useful tool for the quantification of the labelled structures. It is also demonstrated that the result of the measurements (the fluorescence intensity) is dependent on several factors in the experimental system such as the setting of the background threshold. The choice of the threshold value is a crucial point for quantitative analysis as this discriminates between true staining and background. This is clearly seen when comparing two different thresholds for the same analysis (Tables II and III). The variations in the experimental (instrumental and immunohistochemical) settings were eliminated by using controls from the same individual and by staining and performing the analysis of all samples from one individual at the same time and with the laser beam and intensity well configured.

The study started out with the analysis of the mean fluorescence intensity in the optical sections (method 1, persons 1 and 2). By applying thresholding, we obtained a better detection of labelled structures and this technique was therefore applied for all specimens.

Another important question is which part(s) of the histological sections that should be used. Scanning several images below each other introduces possible errors such as absorption and scattering in the specimen, fading of the staining and an uneven penetration of the antibody. The obtained results in the present study are presented as the proportion of specimen area having a fluorescence intensity above the threshold and represents the mean of measurements from the 4 images scanned at different levels below the surface of the section. It was found by using different sampling techniques (see Material and methods) that the fields at the ends of the histological sections, revealed different fluorescent values than the more central part of the sections and these areas were thus excluded. This is in accordance with the findings of Mossberg et al. (17). The importance of adding PPD to the sections to reduce the fading of fluorescence under the laser beam has previously been shown (14, 17).

The enumeration of positively stained cells in the light microscope, e.g. Langerhans' cells, has often been used to describe changes in epidermis under pathological conditions such as contact dermatitis. The methods of counting cells in epidermis are connected with possible misinterpretations due to the technical limitations of conventional light microscopes and the thickness of the specimen sections (18, 19).

It is interesting that the quantification of the CD1a positivity can be performed within a specific tissue volume of epidermis using the CLSM and that this technique demonstrate the dose dependent variations in CD1a+ after application of SLS and NAA.

The CLSM provides a technique where the sampling of data can be performed as an automatic process. The data obtained permits both a quantitative and a structural analysis in the same tissue volume. This is an improvement compared with the conventional use of light microscopic techniques. This opens up new possibilities for the investigation of the time, volume, spatial and quantitative variations in the immune responses of the epidermis. It is notable that in some biopsies there seems to be a discrepancy between the number of CD1a+ cells and the relative amount of CD1a+ (10). This implies that alterations in the Langerhans' cell population defined as number of cells, might not reflect the true alteration in the amount of available surface structures.

In conclusion, it is possible to perform a quantification of the amount of CD1a positivity within a specific epidermal volume by using the CLSM. By combining the quantitative analysis with the possibility of performing three-dimensional reconstructions of cellular structures (12, 20) the CLSM provides a new and powerful technique for studies of immune reactions in the skin.

ACKNOWLEDGEMENTS

Excellent technical assistance was given by Ms. Catharina Johanson. This project was supported by grants from the Swedish Work Environment Fund, the Swedish Medical Research Council, the King Gustav V Foundation and the Edvard Welder Foundation.

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Comparison of Muscle-derived Serum Carbonic Anhydrase III and Myoglobin in Dermatological Patients: Effects of Isotretinoin Treatment

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The serum levels of muscle-specific serum carbonic anhydrase III (S-CAIII) and myoglobin (S-Myo) were analyzed in various male dermatological patients of the same age. The mean levels of S-CAIII and S-Myo were essentially similar in patients with acne, psoriasis vulgaris, atopic eczema and tinea, suggesting that common dermatological diseases do not affect the serum levels of the muscle markers. Increased levels of S-CAIII, which is specific for skeletal muscle cells, were found in the acne patients who had been treated with isotretinoin. However, when S-CAIII and S-Myo were studied in 24 patients (16 males, 8 females) before and during isotretinoin treatment, no constant increases in these markers could be observed. When individual patients were followed for several months, transient increases or decreases could be observed. The changes in S-CAIII, or S-Myo, did not correlate with the dose of isotretinoin, nor with the duration of the treatment. The results suggest that systemic isotretinoin does not specifically affect skeletal or myocardial muscles. The increases in these markers observed in the course of dermatological diseases and isotretinoin treatment are obviously due to other factors, such as exercise.

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Retinoids are extensively used for the treatment of various skin disorders. In particular, isotretinoin has been widely used for the treatment of severe acne. Treatment with isotretinoin produces serious and numerous side effects which have been well documented in clinical trials. One of the side effects is muscle pain, with mild to moderate increases of creatine phosphokinase activity (1–5).

Recently, the development of new assays for following changes in muscle metabolism has proven to be particularly useful. These assays utilize the accurate, simultaneous measurement of serum carbonic anhydrase III (S-CAIII) and myoglobin (S-Myo) levels (6). Isoenzyme III of CA has been shown to exist in appreciable amounts only in type I (slow-switch, or red) skeletal muscle fibers (7, 8). Myoglobin is found in skeletal and cardiac muscles, but not in smooth muscle. The simultaneous measurement of S-Myo and S-CAIII can be used to evaluate the origin of muscle-derived proteins in serum and, thus, to differentiate between myocardial and skeletal muscle damage (6). Especially CAIII has been shown to be a more sensitive marker of muscle damage than creatine kinase in various neuromuscular diseases such as polymyositis (9). S-Myo and S-CAIII can also be used to study the intensity of physical exercise and both are decreased relatively rapidly (within 24 h) after exercise (10). In the present study, S-CAIII and S-Myo were measured in acne patients. The levels of S-CAIII and S-Myo were compared to those found in other common skin disorders and to those of patients receiving isotretinoin treatment.

PATIENTS AND METHODS

Morning serum samples were collected from young male dermatological patients (109 subjects) with various common skin diseases. In the second part of the study, serum samples were taken before and during isotretinoin (Roaccutane®, Roche) treatment, from 16 male patients and 8 female patients. Clinical details of these patients are shown in Table I. The serum samples were kept at −20°C until assayed.

S-CAIII and S-Myo were assayed using a dual-labelled time-resolved fluorimunoassay (11). The control level of S-CAIII for males and females is 21 ± 8 µg/L, while those of S-Myo are 31 ± 11 µg/ml and 25 ± 10 µg/L for males and females respectively. Statistical analyses: Student’s t-test and linear regression were used.

RESULTS

S-CAIII and S-Myo levels were measured in sera from 109 patients with acne, atopic eczema, psoriasis vulgaris, tinea, or some other common skin disorder (verruca vulgaris, condyloma, lichen ruber planus, various eczema, urticaria). All these patients were males and of about same age, in order to eliminate variations due to age and sex. The individual levels of S-CAIII and S-Myo were variable, as shown in Figs. 1A and B. With respect to the various diagnostic groups, S-CAIII levels were superior to the mean ± 2 SD of the control values in one patient having atopic eczema (50 µg/L), in one psoriasis vulgaris patient (38 µg/L), in two tinea patients (98 and 50 µg/L).

Table I. Clinical details of acne follow-up patients treated with isotretinoin

<table>
<thead>
<tr>
<th></th>
<th>Age (range)</th>
<th>Weight (range)</th>
<th>Isotretinoin (mg/kg)</th>
<th>Total dose (mg)</th>
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<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>Males</td>
<td>16</td>
<td>19.5</td>
<td>(16-29)</td>
<td>71.5</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>21.5</td>
<td>(15-31)</td>
<td>59.9</td>
</tr>
</tbody>
</table>

*Total dose corresponds to the cumulative isotretinoin dose (mg) that the patients had received until the serum carbonic anhydrase III and myoglobin were determined during treatment (see Fig. 3).
Effects of isotretinoin treatment

Fig. 1. Levels of serum carbonic anhydrase III (S-CAIII) (A) and myoglobin (S-Myo) (B) in various dermatological patients. The two solid horizontal lines indicate the mean ± 2 SD of S-CAIII and S-Myo in controls. The mean levels of S-CAIII and S-Myo in various diagnostic groups are shown by short lines. A = acne; A + I = acne plus isotretinoin; AE = atopic eczema; PV = psoriasis vulgaris; T = tinea. Open dots in acne patients indicate those who were treated with oral tetracycline. Open dots in tinea patients indicate those who were treated with griseofulvin. One tinea patient was treated with ketoconazole (△).

μg/L), who were treated with ketoconazole or griseofulvin respectively, and in one patient having eczema cruris (143 μg/L). S-Myo was superior to the mean + 2 SD in one psoriasis vulgaris patient (70 μg/L), in one acne patient (62 μg/L) and in one patient receiving ketoconazole treatment (203 μg/L). In acne patients who had been treated with isotretinoin, S-CAIII levels were superior to the mean + 2 SD in 6 out of 29 patients (20.7%), while S-Myo was high in 4 out of 29 patients (13.8%).

The ratio of S-CAIII/S-Myo was lowest in the acne group, but there were no statistically significant differences between the various patient groups. When the acne patients who had received isotretinoin, and who exhibited elevated levels of S-CAIII, were analyzed more carefully, no correlation with other parameters was found. In particular, the dose of isotretinoin (mg/kg/d) was almost identical in this group (0.51 ± 0.11 mg/kg/d), when compared to those presenting normal levels of S-CAIII (0.46 ± 0.14 mg/kg/d).

Follow-up of acne patients receiving isotretinoin

Since it seemed that systemic isotretinoin might increase the levels of S-CAIII, and S-Myo in some patients, S-CAIII and S-Myo levels were measured in 24 patients before and during treatment (see also Table 1) (Fig. 2). In some patients, assays were carried out several times during isotretinoin treatment (Fig. 2). As shown in Fig. 2, isotretinoin did not have any constant effect on S-CAIII in males or females. S-Myo did not change markedly during isotretinoin treatment (not shown). When individual patients were followed for 3−4 months, changes in S-CAIII were, in general, relatively small (Fig. 3). In only one patient was S-CAIII elevated over the mean + 2 SD of the controls (patient 4). S-Myo changes were more pronounced (Fig. 3), but, here again, no constant increase or

![Graph](image)

Fig. 2. S-CAIII in acne patients treated with isotretinoin. Samples were taken before and during the treatment in males and females (first determination). Mean levels are shown by the short horizontal lines.

![Graph](image)

Fig. 3. S-CAIII and S-Myo in 6 representative patients who were followed for variable periods. Patient 1: a 16-year-old male, 0.63 mg isotretinoin/kg/d; Patient 2: a 15-year-old female, 0.78 mg isotretinoin/kg/d; Patient 3: a 19-year-old female, 0.63 mg isotretinoin/kg/d; Patient 4: a 20-year-old male, 0.52 mg isotretinoin/kg/d; Patient 5: a 17-year-old male, 0.47 mg isotretinoin/kg/d; and Patient 6: a 20-year-old male, 0.61 mg isotretinoin/kg/d. Symbols: ● = S-CAIII; ○ = S-Myo.
decrease could be observed during isotretinoin treatment (Fig. 3). In some patients, S-Myo was constantly high during treatment (see patients 2, 5 and 6), while in others, transient increases could be observed (patients 1 and 3).

**Correlation between S-Myo and S-CAIII**

There was a significant correlation between S-Myo and S-CAIII in acne patients both with (Fig. 4) \( r = 0.703, p < 0.001 \), and without isotretinoin treatment \( r = 0.715, p < 0.001 \). Thus, the isotretinoin treatment did not change the relative ratio of S-Myo and S-CAIII. S-Myo and S-CAIII levels did not correlate with the daily dose of isotretinoin used (mg/kg/day), nor with the total amounts of isotretinoin given to the patients.

**DISCUSSION**

In the first part of the study, increased levels of S-CAIII, a highly specific marker for skeletal muscle cells, were found in randomly collected serum samples from various dermatological patients, especially in those who had received systemic isotretinoin. Over 20% of these patients presented abnormally high S-CAIII values (superior to the mean + 2 SD of control levels). This suggested that systemic isotretinoin might increase serum levels of S-CAIII. High levels of S-CAIII were also found in some other dermatological patients not receiving any isotretinoin treatment. These included, for example, cases of psoriasis vulgaris, atopic eczema, eczema cruris, as well as two patients who had been treated with systemic griseofulvin, or ketoconazole. In the second part of the study, S-CAIII and S-Myo were followed in 24 patients receiving isotretinoin treatment. Surprisingly, levels of S-CAIII in males were even lower during isotretinoin treatment than those found in the same patients before treatment (statistically not significant).

Previously, transient increases in creatine kinase (CK) had been observed during isotretinoin treatment (1–5). In some patients, isoenzyme fractionation has suggested that elevated CK levels were derived from skeletal muscles (4). However, accumulated data indicate that a direct effect of isotretinoin on muscles is not evident in most patients presenting elevated levels of CK, whereas the status and intensity of exercise are more important causal factors (12). Our results are in agreement with these previous studies, even though we have used different muscle markers. Since S-CAIII is the most specific marker of skeletal muscle cells, the failure to find constant increases in this marker during isotretinoin treatment indicates that isotretinoin does not generally affect skeletal muscles. This suggests that routine measurements of carbonic anhydrase III and myoglobin are not necessary during isotretinoin treatment.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

Sequential Concentration of Chloroquine in Human Hair Correlates with Ingested Dose and Duration of Therapy*

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Human scalp hair was analyzed for chloroquine using gaschromatography. In 5 patients it was demonstrated that the amount of uptake of chloroquine into the hair varied proportionally with the dosage (from 500 mg/week to 10 g single dose) and with the time of administration. The chloroquine concentrations ranged from 8 to 1100 μg/g hair. Chloroquine could be determined quantitatively after a single toxic dosage used in a suicidal attempt and also after low therapeutic doses. The sequential examination of the hair shaft allows an assessment of the chloroquine amount taken over time, the individual dosage, the initiation and termination of therapy. As hairs can be collected easily, they are a unique specimen for investigation, and it is suggested that they can virtually be used as a “tachogram” of chloroquine drug-therapy or intoxication. Key word: Gaschromatography.

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Human hair is a unique specimen for the detection of metals, addictive narcotics and drugs. Since incorporated substances can be detected retrospectively, even after months or years, hair is a valuable tool for forensic medicine. This holds true for metals such as arsenic, thallium and lead, which can already be detected in the picogram range, and, in recent years, also for organic substances such as therapeutic and narcotic drugs (1).

Chloroquine can be detected in hair, not only in therapeutic doses, but also after overdosage in white hair (2, 3). Previous studies have examined total hair in full length. The objective of this study was to determine whether the sequential analysis of hair allows conclusions as to the doses taken and the time of treatment. For this purpose we examined 5 patients with different models of chloroquine-intake.

MATERIAL AND METHODS

Patients and dosage

Five patients with five different models of chloroquine intake were studied (2 males, 3 females; single chloroquine dose between 70 mg and 10 g). None of them had evidence of pathological hair loss.

Patient 1: Continuous dosage; 23 years, female, chronic discoid lupus erythematosus (CDLE), chloroquine therapy for 90 days, daily dosage 250 mg/day.

Patient 2: Intermittent weekly low dose for malaria prophylaxis; 29 years, male, healthy, malaria prophylaxis, chloroquine therapy for 60 days, 500 mg once weekly corresponding to 70 mg/day.

Patient 3: Long term therapy with varying dosage; 69 years, female, CDLE, chloroquine for 270 days; the first 120 days 125 mg/day were prescribed, the next 120 days 250 mg/day. After confronting the patient with the results of the chloroquine concentration in her hair, the patient admitted to having actually taken only 60 mg/day for 120 days, 100 mg/day for the next 30 days and 125 mg/day for the last 120 days.

Patient 4: Intermittent therapy; 23 years, male, CDLE, chloroquine for 244 days; initially 500 mg/day for 51 days, no therapy for 176 days, 17 days 500 mg/day.

Patient 5: Suicidal attempt; 24 years, female, healthy, single dose of 10 g chloroquine 1 year before taking the hair.

