

IMMUNOFLUORESCENCE PATTERNS IN SUN-EXPOSED AND NOT-SUN-EXPOSED SKIN OF HEALTHY INDIVIDUALS

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Abstract. Immunofluorescence studies were performed on the sun-exposed (S) and not-sun-exposed (N) skin of 25 healthy volunteers regarding the incidence of the five Ig classes, albumin, fibrinogen, C1q, C4, C3B, C3c, C3d, C5, C3PA and properdin. In addition a comparative study of two so-called anti-complement antisera was made, one (C I) containing anti-(C4+C3a+C3d), the other (C II) anti-(C3B+C3c). In all biopsies the epidermal basement membrane (BM) was stained positively with anti-C3d in a fibrillar, interrupted linear, or granular pattern. A band-like picture of the BM was observed with anti-IgG, anti-albumin and anti-fibrinogen in three biopsies of the S skin. In two other S skin biopsies a lichenoid pattern was found with anti-fibrinogen, anti-C3d and IgM. The latter antiserum caused a weakly positive band-like picture of the BM, occasionally together with granular deposits, more often in the S skin (11) than in the N skin (4). In the capillary walls, granular deposits of IgM, C3d and occasionally C3c were seen in both the S skin (7) and the N skin (13). In the subjacent vessels, globular deposits positive with anti-C3d, C5 and C3c were found predominantly in the S skin. In the M. arrector pili, granular deposits of C3d and, less obviously, C5, properdin and IgM were frequently observed. A band-like, linear, or granular picture of the BM of the adnexal structures was caused by anti-C3d. IgG was found around the sweat gland in about 30%, but the other serum proteins were only rarely present. The IF patterns of the two so-called anti-complement sera showed marked differences, which were apparently caused by the presence of anti-C3d in the C I serum. The significance of these findings for the diagnostic and pathogenetic interpretation of IF results in pathology are outlined briefly.

Key words: Immunofluorescence; Normal skin; Basement membrane; Walls of vessels; Adnexal structures; Complement

Since the first results of immunofluorescence (IF) studies in dermatology were published (4), an overwhelming number of publications in this field have appeared. The application of this immunologi-

cal method appeared to be of great value for the approach to both diagnostic and pathogenetic problems of skin diseases. However, there is a surprising gap in our knowledge of the IF pattern of the skin of healthy individuals.

Hitherto, only one study has been performed which deals seriously with this problem (1). These investigators examined the skin of the extensor site of the forearm, i.e. the sun-exposed skin. Amongst other things, they found a 100% incidence of "complement" (C3/4 and C3d) along the epidermal basement membrane (BM), together with deposits of IgM in about 20%. In addition, granular and globular structures, positively stained with antisera directed against IgM, C3d and C5, were found in the walls of the papillary capillaries and dermal vessels. Subsequently, these results were disputed by a group of prominent authors (2), who, however, based their arguments on their experiences with the not-sun-exposed skin. More recently, Blenkinsopp et al. (3) reported the results of IF studies on normal skin adjacent to non-inflammatory lesions on the face and other parts of the body. They also found granular deposits of "complement", IgM and—in the face—of IgG and IgA along the BM, but with a lower incidence than Baart de la Faille-Kuyper et al.

It is evident that a knowledge of the IF pattern in the skin of healthy individuals is indispensable for a reliable assessment of IF findings in both diagnostic and pathogenetic investigations. Because of the conflicting results, as mentioned, we decided to investigate the sun-exposed (S) and not-sun-exposed (N) skin of 25 volunteers free from skin disorders. In addition, as "complement" was claimed to be present in all cases, a comparative study was

Table 1. Incidence of positive staining patterns in the superficial layers of the sun-exposed (S) and not-sun-exposed (N) skin of 25 healthy individuals

C I = anti-(C4+C3c+C3d); C II = anti-(C3B+C3c)

