

AN IMMUNOHISTOCHEMICAL STUDY OF THE SKIN OF HEALTHY INDIVIDUALS

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Abstract. By means of the immunofluorescent (IF) technique, IgG, IgM, albumin, and C (especially the late acting-components) have been demonstrated in the epidermal basement zone (EBZ) and in the walls of superficial blood vessels in the skin of healthy individuals. It has been suggested that the homogeneously staining deposits of IgG and albumin may result from an enhanced passage of serum proteins through the endothelium of these blood vessels. The frequent occurrence of late-acting C components or their fragments, especially C3d, may indicate that these proteins play some role in physiological mechanisms. The IgM deposits may represent immunoglobulins bound to fixed C.

IF studies are in vogue in numerous areas of medicine, including dermatology. In the cutaneous tissues, various IF staining patterns have been recognized as diagnostic hallmarks of a number of diseases, e.g. lupus erythematosus, bullous pemphigoid, pemphigus, and dermatitis herpetiformis. Usually, the investigations aim at the localization of immunoglobulins and C components in diseased and uninvolved skin of patients only. Data concerning the presence of serum proteins in the skin of healthy individuals are as sparse as fragmentary. They are necessary, however, for an accurate interpretation of the findings in disease. The present study has been undertaken to obtain a more detailed knowledge of the immunohistochemistry of the skin of healthy individuals.

METHODS

Biopsy

Under local anaesthesia with aethylchloride, tissue specimens were obtained by punch biopsy from the extensor surface of the forearm of 12 female and 11 male healthy volunteers. The biopsies were snap-frozen in liquid nitrogen, and kept in cold storage (-85°C) until further processing. Cryostat sections were prepared at -24°C , and kept at -30°C until they were used for IF procedures (2, 11).

Immunofluorescent studies

The tissue sections were stained according to the indirect IF technique (2, 11) and examined with a Leitz Orthoplan microscope with epi-illumination and interchangeable dichroic mirrors, equipped with a Xenon arc (XBO, 75W) (12). We used position 3 of the vertical illuminator, and K515 as extra secondary filter. Micrographs were made on Kodak tri-X (27/10 Din.).

Antisera and conjugates

Rabbit antisera to human IgG, IgA, IgM, albumin, C1q, C4, C3b, C3c, C3d, C5, and a FITC-labelled horse anti-rabbit globulin serum were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. The methods of preparation, and the specificity of the antisera to the complement components and fragments have been described by Engelfriet and colleagues (4), and by Van Joost and collaborators (7). The characteristics of the other antisera and the conjugate have been described elsewhere by one of us (8).

RESULTS

Granular deposits of IgM, usually interrupted by negative areas, were found along the EBZ in 5 of the 23 biopsies examined. In the same cases, as well as in 10 others, conspicuous, finely granular staining was also observed in the walls of papillary capillaries (Fig. 1).

Homogeneously staining IgG deposition was encountered along the EBZ in 3 cases. The fluorescence was usually not sharply delineated at its cutaneous side. Albumin was concomitantly present in these, and in another 4 cases, in a pattern identical to the one of IgG. Homogeneously staining blood vessel walls (especially the sub-papillary plexus) denoting the presence of IgG and albumin (Fig. 2) were also observed in these 7 cases. In 2 other cases, only albumin was demonstrable in the vessel walls. A rather diffuse staining of IgG and albumin in the



Fig. 1. Granular deposits of IgM in the walls of superficial blood vessels (arrows). E=epidermis. Oil immersion, $\times 54$.

extra-vascular compartment was a rather common finding.

Deposits of IgA were not encountered in the cutaneous tissues.

In all 23 cases, C3d appeared to be present along the EBZ in a partly granular, partly linear pattern. The impression was gained that the linear fluorescence was localized along the rootlets of the basal epidermal cells. Trace amounts of C3b, C3c, and C5 were only found occasionally in the EBZ; C1q, and C4 were not detectable in this region.

Using the anti-C3d serum, we observed 3 different IF staining patterns of the blood vessels: The walls of capillaries stained homogeneously (Fig. 3) in 8 cases, and finely granular in 9, whereas globules of C3d were encountered (especially in the sub-papillary plexus) (Fig. 4) in 18 cases.