Method

The examinations were made on patients 1–4 still under therapy, in patient 5, 1 year after a single overdose. About 50 occipital hairs were plucked (patients 2–5); on patient 1 they were cut just over the scalp’s skin. After partitioning them into segments of 1 cm length, 45 single samples were analyzed.

The specimens were weighed, then dissolved with 1.0 ml of 15% potassium hydroxide in a waterbath for a maximum of 2 min. The samples were immediately cooled, diluted with 4 ml aqua dest., and extracted, twice each, with diethylether and subsequently a mixture of dichloromethane and ether (70:30v/v). The extracts were analyzed by gaschromatography with a nitrogen specific detector after separation on a packed glass column, filled with 5% SE 30 on chromosorb W-AW DMCS, 80–100 mesh (gaschromatograph: Hewlett-Packard, type 5880); gas: helium 20 ml/min; temperature-programme: 160°C 1 min isotherm, increase-rate 10°C/min, end-temperature 280°C). The quantitative analyses were repeated at least twice. The lower detection limit was 1–2 ng absolutely. As we examined specimens from clinical patients, the results could not be corrected in respect to yield.

To confirm the results of gaschromatography, single extracts were analyzed by gaschromatography/mass spectrometry with a GC-MS

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**Fig. 1. Results of hair analyses in patients 1–4, for dosages see "Materi- al and Methods".**

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high growing fraction and the continuous hair-production, circulating substances are incorporated into the hair-shaft. These remain in the shaft — in contrast to blood and urine — over months and years. In this respect, hair is not only in its structure a kind of tachogram (5), more importantly it is a pharmacological and toxicological tachogram.

Since samples of hair can be taken at any time, stored without problems and examined at any time, they are of experimental, toxicological and forensic importance. Especially by sequential examination of single hair sections, it is possible to assess the intake of the substance retrospectively.

Chloroquine is incorporated into and preserved in hair. The aim of our study was not to compare absolute chloroquine concentrations in serum and hair but to investigate the relations of different concentrations of chloroquine in hair to different models of intake. The following characteristics were found:

1. Even low chloroquine dosages, e.g. typical malaria-prophylactic doses (500 mg once a week), can be detected in hair (Fig. 1).
2. Higher chloroquine dosages lead to higher concentrations in hair (Fig. 1).
3. A characteristic time course was found: at the beginning of therapy there was a continuous rise; after the end of therapy, however, a protracted decline occurred. This characteristic of the chloroquine concentration in hair correlates with the pharmacokinetics of the substance and its tendency to be stored in deep compartments (Fig. 3).
4. The chloroquine concentration incorporated in hair correlates with the temporal course of the chloroquine dosage ingested (Fig. 1).
5. Even after 1 year a single chloroquine dosage can be detected and placed in time (Fig. 3).

The chloroquine concentration in hair apparently depends on the serum concentration over the time. The concentrations found correspond to the pharmacokinetics of chloroquine. Initially, deep compartments are slowly filled. Due to a long half-life of 6–33 days, a steady state is not reached before 3
weeks, i.e. not before 3 to 4 half-lives. After withdrawal of the drug, the serum concentration declines very slowly, i.e. over months (Fig. 1), due to the accumulation in deep compartments (distribution volume 116–880 l/kg bodyweight) (6–10). After reinstallation of the drug the concentration in serum and hair rises much faster, as the deep compartments are still filled to a large extent (Fig. 1).

After a single application of a toxic dose chloroquine is incorporated into hair and can reliably be detected after 1 year (Fig. 3). The concentration in hair, however, is rather low. This is the result of a fast and successful therapeutic intervention, leading to extremely low serum concentrations. The position of the chloroquine maximum, found 1 year after intake of the drug, corresponds to the growth rate of human hair of about 0.36 mm/d. The incorporation phase, which lasts 4 months, shows that chloroquine is accumulated in deep compartments even after a single intake, from where it is released over several months.

The sequential examination of scalp hair allows retrospective conclusions concerning the initiation, duration and dose of chloroquine intake. As the chloroquine concentration measured over time depends on the pharmacokinetic profile of the substance, hair can serve as a semi-quantitative pharmacokinetic indicator for chloroquine. Chloroquine serum concentrations can be monitored retrospectively in hair by highly specific and sensitive analytical methods. Thus hair analysis gives retrospective evidence for unreliable intake, overdose in attempted suicide or poisoning.

REFERENCES
Lichenoid Eruption Induced by Low Dose Captopril

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A patient with congestive cardiac failure developed a rash following captopril treatment. The clinical and histological features were consistent with a lichenoid eruption. The rash spontaneously resolved without any treatment three months after captopril was discontinued. Key words: A. C. E. inhibitors; Sulphydryl group; Papules.

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Rashes occurring during the first few weeks of treatment have been the commonest of the side effects reported with captopril. Lichenoid eruptions produced by captopril are uncommon. We report the first case of a lichenoid eruption induced by low dose captopril.

CASE REPORT

An 83-year-old woman with long-standing aortic stenosis presented in March 1991 with left ventricular failure and atrial fibrillation. She was treated initially with diuretics and digoxin. Captopril 6.25 mg three times daily was added to her treatment one week later to further control her heart failure. She improved significantly and was discharged on captopril 12.5 mg twice daily. She presented in June 1991 with an itchy rash affecting her arms, upper chest, back and the sides of the neck. Captopril was thought to be the cause of the rash, and she was admitted to hospital for monitoring of her heart failure whilst the drug was withdrawn.

Examination at this time revealed multiple violaceous papules and plaques with some scaling on her arms, upper chest, back and neck. There were no blisters or mucosal lesions. A skin biopsy from a papule on the upper chest showed superficial perivascular and interal chronic inflammation with focal disruption of the basal layer with epidermal atrophy. There was patchy hypergranulosis, and necrotic keratinocytes were seen within the epidermis and in the basal layer (Fig. 1). Direct immunofluorescence showed IgG and C3 intrapidermal cytoid bodies with a fibrin band along the dermo-epidermal junction. The rash began to fade within 48 h of stopping the drug. She developed heart failure one week after drug withdrawal and enalapril 5 mg daily was substituted for captopril. There has been no exacerbation or recurrence of the rash, and the rash faded completely 3 months after drug withdrawal.

DISCUSSION

Up to 62% and 15% of patients treated with enalapril and captopril, respectively, were reported to develop a rash during the first few weeks of treatment. (1) Maculopapular, urticarial (2), pityriasis rosea-like (3), erythrodema (4), psoriasisform (5), lupus erythematosus-like (6) and pemphigus (7) have all been reported with captopril.

Two types of lichenoid eruptions have been described with captopril with different clinical and histological features (8). The first type of lichenoid eruption was preceded by a pityriasis rosea-like rash and was reported in four patients taking low doses of captopril (12.5-100 mg daily) (8, 9), and the rash evolved to leave hyperpigmented macules in two patients and small lichenoid papules in the other two patients before clearing after 1-2 weeks of drug withdrawal. The second type of lichenoid eruption occurred without any preceding rash and consisted of large lichenoid flat-topped papules which coalesced to form plaques. This type of rash was found in three patients taking large doses of captopril (100-450 mg daily), and the rash took 3-6 months to fade completely. The oral cavity was spared in all seven patients described. Our patient had similar clinical and histological features to the second type of lichenoid eruptions but differed in that she was taking a much lower dose of captopril comparatively (25 mg daily). The pathogenesis of captopril-induced lichenoid eruption is not well understood, though the presence of the active sulphydryl groups of captopril have been suggested to play an important role in the cell-mediated immunological reactions which lead to the development of the rash (10, 11). Our patient required captopril and benefited from using enalapril though cross sensitivity between these two drugs have been reported (12).

REFERENCES

Pityriasis Alba in a Psoriatic Location

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Three patients with pityriasis alba whose lesions were confined to the knees only are reported. Such cases can be misdiagnosed as psoriasis. The key to the correct diagnosis lies in the physician's awareness of the existence of this variant of pityriasis alba. Key words: Knees; Psoriasis.

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Pityriasis alba is a relatively common skin disorder, usually seen in pre-adolescent children. In this age group the incidence may reach 30–40% (1). The individual lesion is a round or oval patch, 0.5 to 5 cm in diameter, with fine, loosely adherent scales. The margins are moderately sharp and may be slightly elevated. There may be an initial stage in which the lesions have an erythematous appearance, but patients usually seek doctor's advice when the lesions become hypopigmented. Lesions are limited to the face in 50–60% of the cases. In a further 20%, the neck, arms and shoulders are also involved. Adamson (2) described an uncommon clinical variant in which the lesions are distributed symmetrically over the buttocks, upper thighs, lower back and extensor aspects of the arms. Widespread involvement occurs more frequently in older children and young adults. This condition has been referred to as "extensive pityriasis alba" (3). This uncommon variant of pityriasis alba has been further categorized into the idiopathic form and the atopic form (4).

We here described 3 patients with pityriasis alba whose lesions were limited only to the knees. This variant of pityriasis alba is probably the same as R. L. Sutton Jr. described some years ago (5), called "summertime pityriasis of the elbows and knees".

CASE REPORTS

Case 1

A 10-year-old boy presented with a 6-month-history of hypopigmented scaly plaques on his knees. He had been diagnosed as suffering from psoriasis and had been treated unsuccessfully with steroid ointments. On physical examination two symmetrical oval plaques were seen on his knees, each 4x5 cm in diameter. They were hypopigmented and appeared lighter than the surrounding skin. Fine adherent silvery scales covered the plaques. Since the patient was asymptomatic, he was told to use only emollient creams after bathing. No change has been noted over 6 months' follow-up.

Case 2

A 9-year-old boy presented with a history of white scaly plaques of 2 months' duration, on his knees. As in the previous case two sharply demarcated, hypopigmented, scaly plaques were seen on both knees. The plaques resolved spontaneously 4 months after their appearance.

Case 3

A 19-year-old male presented with a large, scaly plaque on his right knee and a smaller and flatter plaque on his left knee. He had been treated for several months, with steroid ointments, by a dermatologist who had diagnosed psoriasis. A large, scaly plaque, lighter than the surrounding skin, was seen on his right knee. It was sharply demarcated, and the edges were erythematous and slightly elevated. A smaller lesion seen on his left knee was actually a white macule with smooth surface. The patient was told to stop any treatment. No change has been noted over 1 month follow-up. A punch biopsy of the lesion showed mild hyperkeratosis, parakeratosis of the epidermis and slight edema of the upper dermis, with a mild chronic inflammatory infiltrate.

DISCUSSION

Psoriasis must be considered as a differential diagnosis when pityriasis alba is located on the knees. The plaques of pityriasis alba are, however, flatter and smoother, and they are of a softer consistency than the psoriatic plaques. In addition, in the psoriatic plaques the Auspitz sign can be elicited, while in pityriasis alba plaques the sign does not appear. The fact that we saw two cases (1 and 2) in the same week, and the third case one half year later, supports our belief that we are not dealing with a rare disease.

We suggest the name "Psoriasis-like pityriasis alba" for this variant of the disease or according to Sutton "pityriasis alba of the elbows and knees".

REFERENCES

Methotrexate Hepatotoxicity in Psoriatic Patients Submitted to Long-term Therapy

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Departments of 1Dermatology and 2Pathology, Hospital de Santa Maria, Lisbon, Portugal

Eighty-four patients with severe psoriasis who required methotrexate (MTX) therapy have been reviewed. A total of 134 liver biopsies were performed. The lack of correlation between alcohol intake and the use of potential hepatotoxic drugs with pretreatment liver biopsies is noted. A review of 30 patients who had liver biopsies performed before and after MTX treatment showed no statistically significant difference between the histological grades before and after treatment. Nor was there any absolute correlation between the cumulative MTX doses and the histological changes of follow-up biopsies. In this group of patients, 15 (50%) developed fibrosis, which was severe in 2 patients, after 3,431 mg MTX average dose. Cirrhosis was observed in 3 patients (10%) after 1,667 mg MTX average dose. Follow-up liver biopsies are recommended for patients treated with MTX. Key words: Cirrhosis; Fibrosis; Liver biopsy.

( Accepted February 3, 1992.)


Table I. Group I.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hepatotoxic or liver disease</th>
<th>Alcohol</th>
<th>Liver biopsy pre MTX (grades)</th>
<th>MTX dose (mg) at follow-up biopsy</th>
<th>Follow-up liver biopsy (grades)</th>
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</tr>
<tr>
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<td>4000</td>
<td>I</td>
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<tr>
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<td></td>
</tr>
<tr>
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</tbody>
</table>

Hepatotoxicity from long-term methotrexate (MTX) therapy is well documented (1–6). Fibrosis and, less frequently, cirrhosis have been attributed to it. Recent studies, however, have shown that MTX-induced cirrhosis is nonaggressive. There are reports of patients whose hepatic disease, evaluated by liver biopsies, did not deteriorate with maintenance of the treatment (7). Others (8) think that fibrotic alterations observed during MTX therapy are potentially reversible after discontinuance of the drug.

A protocol for MTX usage in psoriasis was elaborated in 1972 (9) and reviewed in 1973 (10), 1982 (11) and 1988 (12). It preconizes liver biopsy before initiating the treatment, or in
Table II. Group 2.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hepatotoxic or liver disease</th>
<th>Alcohol</th>
<th>MTX dose (mg) at follow-up biopsy</th>
<th>Follow-up liver biopsy (grades)</th>
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</thead>
<tbody>
<tr>
<td>31/M/56</td>
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<td>3600</td>
<td>III</td>
</tr>
<tr>
<td>32/F/14</td>
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<td>++</td>
<td>960</td>
<td>I</td>
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<tr>
<td>33/M/54</td>
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<td>++</td>
<td>2400</td>
<td>I</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>7000</td>
<td>I</td>
</tr>
<tr>
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<td>3000</td>
<td>I</td>
</tr>
<tr>
<td>47/M/40</td>
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<td>++</td>
<td>4800</td>
<td>I</td>
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<td>48/F/58</td>
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<td>++</td>
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<td>49/F/11</td>
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<td>51/M/43</td>
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<td>1500</td>
<td>I</td>
</tr>
</tbody>
</table>

the first months after it has been initiated, and follow-up biopsies after 1.5 gm intervals of cumulative MTX doses if there are no alterations in liver chemistry values or risk factors. If there are significant alterations in these parameters, it is recommended that follow-up liver biopsies are performed at 1.0 gm intervals of cumulative MTX doses. The purpose of the present work was to review MTX hepatotoxicity in a population of psoriatic patients submitted to long-term treatment with this cytotoxic drug.

PATIENTS AND METHODS

A review of the medical records between 1965 and 1990 was performed in the Department of Dermatology at the Hospital de Santa Maria, in Lisbon, concerning all psoriatic patients treated with amnioprotein and MTX.

The data of 84 patients were examined focusing on the disease evolution time, type of cutaneous lesions, previous treatments, MTX cumulative doses, laboratory data and liver histology, before and during the treatment, and history of hepatic disease and risk factors, such as diabetes, alcohol abuse and use of retinoids, nonsteroidal anti-inflammatory drugs, barbiturates and arsenic.

Sixty-three patients were males (75%) and 21 females (25%). The ages ranged from 11 to 79 years old (average 49.5). Thirty-five patients had extensive psoriasis, refractory to standard topical therapy, 30 had arthropathic psoriasis, 8 pustular psoriasis and 11 erythrodermic psoriasis. The diseases evolution time ranged from 1 month to 67 years (average 18.4 years).

All patients had previously been given topical treatments. Fifty-three had had combined treatments: 11 had made UVB phototherapy, 8 had been treated with PUVA, 5 with REPUVA and 4 had been submitted to localized superficial X-ray therapy. Ten patients had received systemic corticosteroids, 15 patients etretinate. The MTX cumulative doses, at the moment of the last liver biopsy, ranged from 200 to 10,660 mg, the average dose being 3,374 mg.

Past medical history included hepatitis in 4 patients and diabetes in 8.

Alcohol intake was considered high when there was a consumption equal or higher than 80 gm of ethanol per day, moderate when it did not exceed 60 gm and mild when it did not exceed 40 gm per day.