Antiserum	Epidermal BM		Papillar capillary walls		Subjacent vessel walls	
	S	N	S	N	S	N
IgG	3 ^a	—	4 ^d	—	12 ^d	—
Albumin	3 ^a	—	4 ^d	—	17 ^d	2 ^d
IgA	—	—	—	—	8 ^d	—
IgM	11 ^{a, b}	4 ^{a, b}	7 ^b	12 ^b	1 ^b	—
C3d/C I	25 ^{a, b, c}	25 ^{a, b, c}	7 ^{b, d}	13 ^{b, d}	7 ^{b, d}	2 ^{b, d}
C3c/C II	2 ^b	8 ^b	5 ^{b, d}	7 ^b	5 ^{b, d}	—
C5	—	—	1 ^b	1 ^b	8 ^b	1 ^b
Properdin	—	—	1 ^b	1 ^b	—	—
Fibrinogen	5 ^{a, c}	—	6 ^d	4 ^d	17 ^d	2 ^d

^a Band-like. ^b Granular. ^c Fibrillar. ^d Homogeneous.

performed on the staining patterns of two so-called anti-complement sera, one (C I) including anti-C3d, the other (C II) without.

MATERIALS AND METHODS

The group of healthy individuals consisted of 10 female and 15 male volunteers, varying in age from 23 to 55 years. They did not use medicaments and had been free from any kind of infection for at least one month. Punch biopsies were taken from the ethylchloride-frozen skin of the extensor of the forearm (S skin) and of the region just below the spina iliaca anterior superior (N skin). The biopsies were snap frozen and stored in liquid nitrogen. Cryostat sections (4 µm) were processed for IF.

The conjugates and antisera were obtained commercially from the Netherlands Red Cross Blood Transfusion Service (CLBD), Amsterdam, except for rabbit anti-human IgG-FITC (Miles), goat anti-human properdin (Flow Laboratories), rabbit anti-human C3-proactivator (C3PA) (Behringwerke AG, Marburg/Lahn) and rabbit-anti-“complement”, C II (DAKO).

The characteristics of the conjugates were: Goat anti-human IgG, F/p ratio 3.9, prec. titre 1:32 (61.041-S452, Miles); Sheep anti-human IgE, F/p ratio 2.9, prec. titre 1:32, prot. conc. 5 mg/ml (SH 25-02-F05); Horse anti-rabbit Ig, F/p ratio 3.5, prec. titre 1:32, prot. conc. 12.6 mg/ml (PK 17-2-F8); Rabbit anti-Goat Ig, F/p ratio 3.1, prec. titre 1:32, prot. conc. 8 mg/ml (KG 17-2-F1); Rabbit anti-human IgM, F/p ratio 2.3, prot. conc. 5.3, prec. titre 1:32 (KH 15-18-F01); Rabbit anti-complement (C3c+C3d), C I, F/p ratio 2.7–3.5, prec. titre 1:64, conj. conc. 7.4 mg/ml, dil. 1:100 (KH 47-12-F027); Rabbit anti-complement (C3B+C3c), C II, dil. 1:40 (F 1062-lot 010A, DAKO).

Unlabelled antisera, whose specificity was proven in precipitation and agglutination tests, used in the first step of the indirect technique, were: Rabbit sera directed against human IgA, dil. 1:20 (KH 14-115-A1); IgM, dil. 1:50 (KH 15-18-A02); IgD, dil. 1:25 (KH 20-07-A03);

Albumin, dil. 1:100 (KH 02-15-A01); Fibrinogen dil. 1:75 (KH 18-04-A02); complement (anti-C4+C3c+C3d), C I, dil. 1:50 (KH 13-113-A1); and complement (anti-C3B+C3c), C II, dil. 1:80 (10-062, Lot 109B DAKO).

Unlabelled antisera whose specificity was proven in precipitation tests only, used in the same technique, were: Rabbit sera directed against human C1q (KH 43-02-P02), C4 (KH 12-08-P02), C3B (KH 11-16-P01), C3c (KH 41-14-P01) and C5 (KH 44-03-P03), all diluted 1:50; rabbit serum directed against human C3PA, dil. 1:10 and 1:20 (6108 E 053, Behringwerke); and a goat anti-human properdin, dil. 1:40 (108001, Flow Laboratories).