Forty-eight patients (57.1%) referred alcohol intake, 42 being males (66.6% of the men) and 6 females (28.6%). According to the criteria previously mentioned, habits were considered severe in 15 patients, moderate in 23 and mild in the remaining 10 patients.

Forty-nine patients (58.3%) used potentially hepatotoxic drugs, some of them having taken more than one. The nonsteroidal anti-inflammatory drugs had been used by 34, systemic corticosteroids by 10 and etretinate by 15. Seven patients used other drugs like immunosuppressors, arsenic or diphenylhydantoin.

Concerning laboratory data, special attention was paid to full blood cell count, liver and renal function tests.

Liver biopsy specimens were obtained by the percutaneous Menghini technique, using the Jamshidi needle. Sections were stained with hematoxylin-eosin, Masson trichrome and reticulin stains. All biopsy specimens were reviewed by the same pathologist. The histological parameters were classified in 4 grades (12): grade I, normal; grade II, fatty infiltration; grade III, nuclear morphology - mild alterations; grade IV, moderate and severe alterations; grade V, fibrosis (Klampfer's classification); grade VI, cirrhosis.

The patients were divided into three groups, depending on the moment of the liver biopsies in relation to the treatment (Tables 1 and 2): group 1, 30 patients with biopsies before and during MTX treatment; group 2, 21 patients with biopsies during the treatment only; group 3, 33 patients with pretreatment biopsies only.

The groups were compared by the χ² test modified for small series.

RESULTS

The laboratory data were normal in all patients before taking MTX. In 19 patients (22.6%) significant abnormalities were found during therapy. From these, 17 had alterations in liver chemistry values, which normalized after discontinuing MTX. The remaining 2 patients had bone marrow depression after cumulative doses of 390 and 3,600 mg.

The total number of reviewed biopsies was 134, with 10 patients having 2 follow-up biopsies, 3 patients having 3 follow-up biopsies and one patient having 5 follow-up biopsies.

In group 1, constituted by 30 patients with biopsies both
Table III. Group 3.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hepatotoxic or liver disease</th>
<th>Alcohol</th>
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<td>54/M/38</td>
<td>++</td>
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<td>III</td>
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<td>56/M/70</td>
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<td>II</td>
</tr>
<tr>
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<td>II</td>
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<td>III</td>
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<td>III</td>
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Table IV. Group 1.

<table>
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<th>Pre MTX liver biopsy</th>
<th>Follow-up liver biopsy</th>
<th>% Grades</th>
<th>Patients</th>
<th>% Grades</th>
<th>Patients</th>
<th>MTX (mg) average</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36.7</td>
<td>I</td>
<td>11</td>
<td>13.4</td>
<td>I</td>
<td>4</td>
<td>200-2,500</td>
</tr>
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<td>11</td>
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<td>II</td>
<td>8</td>
<td>370-7,000</td>
</tr>
<tr>
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<td>III</td>
<td>8</td>
<td>50.0</td>
<td>III</td>
<td>15</td>
<td>440-10,050</td>
</tr>
<tr>
<td>00.0</td>
<td>IV</td>
<td>0</td>
<td>10.0</td>
<td>IV</td>
<td>3</td>
<td>1,600-1,700</td>
</tr>
</tbody>
</table>

histological findings improved to grade II after MTX cumulative doses of 3,750 and 370 mg, respectively.

In group 2, constituted by 21 patients with liver biopsies during MTX treatment only, 8 (38.1%) were in grade I, 4 (19.1%) in grade II, 7 (33.3%) in grade III and 2 (9.5%) in grade IV. The MTX cumulative doses ranged from 960 to 7,000 mg (average 3,287 mg) in patients with grade I, from 1,420 to 4,170 mg (average 2,648) in patients with grade II, from 3,143 to 9,360 mg (average 6,039 mg) in patients with grade III and after 3,000 mg in the 2 patients with grade IV.

Finally in group 3, constituted by 33 patients with pretreatment liver biopsies only, 16 (48.5%) had histopathological findings of grade I, 5 (15.2%) of grade II and 12 (36.3%) of grade III.

It is important to note that in the 42 grade III liver biopsies, the histological findings disclosed moderate to severe fibrosis, only in 3 patients (nos. 5, 13, 31).

DISCUSSION

MTX is an efficient therapy in the treatment of severe forms of psoriasis. Hepatic fibrosis and cirrhosis are the major problems related to its long-term administration (1-6). Some risk factors, like patient age (14), abnormalities in renal function (11), obesity and diabetes (1, 15) and alcohol intake (2), increase the incidence of hepatotoxicity from MTX. In this study, 26% of the patients in group 1 (previous or follow-up biopsies) and 36% of group 3 (previous biopsy), had mild grade III liver alterations (liver fibrosis) before beginning treatment, which compares with the figures reported by Weinstein et al. (22%) (1), Reese et al. (20%) (4) and Newman et al. (18%) (8). However, there was no correlation between the observed alterations and the amount of alcoholic beverages consumed, or the use of potentially hepatotoxic drugs. For that reason, the pretreatment liver biopsy is essential for therapeutic decision, since there is not a significant correlation between liver function tests and histological findings (1, 2, 4, 5, 7, 8, 15). According to some authors (13, 16) the tendency to fibrosis and cirrhosis is higher in patients whose pretreatment liver biopsies have abnormal histological findings. In group 1 there was no statistically significant difference in paired observations of the same patient between the histological grades before and after the treatment previous and fol-
low-up biopsies (13). There was no significant correlation between the cumulative MTX doses and the histological grade of the follow-up biopsies (13).

However, the difference is significant (13) when comparing the percentages of the different grades in the whole group in previous and follow-up liver biopsies (Table IV).

The risk of developing fibrosis and cirrhosis with increased MTX cumulative doses is not unanimously accepted by all authors. Roenigk et al. (15) think that there is no correlation between MTX total dose and liver histological findings. On the other hand, Zachariae et al. (6), who reported 13.5% of cirrhosis after 2,200 mg MTX average dose and 25.6% with a cumulative dose superior to 4,000 mg, support the idea that there is a cirrhosis-increasing risk with the cumulative dosage. Also Nyfors (17), who found 24% of patients with fibrosis and 21% with cirrhosis after 4,000 mg MTX average dose, thinks that fibrosis and cirrhosis frequency increases considerably with cumulative doses between 2,000 and 4,000 mg MTX and, for that reason, defends the need of follow-up biopsies at those levels.

We agree with the great majority of authors that liver biopsies are recommended in follow-up of MTX-treated patients. However, Miller et al. (18) support the view that follow-up liver biopsies can be lengthened if ultrasound scans remain normal. Zachariae et al. (19, 20) concluded that serial measurements of serum PIINP (aminoterminal propeptide of type III procollagen) is a valuable non-invasive test for liver function in MTX-treated patients. Normal levels of PIINP allow a reduction in the follow-up liver biopsies. For O’Connor et al. (21) the high sensitivity of the liver function tests may reduce the follow-up liver biopsies in the absence of abnormal results.

In our series we observed a great individual variability between the MTX cumulative dosage and the histological findings in posttreatment biopsies. In group 1, 15 patients maintained the same level of liver histological changes after 3,413 mg MTX average dose, while in 13 patients there was a worsening of the liver biopsy after 2,180 mg MTX average dose; in 2 patients there was even an improvement of the histological findings after MTX doses of 370 and 3,750 mg. There was no absolute correlation between liver alteration level in posttreatment biopsy and MTX cumulative dosage. The occurrence of liver disease seems to depend mostly on individual susceptibility that cannot be anticipated.

MTX, when used according to internationally established criteria (12), carefully weighting the risk/benefit relation and informing the patients about its interactions, especially with regard to alcohol intake, is a valuable drug in the treatment of severe psoriasis.

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REFERENCES

Elimination Diet in Young Children with Atopic Dermatitis

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Thirteen children with severe current atopic dermatitis unresponsive to topical treatment were started on an elimination diet. One child was excluded because she could only keep to the diet for 3 days. Twelve children aged 0.8–4.1 years maintained the diet for 2–4 weeks. In six children the dermatologist’s score showed a clear improvement while on diet, in 2 children there was a minor improvement and in 4 children the dermatologist’s score did not change during elimination diet. Challenges were performed with egg, milk and wheat in 6 children and with milk and wheat in 2 children. The challenges were done in an open way except for the dermatologist, who was unaware of which food the child had received. No child in the study had an immediate reaction but 3 children had late reactions, one after egg, one after milk and one child after challenge with wheat.

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Uncontrolled dietary manipulations are common among children with atopic dermatitis (AD) (1). Clinically, we often meet parents who are more eager to try their children with various elimination diets than with basic topical treatment despite a negative history of food allergy and without evidence of improvement while on diet. Allergists and dermatologists have different opinions about the prevalence and importance of food allergy in AD (2). Despite many studies, the true prevalence of food allergy among AD children remains unclear (3–12). It is emphasized by many authors that, before giving complex dietary instructions, the physician should be certain about compliance with topical corticosteroids and emollients (10–15).

In this article we report our experiences with a selected group of AD children who were treated with a strict elimination diet (ED) and, if improving, were subjected to open challenges in the hospital. At inclusion, all children had severe AD in spite of adequate topical treatment and elimination of the food items to which the child was suspected to be allergic.

MATERIAL AND METHODS

Patients

Thirteen children, 8 girls and 5 boys, aged 10 months to 4 years, with active severe dermatitis were included. The diagnosis was based on the criteria of Hanifin & Rajka (16). All the children were patients at the outpatient clinic of the Department of Dermatology, Sahlgrenska sjukhuset, and were selected because they did not respond well enough or had severe flare-ups despite treatment with emollients, hydrocortisone, intermittent triamcinolone, oral antihistamines and, when indicated, oral antibiotics. Most parents had received instructions about the topical treatment from a trained nurse at a special visit in what we call an “eczema school”, described elsewhere (17).

Only families in which parents were interested in trying a strict elimination diet, even after having been given a thorough explanation about possible problems during the trial, were included.

Methods

A period of 1 month’s baseline scoring was carried out before the children were started on the ED (vide infra). During this period, parents were asked to continue the eczema treatment as optimally as possible. The parents also recorded the eczema symptoms on a diary card. Following the baseline scoring, ED was started for 1 month. The children were examined before and after the strict ED and the physicians and parents together decided whether to continue with the challenge or not. The challenges were carried out at 1- or 2-week intervals with cow’s milk, egg and wheat. If the child could tolerate the test food, this was added to the ED. We did not challenge any child with food to which there was a clear history of immediate allergy. During the challenge period, the child was checked weekly by the dermatologist, before each new challenge by the pediatrician and the dermatologist, and in case of a reaction while in the hospital by the pediatrician.

Parents’ scoring

Throughout the study, parents were asked to record daily scores for eczema (0–4), pruritus (0–4) and disturbance of night sleep (0–3). The scores were averaged and a mean daily score for the final 10 days of the elimination period was used.

Dermatologist’s scoring

An eczema score was assigned at each visit, based on the type, intensity and distribution of the lesions. A mean score for intensity was graded separately for erythema, lichenification, vesication, excoration, papules, and dryness, and the scoring was as follows: 0 = no symptoms, 1 = mild, 2 = moderate, 3 = marked, 4 = severe.

The distribution of the lesions was scored from 0 to 4 as follows: 0 = no eczema, 1 = one local site affected (symmetrical lesions always counted as one locus), 2 = two local sites affected, 3 = three local sites affected, 4 = four or more local sites affected. The six different intensity scores were multiplied by the distribution score and the sum of these became the total eczema score. The maximum score was 96. Photographs were taken at each visit.

Elimination diet

The ED contained only the following items: caseinhydrolysate (Nutramigen®), lamb’s meat, rice, corn, corn oil, potato, cucumber, melon, bilberries, salt, sugar, and gluten and milk-free bread. Instructions about ED were given by a dietician to the parents and when indicated also to day-mothers or personnel at the day-care centres. The information was given orally as well as in writing on a handout with menu suggestions for different age-groups. The dietician participated at all return visits and usually had telephone contact with the families between visits. Parents were instructed to give the children calcium supplementation and vitamins if the children did not take the caseinhydrolysate.

Challenge

The challenges were carried out in an open way. Only the dermatologist was unaware of which food the child had received. The first 2 days of challenge were performed in the hospital. In most cases the child was kept as an inpatient, and if immediate reactions appeared these were treated by the pediatrician. The children stayed in the
Table 1.

<table>
<thead>
<tr>
<th>Case #</th>
<th>Age (years)</th>
<th>Ige (Ku/l)</th>
<th>SPT ≥2+</th>
<th>RAST ≥2</th>
<th>Positive history</th>
<th>Parents' scorea</th>
<th>Physician's scoreb</th>
<th>Challenged with</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.2</td>
<td>210</td>
<td>Soybean</td>
<td>Soybean</td>
<td>wheat</td>
<td>6.3/8.9</td>
<td>36/20</td>
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<td>60</td>
<td></td>
<td></td>
<td></td>
<td>4.4/2.9</td>
<td>15/13</td>
<td>Wheat, milk</td>
</tr>
<tr>
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<td>90</td>
<td>Egg, fish</td>
<td>Egg, fish</td>
<td>Egg, fish</td>
<td>4.9/5.3</td>
<td>26/75</td>
<td>Wheat, milk</td>
</tr>
<tr>
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<td>3.8</td>
<td>990</td>
<td>Egg</td>
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<td>36/34</td>
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<td></td>
<td></td>
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<td>12/2</td>
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</tr>
<tr>
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<td>2.3</td>
<td>30</td>
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<td></td>
<td></td>
<td>7.4/4.3</td>
<td>49/5</td>
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<td>2.6</td>
<td>100</td>
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<td></td>
<td>10.2/9.0</td>
<td>44/8</td>
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<tr>
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<td>35</td>
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<td></td>
<td></td>
<td>7.3/9.9</td>
<td>24/20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.9</td>
<td>50</td>
<td></td>
<td></td>
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<td>7.5/8.4</td>
<td>52/34</td>
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<td>70</td>
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<td>Wheat</td>
<td>6.6/6.0</td>
<td>9/8</td>
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<tr>
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<td>4.2</td>
<td>Egg</td>
<td></td>
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<td>8.7/5.4</td>
<td>5/42</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.3</td>
<td>4.2</td>
<td>Fish</td>
<td></td>
<td></td>
<td>6.6/4.3</td>
<td>34/2</td>
<td>Egg, wheat, milk</td>
</tr>
</tbody>
</table>

000 = > 2 SD (24). *before diet/after diet, †positive to challenge.

hospital until 4 h after challenge the second day, and if no reaction occurred the new food was included in the diet. On the first day of challenge, each new food was given at 30-min intervals as follows: whole egg was given as a gluten- and milk-free cake containing 0.15 g egg per g cake, as follows: 1 g, 5 g and 10 g of the cake and on the second day one boiled egg. Wheat was given as a milk-free bread, and this was given as follows: 1 g, 5 g and 10 g of the bread and on the second day a free amount. Nonfat cow's milk was given as follows: 5 ml, 10 ml and 100 ml. On the second day, the child received a free amount of milk.

Laboratory Specific circulating IgE antibodies to cow's milk, egg, fish, soybean, and wheat were determined before ED by means of the radio-allergosorbent test (RAST, Pharmacia). Total IgE was also determined.

Skin prick tests (SPT) were performed with green pea, egg, cow's milk, fish, soybean, and wheat (ALK, Allergologisk Laboratorium, Hellerup, Danmark) separately. Histamine (10 mg/ml) was used as positive control. Wheals greater than or equal to half of the histamine reaction were considered positive, provided that the vehicle control was negative and that the diameter of the histamine wheal was at least 3 mm.

RESULTS

Twelve of the 13 children completed the study. One child could only keep to the diet for 3 days and was therefore excluded. The data of the 12 children who completed the study are presented in table I. In one patient (no. 5), the parents found it difficult to adhere to the restricted diet for more than 2 weeks, but since the child had improved so much, they were motivated to agree to the challenges.

In 6 of the 12 children, the dermatologist's scores showed a clear improvement (cases no. 3, 5, 6, 7, 11 and 12), in a further 2 cases (no. 1 and 9) there was a less marked improvement and in 4 children (cases no. 2, 4, 8 and 10) the dermatologist's score did not change during ED. The parents' scores did not always tally with the dermatologist's score. In 3 cases considered improved by the dermatologist the parents' score did not show any improvement (no 1, 3 and 9). In patient no. 3 the reason was obvious, whooping-cough which made her wake up several times every night.

Challenges were performed in 8 of the 12 children. One child (no. 9) who improved according to the dermatologist's score was not challenged because the mother was not motiva-

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ted to continue with the diet. One child (no. 4) was challenged in spite of no improvement in the dermatologist's score. This child had a very unstable eczema before and during the study but the mother's assessment was that the flare-ups were fewer during the ED. In 2 other children (no. 1, 7) eczema deteriorated during the study in connection with infection. No child reacted with immediate symptoms when challenged. Three children reacted with one item each. The positive cases reacted as follows:

Case no. 1

Eczema worsened slowly during the first week when milk was introduced. The other challenges were uneventful. After the challenges, he was eating a diet devoid of cow's milk and had only minimal eczema.

Case no. 7

An itchy erythematous eruption developed mainly on the trunk 8 h after challenge with egg. The other challenges were uneventful. After the challenges, he was given a diet devoid of egg and the eczema disappeared, although he continued to be bothered by itching.

Case no. 11

Abdominal pain, vomiting and flushing in the face developed 24 h after challenge with wheat. Because of difficulty in interpreting this reaction, wheat was withdrawn and a new challenge with wheat was done after 1½ months. After this second challenge, she reacted only with skin symptoms after 2 days, when a severe itchy papular eczema developed on her entire body (Figs. 5, 6). The other challenges were uneventful. After the challenges, she was given a diet devoid of wheat and the eczema improved. An accidental challenge later at home with a wheat-cracker started an extensive papular eruption once more. This girl also had a positive RAST for wheat.

Patients no. 5 and 12 improved during the ED period: the situation remained unchanged during the challenges, and these 2 children were returned to their normal diets, which for patient no. 12 was fish-restricted. In patients no. 3, 4, and 6, the eczema worsened several times during the challenge period but with no clear relation to the challenged food. Of these
Fig. 1–2. Patient no. 11 in Nov-88, before diet. Dermatologist's score 54.
Fig. 3–4. Patient no. 11 in Dec-88 after 1 month's diet. Dermatologist's score 2.
Fig. 5–6. Patient no. 11 in Feb-89 after challenge with wheat. Dermatologist's score 38.
5 improved but challenge-negative children, one deteriorated after reverting to a normal diet (no. 4).

DISCUSSION

Dietary manipulation of children with AD is common. Webster et al. (1) found that 71% of children seen in a dermatology outpatient clinic had had significant alterations made to their diet before the first hospital visit. Many of these children had only mild eczema and only a few parents felt that diets had been helpful.

In spite of many studies on selected groups of children with AD, the prevalence of food allergy among all children with AD is not known, nor is the importance of food hypersensitivity in the pathogenesis of AD clear (11, 18). According to a report from a symposium published in 1986, pediatric allergists and dermatologists clearly expressed different opinions about the role of diet in the treatment of AD (2).

A high prevalence of IgE-mediated food hypersensitivity among children and young adults with AD has been shown in a number of challenge studies. In these studies, 33–96% of patients aged 4 months to young adults reacted to food challenge (8, 10, 12). Unfortunately the children in these studies were selected and thus not reveal the true incidence of food allergy in AD.

However, it is not known how often IgE-mediated hypersensitivity is involved in the pathogenesis of AD. Sampson et al. found that children prescribed an antigen-restricted diet for 2–3 years did significantly better than a group of children who were not found allergic to any foods or who did not comply with the ED (8). Pike et al. found that 36% of 66 children responded favourably to the elimination diet (11). However, only 12 of these 66 children experienced prolonged benefits from the diet.

Based on the available studies, the Task Force on Pediatric Dermatology came to the conclusion in 1986 that elimination diet cannot be recommended as a routine treatment in AD. Instead, the basic therapy (i.e. emollients, topical corticosteroids and antihistamines) should be started before dietary manoeuvres because it offers improvement with less inconvenience to family life than does dietetic management (13).

In our study, we selected children who had responded inadequately to basic treatment, even when instructed by a special nurse, i.e. “eczema school”, in which the compliance with the topical treatment is generally very high. According to earlier studies, hypersensitivity to food is more common among the youngest children (14, 15) and we therefore selected only children who were 4 years of age or younger.

Another criterion used was whether parents could comply with the diet. In this age-group, around 60% of Swedish children have day-care or a day-mother outside the home, and it is thus not always easy to adhere to a strict diet over several months (19). In our study, 6 of the 12 children were taken care of by another person during the daytime. It appears from other studies also that non-compliance with diets is common. In the studies by Atherton (3) and Nield (9), 25% of the patients could not complete the trial. Pike et al. selected only families which they thought capable of managing complex dietary in-

structures and all but one of their 66 patients completed one period of strict elimination diet (11). Two- to six-week diet periods have been used in other studies (7, 9, 11). We chose 4-week periods but shorter or longer periods may be needed depending on the intensity of the eczema from the start. In our study, 11 of the 13 children completed the 4-week strict diet period and one followed it for 2 weeks. There were many practical difficulties along the way, and the unlimited possibility of contacting the dietician was found to be essential for compliance with the diet.

Based on the state of the child at the return visit, and on the parents’ assessment of the eczema during the preceding period, the parents and the dermatologist together decided whether or not the child had improved sufficiently to continue with the challenges; continuing meant several more weeks on the ED. Small variations in the dermatologist’s scores did not always correspond to a clear improvement. The photographs showed a clear improvement but the difference, e.g. between 24 and 20 as in patient no. 8, was not obvious compared to patient no. 11, who scored 54 before the diet and 2 after the diet (Figs. 1–4). In some children, the dermatologist found an improvement but the parents evaluated the period differently and this could be explained by disturbances of night sleep. This shows the difficulty of evaluating the severity of AD over a period of time with the type of subjective criteria we have.

The food items for the challenges were chosen based on earlier findings that egg, cow’s milk and wheat are most often avoided in the management of AD (13). Sampson & McCantskill found in patients with suspected IgE-mediated allergy that egg, peanut, milk, wheat, soy and fish accounted for 90% of the positive food challenges (8). Cow’s milk, egg and wheat were also found to be among the most common foods identified as causing exacerbation of eczema in children with severe AD selected from a dermatology outpatient clinic (11). We challenged all the children with the same basic foods – egg, cow’s milk and wheat – since earlier studies have shown little or no correlation with RAST and SPT results (8, 11). However, the challenges were not carried out if there was a history of immediate reactions to one or more of these foods, confirmed by positive SPTs and RASTs (i.e. in patients no. 3 and 4).

IgE-mediated hypersensitivity and delayed hypersensitivity may play a role in AD (20). The majority of studies have concentrated on IgE-mediated allergy. Van Bever et al. challenged AD children, and all positive reactions were reported within 1 h (12). According to Burk et al. (10) and Sampson et al. (8), all reactions appeared within 2 h; in addition, some of the children in Sampson’s study developed a “late phase reaction” after 6–8 h, but only following initial symptoms.

No child in our study had an immediate reaction but 3 children had late reactions. One child (no. 7) reacted after egg challenge and the other 2 children after milk (no. 1) and wheat (no. 11) after 8 h to 3 days. Immediate severe reactions, however, may be expected even in a selected small group like the present one (21). We thus want to stress the importance of performing food challenges under strict observation in a hospital setting.

We are aware of the difficulty of interpreting these open

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challenges, because many other factors besides foods (e.g., recurrent infections or sleep disturbances) may influence the course of AD. Double-blind food challenges might reduce some of these factors. On the other hand, our design better reflects everyday exposure and can enable us to see reactions that may develop after a prolonged time of antigen ingestion, or reactions in which skin contact may be important (22, 23). It is possible that type I reactions can also elicit type IV-like reactions in the skin, and longer periods of exposure and observation are therefore also needed. Possibly, some of the reactions are just cell-mediated.

It is also difficult to find objective criteria with which to evaluate eczema changes. We used the camera to document the eczema lesions. In case 11, these photographs could clearly capture the change in the extent and activity of the lesions during the study and they thus appeared helpful both in documenting the course of the disease and in providing a correlate with the scoring system used.

In conclusion, we found it worthwhile to work in this way with ED in a highly selected group of patients with severe eczema, not only to identify foods responsible for the worsening of the eczema, but also to allow children who do not respond to ED to return to a normal diet, which for some children meant a diet restricted for one or two foods. This also enabled the parents of non-responding children to focus their time and energy on topical treatment. Working with elimination diets is a team effort, involving patient, parents, pediatrician, dermatologist and dietician, in which all participants are important and essential.

ACKNOWLEDGEMENTS

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REFERENCES

Thirteen patients with a severe adult form of atopic dermatitis (AD) received 3.0 x 10^6 IU of recombinant interferon-α2a (rIFN-α2a) 3 times a week. A satisfactory response was obtained in 5 of them. Serum IgE levels in all 13 patients remained unchanged throughout the study. Flu-like symptoms were common, but clinical or laboratory adverse effects were otherwise slight. The moderately beneficial therapeutic effects observed in this study support a possible role for IFN-α in controlling immunologic deficiencies in atopic dermatitis.

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It is well known that several immunologic abnormalities are present in patients with atopic dermatitis (AD), although it is not known whether they represent a primary defect in the disease. Modulation of the immune system is an attractive approach for therapy of patients with AD, and diverse results have been obtained with levamisole (1), transfer factor (2), thymopentin pentapeptide (TP-5) (3), and cyclosporin A (4, 5). Recombinant interferon-α2a (rIFN-α2a) is a biological response modifier which has been effective in the treatment of diverse dermatologic diseases, such as Behçet’s disease (6), cutaneous lupus erythematosus (7), and some cutaneous tumors (8). IFN-α has been effective in pilot trials of AD patients (9, 10), and rIFN-α was effective in a hyper-IgE patient with eczema (11) and ineffective in 2 AD patients (12). In the present study, the results of a pilot study of rIFN-α2a therapy in 13 patients with AD are reported.

PATIENTS AND METHODS

Patients

Thirteen patients (9 men, 4 women, age 18-31 years), with severe AD for more than 4 years participated in the study. All patients fulfilled diagnostic criteria for AD (13) but were otherwise healthy, and pregnancy was excluded in women.

Treatment

The patients received 3.0 x 10^6 IU of intramuscular rIFN-α2a, 3 times a week, for 4 weeks. This dosage could be doubled for another 4 weeks, if no response was observed at that time, or otherwise continued at the same dosage.

Evaluation criteria

Evaluation of the response to the therapy was made by the same investigator on the basis of clinical examination. We employed the score system proposed by Costa et al. (14), measuring intensity and extension of AD and obtaining a total score. The intensity was assessed using values from 0 to 7 for the following parameters: erythema, oedema, vesicles, crusts, excoriations, scales, lichenifications, pigmentation/depigmentation, pruritus, and loss of sleep, with a maximal score of 70. The extension was assessed by division of the body surface in 10 areas: feet, popliteal fold, remaining legs, hands, arms, buttocks, back, anterior chest and abdomen, face, and scalp. A score of 0 to 3 was assigned for each region regarding the extension of AD, up to a maximum of 30. Thus, a total sum of 100 would mean the highest score of severity. Dermatological examinations were repeated every 2 weeks. At the end of the study, an evaluation of the global therapeutic effect was made by the patient and the investigator using the following scale: no improvement, slight response, moderate response or satisfactory response. Clinical evaluation of adverse effects was made twice a week.

Laboratory tests

Complete blood cell count, biochemical parameters, urinalysis, and clotting studies were performed before and 3 times a week during the first 2 weeks of therapy, and every week thereafter in order to detect any adverse effects promptly. Total serum IgE was measured by radioimmunoassay (RIA) every week. Two weeks after withdrawal of therapy, these determinations were repeated, together with electrocardiogram and thorax roentgenograms. During the assay, only emollient baths were permitted. Oral paracetamol 500 mg was administered together with rIFN-α2a prophylactically to minimize flu-like reactions. In patient no. 10, low potency topical corticosteroids were applied to the most severely affected areas.

RESULTS

The values of scoring prior to therapy and at 2, 4, 6, and 8 weeks are summarized in Table I. All patients completed the 4-week therapeutic trial, while in 6 patients therapy was prolonged for 4 weeks more. No patient had to discontinue therapy due to adverse reactions.

At 4 weeks of therapy a satisfactory response was registered both by the patients and the investigator in patients nos. 1–5, while in the other 8 patients a slight response or no improvement was achieved. Of the 5 patients who improved markedly at 4 weeks, nos. 1, 2 and 3 experienced a prolonged remission without any treatment. Patient no. 4 suffered a new attack of AD when rIFN-α2a was interrupted at 8 weeks, which could be controlled with the same medication at identical dosage; however, a later bout of lesions appeared while he was on rIFN-α2a treatment, which could not be controlled with this medication (score 42). Patient no. 5 worsened at 8 weeks despite continued rIFN-α2a treatment. The treatment was prolonged for patients nos. 10, 11 and 12 at a double dosage and at the same dosage in patient no. 13. None of them improved. Serum IgE levels remained high and unchanged throughout the trial, in both responsive and non-responsive patients. Adverse effects are shown in Table II. All of them subsided during the trial or shortly after finishing.
Table I. Scoring of severity of atopic dermatitis

<table>
<thead>
<tr>
<th>No</th>
<th>Prior to therapy</th>
<th>2nd week</th>
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<th>6th week</th>
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<tr>
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<td>27</td>
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<tr>
<td>2</td>
<td>51</td>
<td>6</td>
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<td>39</td>
<td>17</td>
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<td></td>
<td>Unresponsive patients:</td>
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<tr>
<td>6</td>
<td>56</td>
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<td>13</td>
<td>50</td>
<td>48</td>
<td>38</td>
<td>41</td>
<td>52</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study, 5 out of 13 patients improved markedly after 4 weeks of therapy with rIFN-α2a, while therapy failed or induced only a slight improvement in the other 8 cases.

Strannegard & Strannegard (15) observed a defective capacity to generate interferons in response to viral antigens in AD. Furthermore, peripheral blood mononuclear cells from a significant proportion of patients with AD have an impaired capacity to generate IFN-γ after PHA stimulation in vitro (16, 17). Moreover, small quantities of IFN-γ inhibit the ability of IL-4 to stimulate B-cell growth, enhance IgG1 and IgE production, and induce the expression of low affinity receptors for Fe fragments of IgE (FeR1/CD23) on B cells (18). IFN-α decreases IL-4 induced IgE synthesis as well (11). Lately, both IFN-α and -γ have been shown to block spontaneous IgE production by mononuclear cells of allergic patients in vitro (11). In view of these and additional studies (19), it has been suggested that a decreased production of IFN and an increased production of IL-4 could lead to some primary or secondary immunologic abnormalities in AD.

Previously, Souillet et al. (11) described a patient with hyper-IgE syndrome and eczema in whom treatment with IFN-α resulted in clinical improvement and a gradual decrease of the serum IgE levels. In contrast, MacKie found no benefit from treating 2 AD patients with rIFN-α (12). Parkin et al. (20) reported on 2 AIDS patients with atopic symptoms who improved strikingly after IFN-γ therapy, and two reports noted strikingly beneficial effects with IFN-γ in AD patients (9, 10). The number of patients treated with rIFN-α was too small to draw any conclusions.

The presented data indicate some possible beneficial effect of rIFN-α treatment in AD patients. A placebo group was not considered in our study due to its pilot nature and the resistance of our patients to conventional therapy.