Normal rabbit serum in the first step of the sandwich method was used as a negative control in the indirect method. The occurrence of IgM was investigated by both direct and indirect method. In addition, rabbit anti-human IgM adsorbed to human IgM was used as a specificity control in the case of positive results with unabsorbed rabbit anti-IgM (neutralization test). For the same purpose, sections were incubated with unlabelled, undiluted anti-IgM prior to the incubation with anti-IgM-FITC conjugate (blocking test).

In the comparative study of C I and C II the IF patterns obtained with these antisera were examined on 15 S and N biopsies from 15 of the 25 volunteers, using both direct and sandwich methods. In addition, blocking tests for the C I conjugate carried out by pre-incubation of the cryostat sections with undiluted, unlabelled C I, C II, anti-C3c and anti-C3d.

All sections were investigated according to a protocol in which the staining of the following structures was noted: stratum corneum, epidermal cytoplasm, epidermal intercellular substance, epidermal BM, the walls of the vessels in the stratum papillare, in the plexus subpapillare and in the deeper dermal layers, the BM, cytoplasm and intercellular substance of the glandular adnexal structures, the M. arrector pili, the elastic fibres, and the collagen connective tissue. The examination was carried out in part by two investigators who noted independently the results obtained. The sections were examined with a Zeiss fluorescence microscope, equipped with an incident light and

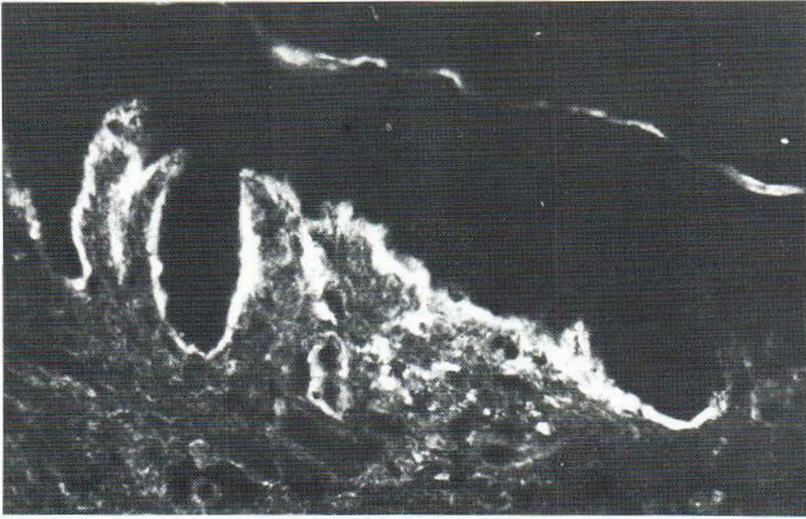


Fig. 1. S skin. Local deposition of fibrinogen in the BM region. Anti-fibrinogen. $\times 225$.



Fig. 2. S skin, same area as in Fig. 1. Globular structures and band-like pattern of the BM region. Anti-C3d. $\times 225$.



Fig. 3. S skin. Fibrillar-linear pattern of the BM. homogeneous staining of the capillary walls. Anti-C3d. $\times 360$.

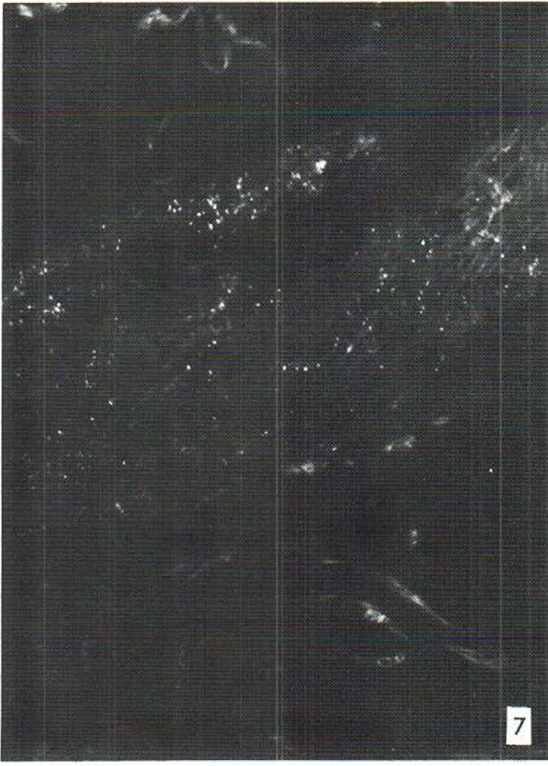
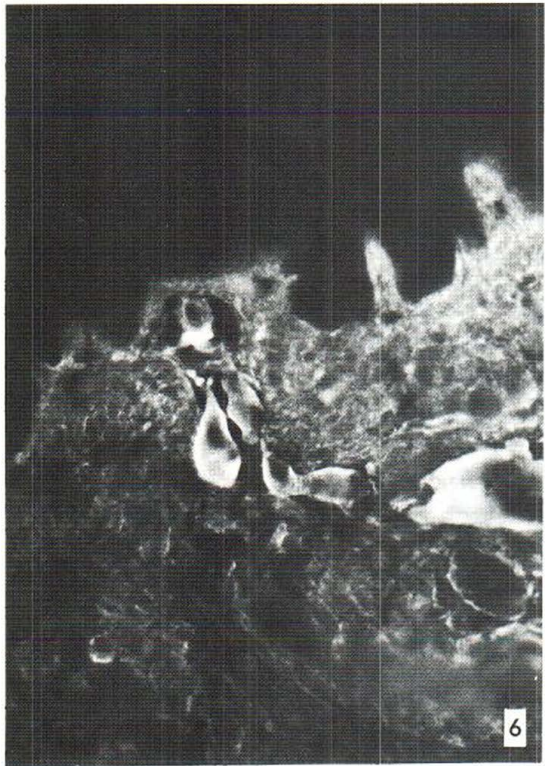
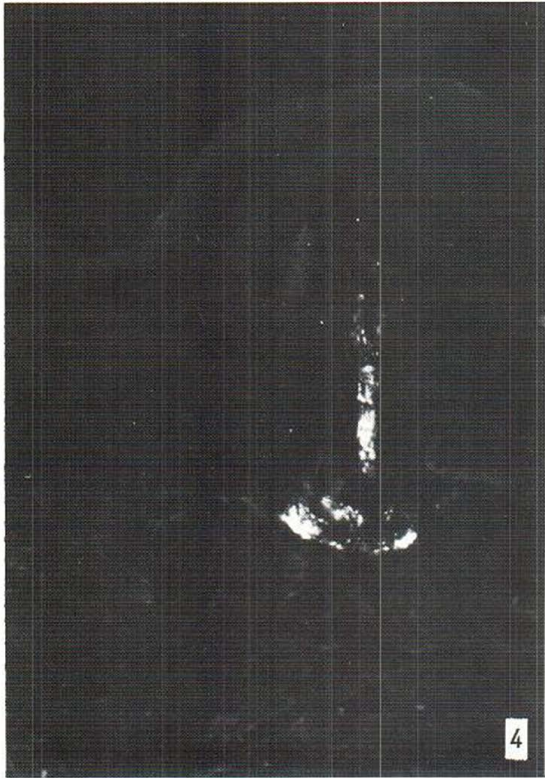


Table II. Incidence and staining properties of globules and particles along the BM in sun-exposed (S) and not-sun-exposed (N) skin of 25 healthy individuals

C I = anti-(C4+C3c+C3d); C II = anti-(C3b+C3c)

Strongly positive	IgM, C I, C3d, IgA
Positive	IgG, C Iq, C4, C3c, C II, C5, properdin
Negative	Fibrinogen, albumin, IgE, IgD, C3B, C3PA, NRS
Incidence	
S	15
N	4

narrow-band illumination system (light source: CS1-lamp 250 W; excitation filters: KP 500 and 600; reflector: 510; barrier filter: LP 528).