Table II. Adverse effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu-like syndrome</td>
<td>12</td>
</tr>
<tr>
<td>Increased serum triglycerides</td>
<td>4</td>
</tr>
<tr>
<td>Increased serum transaminases</td>
<td>4</td>
</tr>
<tr>
<td>Hair loss</td>
<td>1</td>
</tr>
<tr>
<td>Peripheral oedema</td>
<td>1</td>
</tr>
<tr>
<td>Decreased prothrombin time</td>
<td>1</td>
</tr>
</tbody>
</table>

REFERENCES


The Genetic Risk for Alopecia Areata in First Degree Relatives of Severely Affected Patients

An Estimate

PIETER van der STEEN1, HEIKO TRAUBE2, RUDOLF HAPPLE3, JAN BOEZEMAN1, RONNY STRÄTER4 and HENNING HAMM4

Departments of 1Dermatology and 2Human Genetics, University of Nijmegen, The Netherlands; 3Department of Dermatology, University of Marburg and 4Department of Dermatology, University of Münster, Germany

Substantial evidence indicates that genetic factors may have a role in the etiology of alopecia areata (AA). Most studies, however, provide only general information on the familial incidence but fail to specify family relationships. We therefore obtained information on the incidence of AA in first degree relatives of 348 severely affected patients. In 7% one of the parents was affected. Among the siblings of the patients 3% had developed AA, while AA was present in 2% of the children. Taking into account the age of the patients, their lifetime risk was calculated to approach 6%. However, a severe type of AA is to be expected only in about 2% of the children. The degree of involvement observed in the patients did not influence the frequency and type of AA present in their first degree relatives.

Key word: Genetic risk.

(Accepted February 10, 1992.)


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In many studies an increased familial incidence has been found in alopecia areata (AA), indicating that genetic factors may have a causative role (1–10). A high frequency of AA is observed in Down’s syndrome (11–14), suggesting the presence of a relevant gene located on chromosome 21. A second locus possibly involved in AA may reside in or close to the major histocompatibility complex on chromosome 6 (15, 16). However, the relationship between HLA and AA is complex and not undisputed (17). Recently, Duvic et al. demonstrated a negative relationship between the DRB1 allele and AA (16). From this study it may be inferred that the presence of the DRB1 allele protects individuals against developing AA.

Although having a selection bias for the interesting, case reports of AA in twins provide further evidence for a genetic predisposition to AA (18–23). It is of note that in two reports dealing with monozygotic twins, hair loss occurred simultaneously and in identical areas (18, 19).

Pertinent data, however, concerning the genetic risk of first degree relatives of patients with AA are scarce. Most studies provide only general information on the familial incidence but fail to specify family relationships.

Adult patients often wonder how likely it is that their children will develop the same hair disorder. The aim of the present study was to answer this question by giving an estimate of the genetic risk for AA in first degree relatives.

PATIENTS AND METHODS

Three hundred and forty-eight patients were interviewed by use of a uniform questionnaire at the University Centers of Nijmegen (n=194), and Münster (n=154). All patients suffered from a severe type of AA, with a minimum scalp involvement of 40%, and attended a special outpatient clinic for topical immunotherapy. A detailed family history concerning first degree relatives was taken. Moreover, the age at first presentation, the age at onset of AA, and gender were recorded. The extent of hair loss was classified as patchy, totalis or universalis. Fisher’s exact test was used for statistical evaluation.

RESULTS

Of the 348 patients, 208 were female (60%), 140 male (40%); the Nijmegen group had 107 females (55%), whereas the Münster group had 101 females (66%). The median age of the patients was 34 years (Nijmegen: 33 years, Münster: 34 years), the median age at onset of AA was 21 years (Nijmegen: 21 years, Münster: 22 years).

Table I shows the incidence of AA in parents, siblings, and children. In the Nijmegen group, parents were more often affected than in the Münster group (p<0.03).

The frequency of AA in first degree relatives of patients with patchy AA was 13%, and for patients with a totalis or universalis type of AA this was 18% (n.s.).

Fifty-six of the 348 patients (16%) had an affected first degree relative, 51 patients having one affected relative, and 5 patients having 2 affected relatives each. Forty-eight out of 61 affected first degree relatives (79%) had a history of patchy AA, 8 (13%) had experienced AA totalis, and 5 (8%) had had AA universalis. In this subgroup no correlation was found between the degree of involvement of the patients and the type of AA observed in their 61 affected relatives.

Fig. 1 shows the distribution of the age at onset of AA in patients having affected first degree relatives versus the distribution of the subgroup without familial occurrence. The two distributions are similar. The median age at onset of AA in the subgroup with affected first degree relatives was 23 years, for patients without familial occurrence 21 years.

DISCUSSION

In the literature familial occurrence of AA is usually not specified in terms of family relationships. The purpose of this study was to estimate the genetic risk of first degree relatives of severely affected patients. Second and third degree kinships
were excluded because we regarded this information as unreliable.

We identified 25 affected parents in the group of 348 patients (7%). We are aware that the ethnic make-up of the two groups is not identical, and this may explain the difference observed. On the other hand, the two populations showed different risk figures only for the parents, and therefore we decided to base our calculations on the joint study.

The median age of the patients was 34 years. As AA rarely becomes manifest after the age of 50 (Fig. 1), a substantial increase of affected parents in the future is not to be expected.

In this study 9 out of 357 children (2%) were already affected. As discussed above, this figure does not reflect the true lifetime incidence of AA due to the age dependency of the disease. The median age of the children was 18 years. It is noteworthy that 42% of the patients had already developed AA before the age of 19. Thus it may be concluded that the lifetime incidence of AA in the children will approach 6% (i.e. 1/42 × 9/357). It is of note that the incidence among the parents of the patients (7%) corresponds to the calculated lifetime incidence of the children (6%). This may indicate that first degree relatives run the same genetic risk.

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>Nijmegen (n=194)</th>
<th>Münster (n=154)</th>
<th>p value Nijmegen versus Münster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fathers</td>
<td>12/348 (3.4)</td>
<td>9/194 (4.6)</td>
<td>3/154 (1.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mothers</td>
<td>13/348 (3.7)</td>
<td>10/194 (5.2)</td>
<td>3/154 (1.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Parents</td>
<td>25/348 (7.2)</td>
<td>19/194 (9.8)</td>
<td>6/154 (3.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Brothers</td>
<td>16/462 (3.5)</td>
<td>10/289 (3.5)</td>
<td>6/173 (3.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sisters</td>
<td>11/438 (2.5)</td>
<td>5/236 (2.1)</td>
<td>6/202 (3.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Siblings</td>
<td>27/900 (3.0)</td>
<td>15/525 (2.9)</td>
<td>12/375 (3.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sons</td>
<td>2/178 (1.1)</td>
<td>1/85 (1.2)</td>
<td>1/93 (1.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Daughters</td>
<td>7/179 (3.9)</td>
<td>5/95 (5.3)</td>
<td>2/84 (2.4)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Children</td>
<td>9/357 (2.5)</td>
<td>6/180 (3.3)</td>
<td>3/177 (1.7)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Only 7 of the 25 affected parents (28%) had a history of a severe type of AA. We assume that this is because the risk of developing extensive hair loss is in the same range. This means that approximately 2% (i.e. 0.28 × 6.0 = 2%) of all children will eventually develop severe AA.

Neither the number of affected first degree relatives nor their type of AA was influenced by the degree of hair loss in the patients. Immunological and environmental factors may be more important for the clinical course. In the general population the risk for AA is 0.05-0.1% (10). Although there were no mildly affected patients included in the present study, our findings suggest that the genetic risk of developing severe type of AA, even for the offspring of individuals with mild hair loss, is 2%.

The mean age at onset of patients with a positive family history did not differ from those without a familial clustering. This is in contrast with psoriasis vulgaris, where an early onset type showing a high incidence of familial occurrence can be distinguished from a late onset type with predominantly sporadic psoriasis (24). The absence of a separate early and a late onset type in AA may be taken as a further indication that the role of genetic factors is less important in this disease than in psoriasis.

The total number of patients with one or more affected first degree relatives was 56 (16%). This is in agreement with other studies in which the frequency of affected first degree relatives was 10% (2), 12% (4), and 13% (1) but at variance with one report in which the incidence was 6% (3).

Especially those patients who suffer from a severe type of AA are often afraid that their children will develop the same disease. We conclude that, regardless of the type of AA present in a parent, the estimated risk of children for the development of a severe type of AA is approximately 2%.

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![Fig 1. Age at onset of AA in patients with affected first degree relatives (dark bars), and age at onset of AA in the subgroup without familial occurrence (light bars). Both subgroups show a similar distribution.](stock)
A New Case of Zimmermann-Laband Syndrome with Atypical Retinitis Pigmentosa

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Departments of 1Dermatology and 2Pediatric, University of the Saarland, Homburg/Saar, Germany

This paper reports a case study of a 10-year-old girl exhibiting symptoms of a Zimmermann-Laband syndrome (ZLS), including an ocular involvement not previously observed. In addition to the case reported, we have also discovered 21 patients described in the literature. Major clinical findings, defined as being present in more than 75% of the cases under discussion, are presented. Key words: Gingival fibromatosis syndromes; Nail dysplasia; Phalangeal dysplasia.

(Accepted March 23, 1992.)


P. Koch, Hautklinik der Universität des Saarlandes, D-6650 Homburg/Saar, Germany.

The Zimmermann-Laband syndrome (ZLS) is a rare genetic disorder, characterized by gingival fibromatosis with an association of soft tissue, phalangeal and nail anomalies. Two isolated cases were first described by Zimmermann in 1928 (1). The syndrome was later, in 1964, more fully delineated by Laband in a case of a mother and five of her seven children (2). Since that time a total of 22 cases, including the one under present discussion, have been reported in conjunction with other disorders including hypertrichosis, vertebral anomalies and mental retardation (3-9).

This paper sets out to present an additional case of that genetic entity, drawing attention to an ocular disorder not previously reported.

CASE REPORT

The patient, a white female, was born on 9th March 1981, after a full-term uncomplicated pregnancy. The patient's weight at birth was 4,050 kg. No neonatal problems were observed. She is the first born child of healthy, genetically unrelated parents. The parents, grandparents on both sides of the family and the subsequently born 5-year-old brother were or are all free of gingival hypertrophy, dysmorphic syndromes or skeletal malformations. Their family history does however reveal that two maternal cousins of the patient died several days after birth, having exhibited a dysmorphic syndrome which remained unexplored.

The mother of the patient was able to inform us that several members of the family, taken over three generations, had suffered significant loss of hearing. There is no evidence of consanguinity in the family.

Gingival hypertrophy appeared at the time of the breakthrough of the milk teeth. Teething was delayed as a result of the hypertrophy, which worsened over the following years. Diphenhydramon or cyclopromine have never been administered to parents or daughter.

At the age of 4 years, reduced visual acuity was noted for the first time and the impairment has worsened since the patient reached the age of 8 in 1989. This had led in turn to problems with accomplishing school work. No problems with social adaptation have been noted, and the patient is not mentally impaired.

During the clinical examination at the age of 8 years and 9 months a height of 144.5 cm and a weight of 30 kg were registered. The circumference of the head measured 53.8 cm. None of these measurements deviate from the norm. The patient's nose was bulbous with a soft cartilage consistency. The lips were thick and an intense overgrowth of gingival tissue presented the patient's mouth from closing. This affected the whole of both gingiva without extending onto the hard palate (Figs. 1-2). The points of her teeth were visible through the hypertrophied gingiva.

Radiological examination showed that the patient had a full complement of teeth, with persistence of the molar and canine milk-teeth. The finger nails of all digits except the index fingers were small and dystrophic (Fig. 3). Nails on the hallucale resembled spikes set in a shallow pit. When the feet were examined, the toenails of the second and third digit were found to be very small and friable.

Radiological examination of the left hand exposed a hypoplasia of the distal phalanx of the little finger. The terminal phalanges of both the thumb and the index finger were divided; there was no epiphysis. The terminal phalanges of the third finger seemed also to be primarily bipartite but with secondary fusion of the two bone parts (Fig. 4). Bone age was compatible with chronological age. X-rays of the feet were not available.

Neurological examination of the patient revealed no pathological symptoms. Liver and spleen were not palpable. There was no pes cavus. No joint hypermobility could be detected. The skin was dry, soft and velvety. Head hair and body hair were clinically normal. A trichogram of frontal and occipital hairs was normal.

Results of routine laboratory studies including complete blood cell count, serum aminotransferases, alkaline phosphatases and total bilirubin were consistently normal. Radiological evaluations of the vertebral skeleton, skull and chest proved to be normal. An audiometric examination revealed no hearing loss. The karyotype was 46, XX.

Ophthalmological investigations were carried out to investigate the rapid deterioration of vision. On examination, visual acuity was 6/40 and intraocular pressure was 12 mm Hg for both eyes. The fundus examination showed localized peripapillary yellow patches; an optical disc and a macular region that appeared normal. The atypical retinitis pigmentosa was confirmed by performing electro-oculography and electro-retinography. Nuclear magnetic resonance and computed tomography of the brain were normal.

In August 1990 a gingivectomy was performed by the oral surgery department of the University of Münster. All redundant tissue which covered the teeth and interfered with mastication and normal speech was removed. Histological examination was consistent with a diagnosis of gingival fibromatosis with an increase in the number of collagen fibres. As of June 1991 no relapse has been observed.

DISCUSSION

The first report of two affected children in Zimmermann's review article on abnormalities of ectodermal structures is almost certainly not the first observation of that genetic entity. Earlier examples of the syndrome, as indeed suggested by Zimmermann himself and Witkop (10), are very probable.

* Presented in part as a poster at the Journées Dermatologiques de Paris which took place between 6th March and 9th March 1991.
This author related a publication of Hopson (11) who discusses the occurrence of gingival fibromatosis in the case of a father and two children, stating that it was “peculiar that they did not show clubbing defects of the fingers in conjunction to large soft noses so frequently seen in association with gingival fibromatosis”.

Humphry (12) describes a girl with unilateral hypertrophy of the gums, nail defects and apparently without terminal phalanx of the right thumb and the second digit of the left foot, a combination of symptoms suggesting the ZLS.

Among the cases discussed by Laband (2) is a mother of seven children, five of whom were affected. Alavandar (4) investigates an affected mother with three affected sons and an 8-month-old affected grandson. The syndrome was apparently transmitted as an autosomal dominant trait. The other observed patients, including two brothers and a spontaneous case reported in Russia by Il'ina et al. (9) are possibly the result of a new germ cell mutation.

A constant feature of the ZLS is gingival fibromatosis (1, 2, 7, 8), or in cases not histologically examined, gingival hypertrophy (1-6, 9), appearing in early childhood, associated with enlargement of the soft tissue of the nose and ears, absence or dysplasia of the nails and absence, hypoplasia or dysplasia of the terminal phalangeal bones (Table 1).

The clinical findings observed in less than half of all reported cases demonstrate the morphological variability of the syndrome (Table II).

The association between the four major symptoms is not constant but is nevertheless found in 14 of the 22 cases. All of the published cases manifest at least three of the anomalies justifying the diagnosis of the syndrome.

The constant presence of gingival hypertrophy differentiates the ZLS from the tricho-rhino-phalangeal syndromes, associated with a bulbous nose, hair rarification, teeth dysplasia and mental retardation.

Twelve distinct and frequently inherited groups of disorders include gingival fibromatosis as a constant manifestation com-

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Fig. 3. A view of the left hand shows the small and dystrophic fingernails of the digits.