RESULTS

Epidermis

An overall staining of the cytoplasm and horny layer was found with the antisera directed against IgD, C4, C3B, C5 and properdin. A selective staining of the horny layer was observed with anti-C1q, anti-fibrinogen and less extensively with anti-IgM and anti-C3d. The character of this staining pattern, however, was less brilliant and less striated than found in parakeratosis. Occasionally a weakly positive intercellular picture, usually in the basal parts only, was seen with anti-IgG and anti-albumin simultaneously. It was more frequently found in the S skin than in the N skin.

BM-region, papillar capillaries and subpapillar vessel walls (Table I)

The results with the antisera not mentioned in the table were negative. The morphological pattern of the BM staining varied. The band-like, not sharply delineated picture in the S skin with anti-IgG, anti-

albumin and anti-fibrinogen usually did not include the BM region at full length but only a part of it. In two biopsies a lichenoid pattern was found with anti-fibrinogen, together with anti-C3d and anti-IgM (Figs. 1, 2). With the latter antiserum the band-like picture was rather weak, whereas granular deposits were seen in only a minority of the cases (S: 4, N: 1). The staining with anti-C3d was brilliantly positive in nearly all cases. Granular structures in or just beneath the BM level were observed in both the S and the N skin, but the fibrillar and even partly linear patterns dominated with this antiserum (Fig. 3). Care was taken to distinguish a positive band-like staining of the BM region from an enhanced staining of the basal cytoplasm of the stratum germinativum, occasionally seen with anti-IgA, anti-C1q, and anti-C3PA.

Another necessary distinction was that between granular deposits and small elastic globes (Table II). The latter are more irregularly shaped, coarser and more glittering. They can be visualized by an elastin staining method such as the van Gieson-Elastin staining. This was not the case with the granular structures found in the walls of the capillaries (Fig. 4) and vessels (Fig. 5). Moreover, the neutralization and blocking tests, carried out for IgM, resulted in disappearance (capillaries) and considerable loss of positivity (vessels) of these granules. Whereas IgM granules together with those of C3d and occasionally C3c, were found in the capillary walls of both the S and the N skin, they were mainly absent in the subjacent vessel.

By contrast, small globular and granular deposits, positively stained with anti-C3d, anti-C5, and sometimes C3c, were predominantly found in the subjacent vessel walls of the S skin. This prevalence of the S skin was also observed for the homogeneous staining patterns (Fig. 6). The vessel walls in the deeper layers of the dermis were usually negative. However, the walls of the small vessels surrounding the adnexal structures frequently contained globular deposits, positive with anti-C5 and anti-C3d. In addition, a strongly positive picture of the tunica elastica of some of the arterioles was occasionally observed with anti-C3d and anti-C1q. In about 40% of both the S and the N skin a weakly positive, granular or interrupted linear picture of the luminal lining of the endothelium was seen with several antisera but also with the normal rabbit serum. It was therefore considered to be a non-specific phenomenon.

Fig. 4. N skin. Granular deposits in wall of a papillar capillary and a subjacent vessel. Anti-IgM. $\times 225$.

Fig. 5. N skin. Granular deposits in wall of capillaries and vessels. Small globular structures along epidermal BM. Anti-C3c. $\times 225$.

Fig. 6. S skin. Homogeneous staining of walls of capillaries and vessels. Anti-albumin. $\times 225$.

Fig. 7. N skin. Granular structures within the M. arrector pili. Anti-C3d. $\times 225$.