Fig. 4. Radiography of the left hand. (See text for description).
### Table I. Major clinical findings in 22 patients with Zimmermann-Laband syndrome

<table>
<thead>
<tr>
<th>Finding</th>
<th>No of cases and source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival fibromatosis or gingival hypertrophy</td>
<td>21,+ 1-5</td>
</tr>
<tr>
<td>Dysplasia, hypoplasia or absence of the terminal phalanx</td>
<td>20,+ 1-5</td>
</tr>
<tr>
<td>- Hands</td>
<td>12,? 1-3-9</td>
</tr>
<tr>
<td>- Feet</td>
<td>18, 1-3-5-9</td>
</tr>
<tr>
<td>Enlargement of soft tissues of the face</td>
<td>17, 1-3-5-9</td>
</tr>
<tr>
<td>- Bulbous soft nose</td>
<td>4, 1-5-6-8</td>
</tr>
<tr>
<td>- Thick ears</td>
<td>15, 1-5-9</td>
</tr>
</tbody>
</table>

Major clinical findings are defined as those symptoms identified in more than 75% of all reported cases (n = 22). + represents findings in the case reported in this article.

### Table II. Associated clinical findings in 22 patients with Zimmermann-Laband syndrome

<table>
<thead>
<tr>
<th>Finding</th>
<th>No of cases and source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous</td>
<td>3, 1.6-7</td>
</tr>
<tr>
<td>- Skin soft and velvety</td>
<td>2, 7.9</td>
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<tr>
<td>- Hypertrophic of the face</td>
<td>1, 5</td>
</tr>
<tr>
<td>Skeletal</td>
<td>11, 2, 4, 6, 7</td>
</tr>
<tr>
<td>- Hypertrophically of joints</td>
<td>7</td>
</tr>
<tr>
<td>- Vertebral abnormalities</td>
<td>3, 2-5-9</td>
</tr>
<tr>
<td>- Kyphosis</td>
<td>2, 6-9</td>
</tr>
<tr>
<td>- Scoliosis</td>
<td>2, 1.3</td>
</tr>
<tr>
<td>- Spina bifida</td>
<td>6, 2-4-5</td>
</tr>
<tr>
<td>- Pes cavus</td>
<td>3, 2-5-6</td>
</tr>
<tr>
<td>- Hallux valgus</td>
<td>1, 5</td>
</tr>
<tr>
<td>- Genu valgum, cubitus valgus</td>
<td>1-6</td>
</tr>
<tr>
<td>- Clubbed fingers</td>
<td>1, 5</td>
</tr>
<tr>
<td>- Flexion contractures (hips and knees)</td>
<td>1, 7</td>
</tr>
<tr>
<td>- Asymmetry of the lower limbs</td>
<td>1, 7</td>
</tr>
<tr>
<td>Cranio-Facial</td>
<td>4, 1-6-8</td>
</tr>
<tr>
<td>- Large tongue</td>
<td>2, 5-6</td>
</tr>
<tr>
<td>- Tongue furrowed</td>
<td>2, 5-9</td>
</tr>
<tr>
<td>- High-arched palate</td>
<td>2, 9</td>
</tr>
<tr>
<td>- Macrocephaly</td>
<td>1, 7</td>
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<tr>
<td>- Partial anodontia</td>
<td>1, 1</td>
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<tr>
<td>- Prognathia</td>
<td>1, 1</td>
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<tr>
<td>Hepatomegaly</td>
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<td>Spleenomegaly</td>
<td>6, 2-4-7</td>
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<tr>
<td>Mental retardation</td>
<td>3, 1-6-9, 15-7</td>
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<tr>
<td>- Profound</td>
<td>2, 5-7</td>
</tr>
<tr>
<td>Neurological anomalies</td>
<td>1, 6</td>
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<tr>
<td>- Epilepsy</td>
<td>1, 9</td>
</tr>
<tr>
<td>Recurrent viral hypertrophy of the breasts</td>
<td>1, 8</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>+</td>
</tr>
</tbody>
</table>

+ represents findings in the case described in this article.

### Table III. Gingival fibromatosis syndromes

- Isolated generalized gingival fibromatosis affecting all the gingivae.
- Isolated symmetrical gingival fibromatosis (Runhton).
- Gingival fibromatosis with hypertrophy, oligophrenia and epilepsy.
- Gingival fibromatosis with macrocephaly, brachydactyly and genital dysplasia.
- Gingival fibromatosis-phacomatose syndrome.
- Gingival fibromatosis with progressive deafness.
- Cowden syndrome.
- Cross-McKusick-Breen syndrome.
- Murray syndrome.
- Rambou syndrome.
- Rutherford syndrome.
- Zimmermann-Laband syndrome.

ponent with an overlapping of the clinical features between them, but no association with the major clinical findings of the ZLS (Table III). These genetic entities were reviewed in part by Witkop (10). The familial gingival fibromatosis associated with progressive deafness was described later by Jones et al. (13) and Hartsfield et al. (14).

The family history of our patient reveals several individuals with hearing loss but without gingival hypertrophy, who have appeared over three generations, but a parallelism to the observed ZLS can at best be inferred.

The retinitis pigmentosa to our knowledge not previously described, may represent another unusual clinical feature of the syndrome.

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The Relation between Lichen Planus and Hepatitis C: A Case Report

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A case of simultaneous occurrence of lichen planus (LP) and hepatitis C in the same patient is presented. The patient had received treatment with interferon alpha for her chronic liver disease, and the association between LP, hepatitis C and interferon alpha treatment is discussed.

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An association between lichen planus (LP) and chronic active hepatitis (1), as well as between LP and primary biliary cirrhosis, has been described (2), although one study was not able to confirm an association between oral LP and chronic active hepatitis (3). The association between LP and liver disease was sustained in a recent multi-centre study including 577 patients with LP (4). Recently, the simultaneous occurrence of LP and hepatitis C in one patient was reported (5). An additional case is reported here.

CASE REPORT

A 53-year-old woman with chronic active hepatitis since 1965 was referred to our outpatient to our dermatological clinic because of a persisting itch. She had a history of deep venous thrombosis of the legs and lung emboli, and had received antithrombotic treatment, and she also had a history of upper gastrointestinal bleeding. In 1990, she was recognized as anti-HCV-positive and she was treated with interferon alpha from June 1990 to June 1991. No significant changes in liver enzymes were found in relation to the interferon alpha therapy, or in relation to the eruption of LP. Skin symptoms commenced in May 1991 after 10-11 months of treatment with interferon, Brown-vio
taceous papules were symmetrically located on her trunk and extremities, notably on her hands and feet. No mucosal involvement was found. The diagnosis of LP was confirmed histopathologically from a skin biopsy. The results of laboratory studies in July 1991 were as follows: HBs antigen negative, anti-HBs negative, anti-HCV positive (test method Elisa 100 and RIBA 4, Ortho), aspartate aminotransferase (ASAT) 134 U/l, IgG 137 mg/dl, IgM 1.1 mg/dl, Iga 9.4 mg/dl. Liver biopsy specimen from August 1991 showed chronic aggressive viral hepatitis with active cirrhosis.

Because of intensive itching, therapy for LP was needed. Local application of potent steroids did not improve the skin symptoms. PUVA treatment was then attempted under close control of the ASAT values. After 5 weeks of PUVA treatment (3 times weekly) the skin lesions and itch cleared, while ASAT values remained unchanged.

DISCUSSION

To our knowledge, this is the second reported case of LP and hepatitis C occurring in the same patient. A possible relation between these two diseases would partly explain the increased risk of LP in patients with a history of liver disease (4) and would also lead to new considerations about the etiology of LP. It has been reported that treatment with D- penicillamine in patients with primary biliary cirrhosis may lead to development of LP (6). In our patient, an association between the treatment with interferon alpha and the appearance of the skin lesions is a possibility. Recently, a case of LP induced by interferon alpha in a patient with IgG myeloma was presented (7). In that case the skin lesions appeared after 6 weeks of treatment with interferon alpha. Our patient had received treatment with interferon alpha for 11 months before she developed LP. Moreover, LP lesions were not localized to or accentuated at the sites of injection. Our present findings should, however, be seen in the perspective of the prevalence of LP in interferon-treated patients with hepatitis C. In a recent study, 22 patients with hepatitis C were followed for 36 weeks. Fifteen of these received treatment with interferon alpha (8). No case of LP was reported. In another study, 126 patients with hepatitis C, 91 of which received interferon alpha treatment, were followed for 24 weeks, and no case of LP was reported here either (9). More reports and studies are necessary to come to a conclusion about an association between LP, hepatitis C and interferon alpha treatment.

REFERENCES

Dupuytren’s Contracture (Palmar Fibromatosis) Extending over the Arm

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A rare case of Dupuytren’s contracture, with associated fibrosis extending over the arm, is reported.

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CASE REPORT

A 65-year-old man was referred to the Dermatology Department with a 1-month history of fibrosis of the right palmar skin. He suffered concurrently from insidious progression of hardening of the anterior surface of the right arm. He had not experienced any trauma; nor was there any notable past history except for pulmonary tuberculosis. His family history was negative for similar lesions. At his first visit to the Dermatology Clinic, he presented with several indurated subcutaneous nodules measuring not more than 1 cm in diameter and partially forming cords on the ulnar side of the right palm. This was accompanied by flexion contracture of the right ring finger and little finger. The right arm also exhibited flexion deformity and restricted movement at the right elbow joint (Fig. 1). The indurated plaque covered almost the entire anterior surface of the same arm. The biopsy specimens from the lesions of the palm and the proximal portion of the forearm were stained with hematoxylin-eosin (HE), Masson trichrome and Verhoeff-van Gieson. The palmar section stained with HE showed subcutaneous nodular thickening of the fascia (Fig. 2). This tissue was more fibrotic than cellular. Elongated and compressed cells were separated by thick, wavy bundles of collagen. The findings for the HE-specimen of the thickened fascia from the forearm lesion were consistent with those for the palmar lesion, while fibrous thickening of the fascia was also evident. With Masson trichrome, both the tissues stained blue throughout. With Verhoeff-van Gieson’s stain, nodules from both locations stained predominantly red-purple with black sparsely scattered throughout. These findings implied that proliferation of elastic fibers was minimal in the fibrous tissue. Laboratory data were unremarkable, including those for serum LDH and CPK activities, serum anti-nuclear antibody, C3, and antinuclear DNA antibody levels. The case was assumed to have developed from Dupuytren’s contracture and to have manifested over the palm and arm. The patient did not wish to be operated on.

DISCUSSION

Dupuytren’s contracture, also known as palmar fibromatosis, is a fibromatous hyperplasia of the palmar fascia, associated with dimpling of the skin covering the fascia, and usually forms cords extending axially to each of the flexed fingers (1). The condition may provoke insidious progression of flexion contracture at one or more digits. The typical hand deformity was named after Baron G. Dupuytren and shows an incidence of around 2% among the general population (2), while the age of onset is generally between 30 and 50 years (3). The condition occurs more commonly in patients with alcoholic cirrhosis, epilepsy and diabetes mellitus (4, 5). However, the basic cause is obscure. Histopathologically, the palmar fascia, especially on the ulnar side, is thickened with a combination of fibrous tissue and focal nodules (6, 7). The nodules are composed of a dense collection of fibroblasts in various states of maturation and form a heavy reticulum network. As the fibroblasts mature, rather thick acellular collagen bundles develop, although the fibrosis does not involve tendon sheaths. Furthermore, no new formation of elastic fibers takes place. Treatment often consists of partial fasciectomy, although the more nodular lesions may respond to intralesional corticosteroid injections in their early stage (8, 9).

In the present case, the clinical and histopathological findings of the right hand corresponded to the features of Dupuytren’s contracture. The ipsilateral arm clinically manifested hardening of the anterior surface, and the histopathological findings for its fascia were highly compatible with those for Dupuytren’s contracture.

In some patients with this disease, there is an association with plantar fibrosis, usually present over the medial side of the soles, while one or more nodules may become painful (5, 10). Finally, a similar fibroblasting condition involving the dorsum of the penis, known as Peyronie’s disease, is occasionally associated with fibrosis of the palmar fascia (1). In a series of 159 cases with Dupuytren’s contracture, Hueston (11) reported plantar fibromatosis in 12%, knuckle pads in 42%, and bilateral palmar fibromatosis in 89%. In a separate series of 22 patients with Dupuytren’s contracture, he found that 27% had Peyronie’s disease. However, associated fibrosis at other sites has so far been, to the author’s knowledge, quite unknown in the literature.

It is therefore assumed that this represents the first case of Dupuytren’s contracture associated with fibrosis of fascia extending over the arm, or the disease developing over not only the hand but also the arm.

REFERENCES


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Adjuvant Treatment of Recalcitrant Genital Warts with Systemic Recombinant Interferon-alpha-2c

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Departments of 1Dermatology and Venereology and 2Surgery II University of Innsbruck, Austria

Seventeen male patients with recalcitrant genital warts, who had been unsuccessfully treated with classical destructive modalities for 16 months on average, were included in an open uncontrolled trial. The treatment regimen consisted of caustic and/or surgical measures as judged optimally suited in the individual cases, combined with an intermittent systemic low-dose adjuvant interferon-alpha-2c regimen (3 or 6 5-day-courses with intervals of 2 weeks) followed by a 1-year-observation period. At the end of interferon treatment, no patient had clinically visible warts but 10 still had subclinical acetic acid positive lesions. At the end of the 1-year-observation period, clearance of both warts and acetowhite lesions was observed in 4 patients (23.5%), whereas acetowhite lesions persisted in 4 others (23.5%). Recurrence of clinically visible lesions, always within the acetowhite areas, was observed in 9 (53%) patients. Interferon may thus have been effective in suppressing clinical recurrences of genital warts, but its potency to eradicate subclinical papillomavirus infection was disappointing. Key words: Human papillomavirus; Immunomodulation; Acetowhiteness.

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R. M. Höpf, Department of Dermatology and Venereology, University of Innsbruck, Austria.

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Table 1. Combined destructive/systemic interferon-alpha-2c treatment of recalcitrant genital warts

<table>
<thead>
<tr>
<th>Patient age (years)</th>
<th>Site</th>
<th>Duration (months)</th>
<th>Destruct. prior</th>
<th>Treatment present</th>
<th>INF cycles</th>
<th>Response at end of interferon</th>
<th>Status after one year observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Perineal</td>
<td>8</td>
<td>P</td>
<td>P</td>
<td>3</td>
<td>Total</td>
<td>Clearance</td>
</tr>
<tr>
<td>27</td>
<td>Anal penile shaft</td>
<td>11</td>
<td>P</td>
<td>P (3x)</td>
<td>3</td>
<td>Total</td>
<td>Clearance</td>
</tr>
<tr>
<td>42</td>
<td>Perianal</td>
<td>12</td>
<td>P</td>
<td>Surg.(1x)</td>
<td>3</td>
<td>Total</td>
<td>Clearance</td>
</tr>
<tr>
<td>24</td>
<td>Anal penile shaft</td>
<td>13</td>
<td>P</td>
<td>Surg.(2x) P</td>
<td>6</td>
<td>Partial</td>
<td>Subclinical Persistence</td>
</tr>
<tr>
<td>42</td>
<td>Anal penile shaft</td>
<td>12</td>
<td>P</td>
<td>Surg.(1x) P</td>
<td>6</td>
<td>Partial</td>
<td>Recurrence</td>
</tr>
<tr>
<td>22</td>
<td>Penile shaft</td>
<td>24</td>
<td>P</td>
<td>P</td>
<td>6</td>
<td>Partial</td>
<td>Recurrence</td>
</tr>
<tr>
<td>21</td>
<td>Penile shaft</td>
<td>13</td>
<td>P</td>
<td>Surg.(2x)</td>
<td>6</td>
<td>Partial</td>
<td>Subclinical Persistence</td>
</tr>
<tr>
<td>42</td>
<td>Urethral prepuce</td>
<td>10</td>
<td>P TCA</td>
<td>P</td>
<td>6</td>
<td>Partial</td>
<td>Total</td>
</tr>
<tr>
<td>33</td>
<td>Anal</td>
<td>22</td>
<td>P TCA</td>
<td>P</td>
<td>6</td>
<td>Partial</td>
<td>Subclinical Persistence</td>
</tr>
<tr>
<td>29</td>
<td>Prepuce</td>
<td>24</td>
<td>P TCA</td>
<td>P</td>
<td>6</td>
<td>Partial</td>
<td>Total</td>
</tr>
<tr>
<td>44</td>
<td>Prepuce</td>
<td>72</td>
<td>P TCA</td>
<td>P</td>
<td>6</td>
<td>Subclinical persistence</td>
<td>Recurrence</td>
</tr>
<tr>
<td>23</td>
<td>Urethral</td>
<td>10</td>
<td>P</td>
<td>Surg.(2x)</td>
<td>3</td>
<td>Total</td>
<td>Recurrence</td>
</tr>
<tr>
<td>26</td>
<td>Urethral</td>
<td>10</td>
<td>P</td>
<td>Surg.(1x)</td>
<td>3</td>
<td>Total</td>
<td>Subclinical persistence</td>
</tr>
<tr>
<td>25</td>
<td>Prepuce penile shaft</td>
<td>6</td>
<td>P</td>
<td>P TCA</td>
<td>6</td>
<td>Partial</td>
<td>Subclinical persistence</td>
</tr>
<tr>
<td>26</td>
<td>Anal penile shaft</td>
<td>9</td>
<td>P</td>
<td>Surg.(2x)</td>
<td>6</td>
<td>Partial</td>
<td>Subclinical persistence</td>
</tr>
<tr>
<td>25</td>
<td>Anal penile shaft</td>
<td>9</td>
<td>P</td>
<td>Surg.(2x)</td>
<td>3</td>
<td>Total</td>
<td>Recurrence (after 8 months)</td>
</tr>
</tbody>
</table>