Table III. Incidence of positive staining patterns of the adnexal structures in the sun-exposed (S) and not-sun-exposed (N) skin of 25 healthy individuals

Values in parentheses are the numbers of biopsies in which the structures were found. C I=anti-(C4+C3c+C3d); C II=anti-(C3B+C3c)

Antiserum	M. arrector pili		BM Hair follicle		BM Sebaceous gland		BM Sweat gland		BM Sweat ducts	
	S	N	S	N	S	N	S	N	S	N
IgG	– (20)	– (18)	– (11)	– (13)	2 ^c (8)	– (10)	7 ^c (18)	1 ^c (3)	5 ^c (18)	2 ^c (13)
Albumin	– (18)	– (19)	1 ^c (16)	3 ^c (12)	3 ^c (9)	2 ^c (7)	3 ^c (13)	2 ^c (5)	3 ^c (18)	1 ^c (11)
IgA	– (20)	– (21)	2 ^c (12)	1 ^a (13)	1 ^c (9)	1 ^c (11)	2 ^c (14)	1 ^c (5)	2 ^c (17)	1 ^c (15)
IgM	11 ^a (21)	9 ^a (17)	3 ^a ^c (11)	3 ^a ^c (18)	2 ^a (10)	3 ^a (9)	– (12)	1 ^a (7)	1 ^c (17)	– (14)
C3d/C I	22 ^a (24)	21 ^a (22)	13 ^a ^c (16)	15 ^c (16)	10 ^c (11)	11 ^c (11)	9 ^c (20)	8 ^c (11)	19 ^c (24)	18 ^c (22)
C3c/C II	– (19)	– (22)	2 ^c (12)	4 ^a ^c (10)	2 ^c (9)	3 ^a (10)	– (13)	1 ^c (7)	2 ^c (17)	2 ^a ^c (16)
C5	11 ^a (23)	11 ^a (25)	1 ^c (14)	– (11)	– (7)	– (7)	– (16)	– (6)	– (22)	1 ^c (17)
Properdin	6 ^a (20)	8 ^a (19)	1 ^c (16)	– (9)	2 ^c (6)	– (7)	– (15)	1 ^c (6)	– (18)	– (14)
Fibrinogen	18 ^b (21)	8 ^b (15)	2 ^c (13)	2 ^b (15)	2 ^c (9)	2 ^c (8)	2 ^c (12)	1 ^b (5)	1 ^c (17)	1 ^c (12)

^a Granular. ^b Fibrillar. ^c Band-like-linear.

Musculus arrector pili and adnexal structures (Table III)

Whereas the granules found within the M. arrector were delicate with anti-IgM, anti-C5 and properdin, they were somewhat coarser, more conspicuous and more brilliant with anti-C3d (Fig. 7). The fibrillar staining with anti-fibrinogen was preferentially localized around the fibrils at the edges of the muscular tissue.

The staining results of the pilosebaceous unit in both the S and the N skin indicate that C3d is nearly always present in the BM region (Fig. 8). However, the other serum proteins, including C1q and C4, were occasionally found in addition. In three biopsies of the S skin, granular deposits of IgM and C3d were observed within and between the peripherally layered follicular cells (Fig. 9).

A tendency to different staining patterns of the BM of the sweat gland and the sweat duct was observed with anti-C3d (Figs. 10, 11). In several biopsies a positive staining of the ductal BM was not accompanied by a positive picture of the sweat gland. The converse was observed with anti-IgG in some biopsies. Another outstanding feature was the brilliantly positive staining of coarse granular structures, presumably lipofuchsin granules, in the cytoplasm of the sweat gland with anti-IgM, anti-IgE and anti-C1q. With the latter antiserum the luminal cells of the sweat duct were positive in addition.

The collagen fibres of the dermis displayed a strongly positive picture with anti-IgG, anti-albu-

min and anti-fibrinogen, less positive also with anti-C1, CII and anti-IgA. This positive staining masked to some extent the autofluorescence of the elastic fibres. Beyond this autofluorescence a strongly positive elastic staining was caused by anti-IgM, anti-C1q and anti-C3d, especially in the superficial parts of the dermis.