INF = interferon
TCA = trichloracetic acid
P = podophyllin
surg. = surgery
destruct. = destructive
### Table II. Summary of the results of combined destructive/systemic interferon-alpha-2c treatment

<table>
<thead>
<tr>
<th>No. of patients treated</th>
<th>Total response at end of combination therapy</th>
<th>Partial response at end of combination therapy</th>
<th>Clearance</th>
<th>Subclinical persistence</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17</td>
<td>10 (58.8%)</td>
<td>4 (23.5%)</td>
<td>4 (23.5%)</td>
<td>9 (53%)</td>
</tr>
</tbody>
</table>

Objective measurement of disease extent and determination of its subclinical persistence after clinically successful treatment. Hobenleutner et al. (10), e.g., did not routinely use the acetic acid method to detect subclinical disease which consequently remained untreated, leading to a high recurrence rate (81%) after laser/placebo, and a lesser recurrence rate (42%) after laser/interferon-alpha-2b. Erpenbach et al. (9), in contrast, who performed acetic acid testing, registered relapses in only 19%, using a laser/interferon-alpha-2b combination therapy.

Obviously, persistent subclinical infection is the major factor leading to recurrences after destructive treatment of condylomata acuminata (CA). Since it has been claimed that interferon is capable of eradicating latent human papillomavirus (HPV) infection (12), we decided to combine optimal individual destructive treatment (except surgery) aimed at eliminating clinical lesions with adjuvant interferon aimed at prevention of relapses, in a series of cases who had previously proven extremely recalcitrant to classical treatment alone.

### PATIENTS AND METHODS

Between September 1988 and June 1989, 18 male patients with exophytic genital wart (4 with urethral, 7 with anal involvement) were recruited into the present cross-over study (Table I). They were all in good general health, over 18 years of age, and had been unsuccessfully treated with destructive methods methods in individual regimens for at least 6 months (median 16 months). Comparable classical therapy before and subsequently with interferon was our main strategy to draw conclusions about interferon treatment efficacy. The definitive resistance to therapy in our hands in the last 6 months was the criterion for additional interferon administration. Exclusion criteria were: positive HIV serology, signs of immunodeficiency and contraindications for surgical procedures or interferon. Laboratory tests performed prior to therapy included complete blood count with differential and platelets, serum electrolytes and iron, liver and renal function tests, electrocardiograms and quantitative immunoglobulins. Blood count and liver function tests were monitored at the end of each interferon cycle. Cell-mediated immunity was evaluated with the Menerex® "multitest" (skin test with several recall antigens). All patients were screened for comitant sexually transmitted infections including syphilis and HIV infection. Patients were advised to refrain from sexual intercourse or use condoms during therapy. Their partners were evaluated for HPV infection and treated if infected.

At the beginning of combination treatment, clinically visible CA were removed by such classical regimens judged to be best suited for the individual case. This included podophyllin alone in 4 patients (soft evptive CA), podophyllin plus trichloroacetic acid in 2 (hyperkeratotic CA), podophyllin plus electrosurgery in 8 (extensive exophytic CA or CA at specific locations, like intrarurethral or intraanal), and surgical treatment alone in 3 cases (Table I). Acetic acid testing (5% 5 minutes) was performed at the initial treatment and all follow-up examinations (see below). All acetowhite lesions were treated with podophyllin or trichloroacetic acid.

Recombinant interferon-alfa-2c (Berefort®, Berlex) was given according to the following low-dose interval regimen: 1.4 × 10^6 IU/d were administered subcutaneously into the upper thigh for 5 days, followed by an interval of 2 weeks. If necessary, podophyllin was continued at weekly intervals. Three such cycles of interferon were given in all cases; 10 patients who had persisting lesions thereafter received 3 additional cycles amounting to a total of 30 doses (42 × 10^6 IU) interferon at the end of therapy.

Follow-up examinations were performed at weekly intervals throughout interferon treatment, including acetic acid testing and, if necessary, topical treatment of CA and/or acetowhite lesions. Thereafter, patients were followed up in at least 3 monthly intervals for 1 year. 2/4 acetowhite lesions, which were finally judged as subclinical persistence, were examined histologically, both proved to be caused by HPV by the presence of kolyocyes. The remaining 2 were clearly positive, considering the following clinical criteria: the presence of landscape-shaped acetowhite areas with slightly papillomatous surface in contrast to smooth and glossy surfaces seen in scars, the most likely differential diagnosis for acetowhite lesions in our patients.

Absence of both CA and subclinical acetowhite lesions after the last interferon cycle was classified as total response, and their sustained absence throughout the 1-year observation period as clearance. Absence of clinical lesions but presence of acetowhite lesions at the end of interferon treatment was classified as partial response; presence or reappearance of such lesions during the observation period as subclinical persistence; reappearance of CA as recurrence.

### RESULTS

17/18 patients completed the combined local destructive/interferon treatment and the 1-year-follow-up period (Table I and II). One patient discontinued the treatment for unrelated reasons.

Defining the patients as their own control group during the 6 months before interferon with classical modalities alone, a 100% recurrence rate can be assumed and compared with the final success rate. None of the 17 patients had clinically discernible viral warts at the end of the last course of interferon. Only 7/17 (41.2%), all having received 3 interferon cycles only, were also acetic acid negative (total responses). The remaining 10 (58.5%), all having received 6 interferon cycles, were still acetic acid positive (partial responses). Judgement at this time might be influenced by the classical treatment modality used in addition to interferon. During the observation period, relapses of clinically visible lesions occurred in 9 patients (53%)-2 from the complete response and 7 from the partial response group. It is interesting to note that, in the latter group, clinically visible recurrences always arose within acetowhite lesions. If the term relapse is restricted to reappearance of condylomata in those who have achieved complete response, then the recurrence rate was only 2/7 (29%) or 3/7 (43%), if one considers the additional patient discovered to be HPV positive by acetowhiteing. Recurrences occurred within 3 months after the last course of interferon in 8 patients.
within 8 months in 1. At the end of the observation period, only 4 patients (23.5%) qualified as clearance. In 4 others (23.5%), acetowhite lesions had remained stable and resistant to podophyllin throughout the observation period (subclinical persistence).

Interferon side effects implying bio-availability were common, but mild or moderate. Fifteen patients had flulike symptoms at the beginning of therapy which declined after the first week. A reversible mild decrease in white blood cell count was noted in most patients. No local reactions were observed at the injection sites. One patient developed frontal sinuinitis during interferon therapy, which responded promptly to appropriate antibiotics.

DISCUSSION
Prior to entering the present study, all of our patients had been unsuccessfully treated by destructive methods for 16 months on average. Since the response rate to interferon is supposed to be inversely related to the duration of clinical HPV infection (2), and men are thought to respond more poorly to interferon than women (6), their chances of being successfully treated with interferon alone was judged as equally minimal as the mere construction of treatment with destructive methods. Ethical considerations thus precluded the initiation of a controlled study, comparing classical destructive methods, interferon monotherapy and a combination of both. Instead, we decided to initiate a cross-over study combining those topical destructive measures which were felt to be best suited for the individual cases, with a constant interferon adjuvant regimen. Intralosalional interferon was not feasible in our patients because of disease extent, and gel preparations with an exactly defined content of pharmacologically effective interferon were not available in Austria at this time. We thus chose a systemic intermittent low-dose interferon regimen modified from that of Gross et al. (3) (interferon-alpha-2c instead of interferon-alpha-2a, shorter intervals between treatment cycles). The rationale of this regimen is that both high doses and prolonged application of interferon may result in depressed activity of natural killer cells (13), which are expected to play a major role in controlling HPV infection (14).

After a 12-month observation period, the recurrence rate was 53% in our patient series and thus higher than that reported in trials using destructive measures alone (1). 47% of the patients were clinically symptom-free, but one half still had evidence of subclinically persistent HPV infection by the acetic acid technique, resulting in a total clearing rate of only 23.5%. In 3/4 of these patients surgery was the concomitant classical method, which probably contributed to the outcome. Like several other recent reports (4, 5, 15), we were thus unable to achieve as favorable results as Gross et al. (3) with interferon-alpha-2a monotherapy. In this pair-matched cross-over trial, comparing 1.5 × 10^4 IU versus 18 × 10^4 IU interferon-alpha-2a subcutaneously, complete remissions were observed in 8 of 14 subjects (57%) (3). The low-dose regimen with minimal toxicity was favored, since its efficacy appeared superior to that of high doses. In a later study these authors used the low-dose regimen successfully in 11 of 19 patients (57%) and even demonstrated the absence of HPV-DNA from the sites of previous genital warts in 9 of 11 patients with complete remission by Southern blot hybridization (12). In contrast, Steinberg et al., using nonrecombinant alpha interferon 2 × 10^6 IU to 6 × 10^6 IU 3 times weekly for a minimum of 6 months, describe the failure of interferon therapy to eliminate latent virus (16).

Obviously, our disappointing results are consequently to the inclusion criterion of long standing, particularly therapy-resistant genital warts; conversely, they suggest that interferon-alpha-2c, which discloses minimal functional differences to other interferon-alpha 2 subvariants in vitro (17), at least in the regimen chosen by us, offers only moderate benefit in those circumstances where it would be most needed. In particular, elimination of subclinical HPV infection was not a prominent feature in our patient series, although it may be speculated that the recurrence rate in our patients would have been substantially higher without adjuvant interferon. Present data (8-10, 18) suggest that consequent destruction of subclinical lesions, preferably with laser surgery, is at least as efficient in the prevention of recurrences as interferon treatment.

Obviously, adjuvant interferon does not provide the final answer in the treatment of recalcitrant genital warts. New strategies need to be developed, e.g. combinations of interferon with retinoids (15), immunomodulation (19-21) or vaccination (22).

REFERENCES
The Route of Rapid Access of Drugs to the Distal Nail Plate

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University Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

It has recently been shown that antifungal drugs have unexpectedly rapid access to distal nail, which we have suggested occurs through the site of continuous ventral nail formation along the nail bed. To exclude the alternative possibility of diffusion through the nail plate, we have measured the effect of topical terbinafine cream in onychomycosis. Sustained outward movement of the fungi affected distal segment was seen in only 2 of 10 measured nails, and there was no change in the mean severity of clinical involvement in 53 nails under study. This excludes significant diffusion of drugs through the nail plate, and we conclude that the route of rapid access is indeed through the nail bed.

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By measuring the outward movement of diseased nail after a short oral course of the antifungal terbinafine, we were able to show that the onset of the therapeutic effect on distal nail is rapid (1, 2). This demonstrated that drugs have rapid access to the distal nail and not, as previously believed (3), only by the slow outward movement of drug fixed during formation of nail in the germinal matrix. Others have since confirmed that terbinafine (4) anditraconazole (5) can be detected in distal nail clippings within a few weeks of starting oral therapy. We suggested that the route of rapid distal access was by the nail bed, recently shown to form ventral nail continuously along its length right up to the point of nail detachment (6, 7). However, we could not exclude the less likely possibility that drugs reached the distal nail by diffusion along the length of the plate from the zone of initial formation at the germinal matrix, or from surrounding skin. For this to occur, diffusion of terbinafine through the nail must be very rapid, and consequently we would expect ready penetration through nail to allow a therapeutic effect as easily from topical application of the drug as from systemic administration. We have now tested this possibility.

PATIENTS AND METHODS

We studied 10 patients with clinically and microbiologically proven persistent distal subungual onychomycosis unresponsive to treatment with griseofulvin, because it was unlikely that the infection would regress spontaneously and it is well established that such resistant disease will nevertheless respond to terbinafine (8, 9). Nine had had griseofulvin within the previous 3 months and all had current positive fungal cultures from distal clippings. Only toenails were involved, and a total of 53 nails were affected in the 10 patients. Each patient was given 1% terbinafine cream, from which terbinafine is known to partition satisfactorily with the keratinocyte layer (10), to apply daily to the outer surface of each affected nail for 12 weeks. Four of the patients in whom onycholysis was marked were asked in addition to apply the cream to the space underneath the nail. Assessments were made at the start, and after 2, 6, 12 and 24 weeks. In 4 of the patients further assessments were made for 12-24 weeks to exclude a continued response. For convenience of measurement, a single nail was chosen for study in each patient. At each visit the unaffected length of the study nail, between the nail fold and the most proximal clinically abnormal segment, was measured by the previously described transfer method (2). All nails were examined for clinical infection, and clinically graded on a 0-3 scale for each of onycholysis, hyperkeratosis and overall severity of involvement. Distal clippings for mycology were taken from the study nail.

RESULTS

Of the 10 patients, 8 showed no net outward movement of the affected segment of the study nail over the study period (Fig. 1). In 2 patients the study nails, both great toenails, showed continued outward growth and became normal by 48 weeks. The contralateral great toenails showed similar clinical improvement, but neither became completely normal. In all, of the original 53 affected nails only 3, all mildly affected, had become clinically normal at 24 weeks, and 2 more were now affected. The mean severity score of all affected nails was 4.6 ± s.d. 1.9 before treatment and 4.5 ± 1.6 at 24 weeks. There was no difference in the response of nails to which the terbinafine cream had been applied under the nails. The pathogen isolated from the study nail before treatment was Trichophyton rubrum in 9 patients and T. interdigitale in 1. Seven of the 10 study nails became culture negative by the end of treatment period of 12 weeks, although hyphae were still seen at microscopy in 4 of these, and 1 subsequently reverted to positive culture. The 2 study nails in which there was sustained reversal of fungal invasion first became culture negative at 2 and 36 weeks, respectively.

DISCUSSION

In 8 out of 10 patients we found no clinical improvement or outward movement of nail affected by fungal disease in response to 1% terbinafine applied topically to the outside of the nail plate and additinally to the underside in 4; there was sustained improvement in only 2.