The comparative study of C I and C II revealed that the staining with C I resulted in an IF pattern identical with that obtained with anti-C3d. Moreover, these positive reactions with C I conjugate were strongly inhibited by the blocking tests with undiluted C I and anti-C3d, whereas no such an inhibition was obtained after pre-incubation with undiluted C II and anti-C3c. The staining pattern of C II was similar to that of anti-C3c and it differed strongly therefore from the C I IF-pattern (Tables I and III). No difference was observed between the results of the direct method vs. the sandwich method.

DISCUSSION

The controversial opinions mentioned in the introduction, regarding the IF pattern in normal skin can probably be ascribed mainly to some confusion about the term "complement". It appeared in this study that two so-called anti-complement antisera could cause widely differing IF pictures and that this difference depended mainly on the presence of anti-C3d in one of these sera. The term "complement" should apparently be defined more exactly as

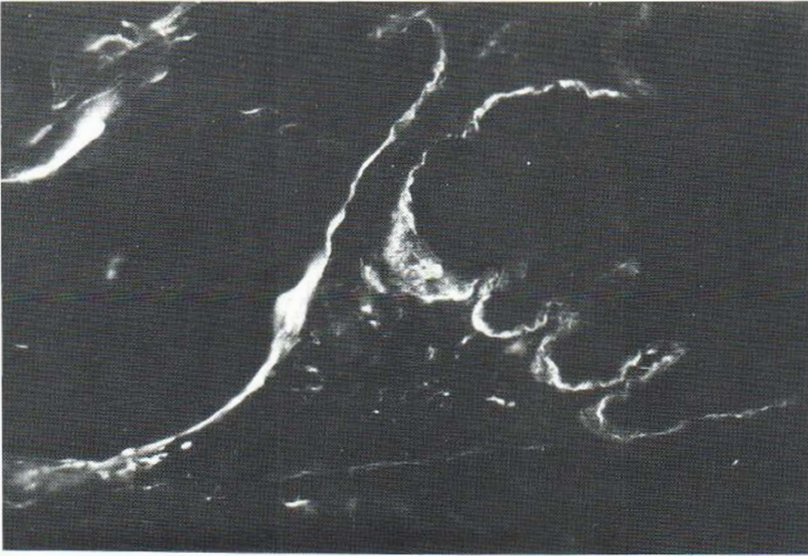


Fig. 8. S skin. Band-like-fibrillar pattern of BM of sebaceous gland. Anti-C3d. $\times 225$.

"C3c" on "C3d" in the reports on IF results, in order to prevent unnecessary confusion. Taking this into account, our results accord well with those of Baart de la Faille-Kuyper et al. (1). In addition, the S skin biopsies show more positive features in the superficial parts of the dermis than the N skin, with the exception of the results of the BM staining

with the antisera directed against C3d and the granular staining of the capillary walls with anti-IgM. No differences were observed between the S and the N skin in the staining patterns of the adnexal structures.

The morphological picture of the positive BM in the S skin with anti-IgG resembles to some extent

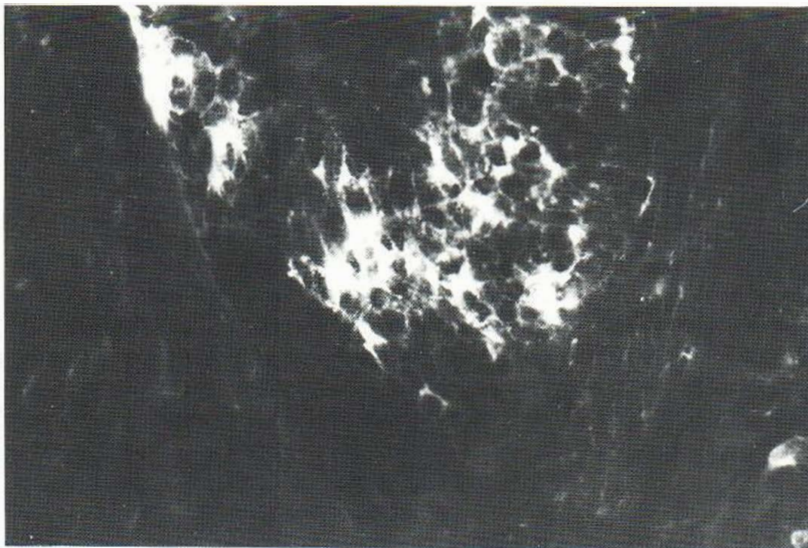


Fig. 9. N skin. Granular deposits within a follicle. Anti-C3d. $\times 225$.