All of the patients had clinically typical disease and the diagnosis was confirmed mycologically. After treatment fewer samples grew fungus, although filaments were still seen in some; these changes may simply reflect the continued presence of topically applied drug on the distal nail sample. Clinical improvement of study nails occurred in only 2 patients (one of whom applied the drug under the nail as well as to its surface), which suggests that diffusion of the drug through the nail from these sites is poor. Outward movement of the affected segment is a more sensitive and earlier indicator of therapeutic effect (2), but even with this method, we likewise failed to detect a response to 12 weeks' topical treatment in 8...
Fig. 1. Unaffected nail length in the nail under study in each of the 10 patients. Terbinafine was applied for 12 weeks (hatched box). Nails not showing possible improvement were not followed after 24 weeks; two great toenails which did show a sustained response are represented by solid lines.

of 10 patients. This finding contrasts with the results of a 2-week oral course of terbinafine, which produced a response in most patients, which was apparent as early as 4–6 weeks (2). Thus, despite this and other evidence that the drug can diffuse through nail (4) and that terbinafine cream partitions effectively with stratum corneum (10), the application of terbinafine cream onto the nail does not achieve therapeutic concentrations of the drug. It is possible that the outer surface of the nail is a greater barrier to diffusion than the rest of the nail, but in subjects who applied the cream under the edge of onycholytic nail, there was no clear added benefit. It follows that when terbinafine is given orally as a short course and rapidly reaches the site of distal nail disease, it cannot be doing so by rapid diffusion through the length of the nail plate from the site of its formation in the matrix, nor from the skin surface at the nail margin. We therefore conclude that the route of rapid passage of drug to the distal nail is indeed as we have suggested (1, 2) from the nail bed, which contributes continuously to nail formation along its length (6, 7). Use of this route of access could allow the development of drugs for onychomycosis which require few, if not single, doses.

ACKNOWLEDGEMENT
We are grateful to Sandoz Pharmaceuticals Ltd for their support.

REFERENCES
LETTERS TO THE EDITOR

Chronic Aquagenic Urticaria

Sir,

Aquagenic urticaria is a rare form of chronic physical urticaria (1), consisting of the occurrence of wheals in the skin in contact with water. Reaction occurs regardless of the temperature of water. We report the case of one patient with chronic physical aquagenic urticaria and discuss the pathogenetic mechanisms involved.

A non-atopic 55-year-old man, having suffered from gout for 5 years, referred to us a 1-year history of itching episodes, erythema and wheal formation every time he was exposed to water regardless of its temperature. Wheezing, difficulty swallowing and subjective respiratory distress also occurred with drinking water. Forced expiratory volume (VEMS) was not measured. Urticarial reaction appeared immediately in the zone of skin in contact with water and tended to generalize involving one limb entirely. Urticarial reaction persisted for minutes even 1 hour after interruption of contact with water. Water used for toilette, sea water, swimming-pool water and even rain triggered wheal formation. His own saliva on the skin provoked the occurrence of wheals. The patient presented moderately positive dermographism. Laboratory tests revealed serum uric acid, 8.80 mg/dl; serum IgA, 670 mg/dl (normal range, 72–232), and serum IgE 137 UI/ml (normal range, 14–120). Skin biopsy taken immediately after wheal induction showed massive degranulation of dermal mast cells.

With the preliminary diagnosis of chronic physical contact urticaria induced by water, a series of examinations were conducted in order to confirm the diagnosis and ruling out other types of physical urticaria. Challenge test consisted of immersion of forearm in water and application of packs soaked in distilled water at room temperature on the forearm. Both tests were strongly positive.

Intradermic injection of methacholine was negative. Application of a glass tube full of water at 2°C induced mild erythema when the tube began to sweat. Application of a glass tube full of water at 40°C induced mild erythema by heat. With the diagnosis of chronic physical contact urticaria induced by water we observed the ability of different organic solvents to reproduce urticarial reaction and the ability of different substances to prevent water contact urticaria (2).

Effective organic solvents, acetone and ethanol, induced wheal formation after 5 min in contact with dry skin areas. Wheals developed earlier if water was added to both solvents. With the purpose of antagonizing the presumed cholinergic component (1–3), scopolamine at 9% was applied during 10 min, on dry skin; although “test for sweating” was not done. Scopolamine did not prevent wheal formation either in petrolatum or in aqueous excipient. The topical application of petrolatum, olive oil, two antihistamines (Atarax® and Hismanal Syrup®, a chemical protective glove (Guante Blanco®) composed by stearic acid, glycerin, propylene glycol, sorbitol and silicone, a water substitute (Cethaphil®) composed by stearyl alcohol, cetetyl alcohol, propylparaben, butylyparaben, sodium lauryl sulphate, methylparaben and finally, clobetasol propionate did not impede (or prevent) urticarial reaction after contact with water. Only intradermal injection of triamcinolone acetonide (40 mg; 1 cm³) prevented the development of wheals after contact with water.

The initial treatment was the combination of prednisone and hydroxyzine hydrochloride. The patient is presently receiving 25 mg daily of hydroxyzine. He is comfortable with routine activities such as washing hands, drinking water or walking in the rain.

Sibbald et al. (3) studied two patients with urticaria localized in those zones of skin contacting with water. In those patients, local release of acetylcholine was shown, suggesting an essential step in the pharmacogenesis of wheals in aquagenic urticaria. Sibbald et al. based their statement in the observation of the suppressor effect of local application of scopolamine in water-induced wheals. We failed to confirm this fact in our patient. Methacholine test was also negative. Application of scopolamine at 9% in petrolatum did not prevent the development of wheals after contact with water.

How the water, on the surface of the skin, initiates wheal formation and itch is unknown (1). The follicular localization of wheals would suggest that some toxic substances, formed by the action of water on sebum or sebaceous glands, through the pilosebaceous unit, would exert an effect of local mast cell degranulation. No urticaria occurred in zones with few and small sebaceous glands.

Active organic solvents (acetone) per se did not trigger wheals. Nor did they prevent wheal formation by water exposure (2). The patient we studied developed wheals after the skin contact with different solvents like acetone or ethanol and lesions appeared earlier when water was added to the solvent. According to the hypothesis of Czarnetzki et al. (3), prolonged application of water on the skin would cause solubilization of an antigen. Acetone is a better solvent than water and could enhance urticaria whereas alcohol, benzene and gasoline would be inefficacious. An unknown substance would penetrate into the epidermis and contact with skin mast cells, which could carry the unknown antigenic substance with specific IgE on its surface. The fact that our patient developed wheals when contacting with acetone and ethanol supports this hypothesis.

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Psoriasis: A Disease of a Thousand Key Words

Sir,

As chronic inflammatory disease, psoriasis involves many different cell types and numerous phenomena can be observed in its course. To mention only the most important: a) psoriatic keratinocytes produce many cytokines (1), b) increased number of mast cells (MC) in the lesions, the MC degranulate and release tryptase and chymotrypsin, prostaglandins, leukotriene B4, histamine, tumor necrosis factor (TNF) (2), etc., c) enhanced activities of neutral proteinases, including plasminogen activator (3), neutrophil cathepsin G and elastase, the latter being bound to the basement membrane zone of psoriatic lesions (4), d) specific clones of helper T cells might start autoimmune response in psoriatic epidermis to still hypothetical autoantigen (5), e) excessive acute regulation of psoriatic keratinocyte proliferation by transforming growth factor alpha and interleukin-6 (6, 7), f) expression of adhesion molecules: intercellular adhesion molecule (ICAM-1) both on lesions keratinocytes and dermal endothelial cells (EC), and endothelial-leukocyte adhesion molecule-1 (ELAM-1) on the endothelium (8), g) EC of high endothelial venule morphology express molecules responsible for promotion of helper T cell migration (9), h) altered function of Langerhans cells in psoriatic epidermis (10).

The limited journal space given for publication of experimental data forces authors to shorten the discussion to the most sound arguments supporting the explanation of the pathological phenomenon under study. The fact that neuropeptides is used as a key word in our paper (11) concentrates the attention of the reader to the field of neuropeptidomodulating effects in psoriasis, only partly explored. As to the distribution of nerve fibers in psoriatic skin (12), they are uneven, and subsequent sections should be studied to collect a sufficient number of fibers for statistical analysis. Immunocytochemical techniques to visualize nerve fibers due to the presence of specific neuropeptides such as substance P (SP) or calcitonin gene-related peptide (CGRP) are more precise. The generation of nerve growth factors in the psoriatic skin should also be taken into account when the elongation and the higher density of nerve fibers are considered.

From the point of view of immunologists, the study of various mediators in psoriatic skin, such as neuropeptides, should include identification of mediator-producing cell, quantitation of mediator, and expression of its specific receptors (number, distribution, changes in affinity, etc). All these factors determine the final effect of mediator on psoriatic skin, i.e. SP. An increased amount of SP in psoriatic skin occurs possibly in nerve fibers but not in extracellular space (13), which after release might be responsible for the desensitization of specific receptors for SP on MC, etc.

I would like to comment on some recent publications on the effect of SP on the skin. An article by Matis et al. (14) describes that ELAM-1 is rapidly induced on postcapillary dermal venules as a direct consequence of experimentally elicited degranulation of adjacent MC. It seems likely that this reaction depends on SP-stimulated release of TNF from degranulating MC. The production of cytokines by keratinocytes in response to SP has also been suggested by Brown et al. (15).

Our recent data on decreased activity of specific skin elastase inhibitor (SCALP) (16) throw some new light on the concentration of various low molecular mediators in psoriatic skin. Reduced activity of SCALP is responsible for the increased activity of neutrophil elastase, which in turn accelerates degradation of polypeptides. Many low-molecular weight mediators and cytokines might be degraded more efficiently and their biological activity might last much shorter in psoriatic skin than in normal skin. This seems to explain at least in part why a higher dose of capsaicin is needed in psoriatics to induce SP-mediated neurogenic erythema (11). SP secreted from nerve fibers is presumably degraded more readily after its release, which reduces its effective concentration in the tissue. Thus, higher doses of capsaicin are needed to release more SP to produce the neurogenic inflammation of the same intensity. In addition, our preliminary studies (17) have shown that serum beta-endorphin in psoriatic patients is about two-fold elevated which may be responsible for the stimulation of opioid receptors on dermal nerves and antiinflammatory effect. It is likely that this in turn may change the effect of capsaicin on the SP release from nerve endings in psoriatic skin.

Summing up, the role of neuropeptides in the pathogenesis of psoriasis is no conclusive explanation. It might be a secondary phenomenon but an important factor modulating inflammatory response in patients. We thank Dr Olle Johansson for high evaluation of our paper (11).

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LETTER TO THE EDITOR

General Pustular Psoriasis: Pathogenetic Relationship between Pustule and Epidermal Sweat Duct

Sir,

We have with interest read the monography Psoriasis (1), in which Wright & Baker in their treatise on the histopathology of pustular psoriasis make the following statement referring to our paper in Acta Derm Venereol 1974 (2).

"It has been claimed that the initial events are at the acrosyringium causing sweat duct obstruction and pustule formation, but this has not been substantiated."

This statement is not correct and misrepresents our results. Discussing the role of the sweat duct, we concluded that: "the difference between pustular miliaria and the spongiform pustule is that the eliciting factor in the former has been shown to be the obstruction of the horny portion of the sweat duct, whereas no such changes have been observed in pustular psoriasis" (2).

It is remarkable that Wright & Baker have omitted other convincing studies on this and related subjects, concerning localization, which have fully confirmed our findings. Shelly et al. (3, 4) affirm in their study of pustular psoriasis "that it was possible by serial sections to find that the focal point for early pustular formation was indeed the epidermal sweat duct unit. The primary lesion of generalized pustular psoriasis has previously been shown to be similar to pustular miliaria but the precise localization has just been discerned by Neumann & Härd. In both pustular and regular psoriasis they found that the primary lesion arises at the acrosyringium. We can confirm that the leukocytes swarm to the terminal sweat duct."

Ogino et al. (5) have found a connection of pustules in 6 out of 7 cases of pustular erythema either with the hair follicle or with the epidermal sweat duct, thus extending the pathogenetic significance of adnexa and pointing to the toxic basis of these pustules.

These are our remarks to a statement, made by Wright & Baker, which we find unjustified and incomprehensible.

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Reply to the Letter by Härd & Neumann
We were interested to read the comments made by Härd & Neumann concerning our discussion of their work relating the formation of spongiform pustules and microabscesses in pustular psoriasis to events at the acrosyringium. We also thank

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them for drawing our attention to their findings in more detail, and for making it clear that they do not believe sweat duct obstruction to be the initiating factor. Nonetheless, their paper certainly does state that initiating events occur at the acrosyringium (1). Their abstract states: “The cells supporting the lining membrane of the sweat duct were found to be involved in the spongiform process. The view is expressed that this causes the sweat duct to collapse, the wedge-shaped or crescent-shaped pustules being thus formed.”

Of the two references by Shelley et al. quoted by Hård & Neumann, the second is a single case report of localized pustular psoriasis in which the pustules surrounded the terminal part of the intraepidermal sweat duct (2). Intraepidermal skin tests using streptococcal antigen produced a toxic pustular reaction in which the intraepidermal pustules were closely related to the sweat duct. A variety of other bacterial antigens produced no reaction. The other reference concerns 2 cases of generalized pustular psoriasis where the pustule bears no relation to the sweat duct (3). For example, the legend to Fig. 5 states “Note the sweat duct on the left side showing independence of pustule and sweat apparatus,” and the legend to Fig. 6 “but the sweat duct continuity could not be established.” We do not believe that these decidedly equivocal case reports comprise “convincing evidence.”

The other work which Hård & Neumann refer to is that of Ogino et al. (4). This was a study of 7 patients with a “generalised pustular toxic erythema” which the authors clearly distinguish from pustular psoriasis. These reports suggest that toxic pustular eruptions rather than pustular psoriasis may well develop in relation to the intraepidermal portion of the sweat duct.

While recent studies of the intraepidermal localization of neutrophil activating peptides (5) give further theoretical support to the concept that events at the acrosyringium may be the initiating factors in pustular psoriasis, we are still of the opinion that the case has yet to be substantiated.

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Stephen Wright and Harvey Baker. The Royal Free Hospital School of Medicine, London NW3 2QG, Great Britain, and The Royal London Trust Hospital, London E11 88, United Kingdom.
Dear Sir,

Recently Björggren & Holst reported an interesting case of reticulate hyperpigmentation of Iijima, Naito and Uyeno, affirming this disease to be previously described only from Japan (1,2). However, we think that other cases of pigmentary disorders corresponding to the form first described by Iijima have already been reported from European and American countries, although with different descriptive names.

In 1988 Kalter et al. (3) reported two cases of “Linear and whorled nevoid hypermelanosii” which, in terms of distribution, natural history, and histology resembled the cases of Iijima. In the same article, the authors, after a review of the literature, discovered other previously reported cases of similar hyperpigmentations of infancy, although associated with congenital anomalies (4,5).

Two cases of “Hyperpigmentation reticuée zosteriforme” were presented at the Journées Dermatologiques de Paris in the same year (6).

At the same time we reported, in a letter to the British Journal of Dermatology (7), a boy who showed a pigmentary disorder with clinical features common both to reticulate hyperpigmentation of Iijima and to progressive crèbriform and zosteriform hyperpigmentation (PCZH) described by Rower in 1978 (8).

The case of reticulate hyperpigmentation (Iijima, Naito and Uyeno type) observed by Björggren (1), occurring in a 15-year-old girl (age typical of PCZH) (8), confirms our point of view about the existence of borderline forms of the disorder described by Rower (PCZH) (8) and of that by Iijima and colleagues (2).

Finally we think that all these cases can be considered as a part of a unique pigmentary disorder and termed “Linear and whorled nevoid hypermelanosii” as proposed by Kalter et al. These authors have well delineated the main characteristics of these anomalies. In addition we think that, on the basis of clinical presentation, these cases can be classified according to forms: a) congenital or acquired; b) unilateral (limited to a hemisome, with sharp midline demarcation) or diffuse; c) isolated or accompanied by other developmental defects. Further case presentations could clarify the importance of these associations as well as those with eosinophilia and nevus spilus.

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In response to the letter by Di Lernia et al.

We read with interest the letter from Di Lernia, Patrizi, Neri and Varotti concerning our report on a case of reticulate hyperpigmentation. We agree with the authors in every respect.

This also means that we approve of the proposed way of classification using the term suggested by Kalter et al. (Linear and whorled nevoid hypermelanosii) and then adding the subgroups as defined by Di Lernia et al.

Their suggesting is logical and should turn out to provide a useful way of identifying and classifying these pigmentary disorders.

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