Fig. 10. S skin. Sweat gland, BM negative; sweat duct, BM positive. Anti-C3d. $\times 225$.



Fig. 11. N skin. Partly granular, partly band-like picture of BM of sweat duct. Anti-C3c. $\times 225$.

the picture of the usually also sun-exposed skin lesions of chronic discoid lupus erythematosus (CDLE). Similarly, the positive features with anti-IgM and anti-C3d might lead to a 'false-positive' lupus band test in cases suspected of systemic LE. Therefore, it is of importance not only to use an antiserum directed against immunologically uninvolved serum proteins such as albumin, but also to investigate the possible presence of C-factors other than C3, such as C1q, C4 and properdin, which are usually found in lupus erythematosus (14). The same care must be taken with the diagnosis of sub-epidermal bullous lesions as for herpes gestationis (9, 10) and for a possible sub-form of bullous pemphigoid (11), in which a linear or band-like deposition of "C3" without Ig along the epidermal BM has been reported.

About the nature of the IgM/"C3" granules one can only speculate. The interpretation of their presence as being "the residual products of the activities of the daily immunological defence" (1),

does not sound unlikely. Attention has recently been drawn to the presence of IgM/"C3" granules in the walls of the papillar capillaries in the case of pityriasis lichenoides (6) and erythema exsudativum multiforme (5, 8, 16). It is evident from our results that a prudent judgement of this phenomenon is necessary (13) even if circulating immune complexes can be demonstrated.

The homogeneous staining of the walls of the vessels in the S skin may reflect an enhanced vaso-permeability due to sunlight. It should be taken into account in the assessment of IF results obtained with skin of porphyrias (7), in which, however, the thickening and staining of the walls is more conspicuous and, in addition, the capillaries are more prominently involved. Particularly in the S skin intramural globular granules were encountered which were positive with anti-C3c, anti-C3d and anti-C5 but usually negative with the anti-Ig antisera. The nature of these deposits is obscure. At any rate, they do not represent small elastic

intramural globuli, for such structures were not seen in parallel sections stained with the van Gieson-Elastin staining.

In the M. arrector pili, granular structures positive with anti-C3d, C5 and IgM were frequently seen. They differ from the granules seen in SLE, which usually contain other Ig, C1q and C4 too (own unpublished observations). Although one might theorize about the possible trapping or aggregating properties of the smooth muscular tissue, both in the vessel walls and in the M. arrector, a more than diagnostic value judgement of this phenomenon cannot easily be given.

The high incidence of C3d, sometimes accompanied by one or more Ig or other C-factors, along the BM of the pilosebaceous unit in normal skin, should be borne in mind when assessing the role of complement in disorders such as acne vulgaris (15). In fact, the binding of C3d at this site can hardly be related to any pathological disorder. The same holds true for similar positive features noticed along the BM of the sweat gland and sweat duct, for instance in cases of pemphigoid (12). The possible predilection of anti-IgG for the glandular BM versus that of anti-C3d for the ductal BM might reflect a difference in composition of these structures. In fact, a difference in antigenicity between them was observed by us, when comparing a bullous pemphigoid serum and an anti-glomerular BM antiserum (unpublished data).

The biological importance of the positive findings reported in this paper is unknown. The only thing which is fairly clear is that there are no signs of complement activation via the classical pathway in normal skin. But the extent to which the presence of C3c, C3d and C5 reflects an activation via the alternate pathway remains a matter of speculation, and so does its significance. Investigative methods more dynamic than the static IF method are required for the solution of these problems.

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