Twice, the vessel walls stained homogeneously for C4, whereas C4 was found in a granular pattern in 2 other cases. Globular staining of C3c, and C5 was

observed in the vessel walls 4, and 10 times, respectively.

The vascular IF patterns of C could be encountered in one biopsy, even in the same vascular structure.

C3c, C3d, and C5 were occasionally demonstrable in the dermal connective tissues, mostly along elastic fibres.

DISCUSSION

The results of the present study indicate that IgG, IgM, albumin, and C components or their fragments can occur extravascularly in the skin of healthy individuals. As tissue proteins and serum proteins are normally in a state of dynamic interchange it is not at all surprising that serum factors are present in the extravascular compartment. This seems to pertain especially to the homogeneously staining depositions of IgG and albumin, which may result from an enhanced passage of proteins through the vascular endothelium. It seems likely that the vessel wall thickening we observed in association with the deposi-

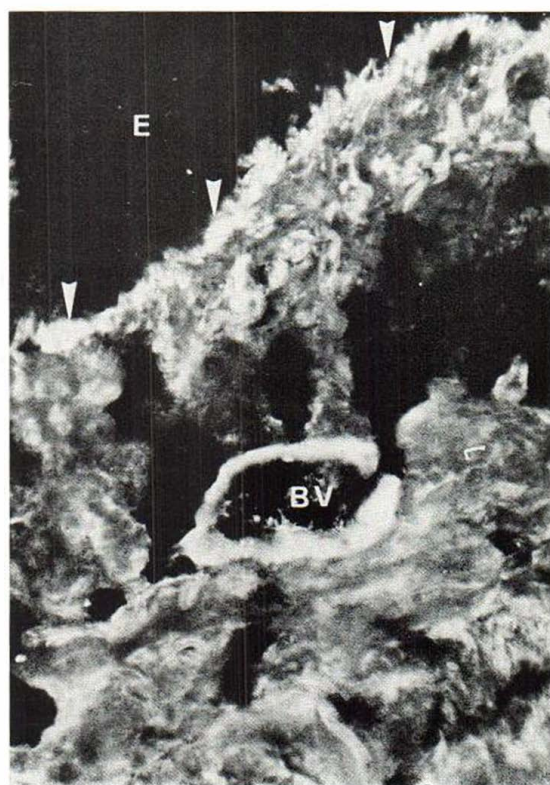


Fig. 2. Homogeneously staining deposition of IgG in the wall of a blood vessel (BV) and immediately below the epidermis (arrows). E = epidermis. Oil immersion, $\times 54$.

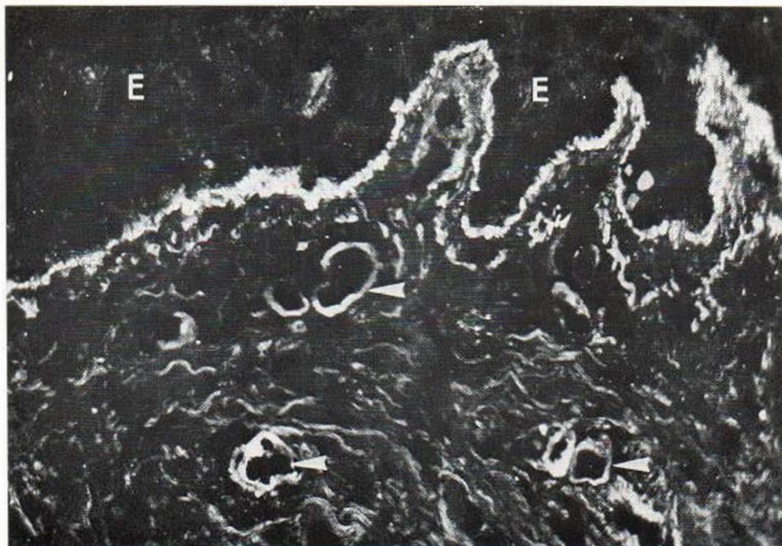


Fig. 3. Healthy skin displaying bright staining for C3d in the epidermal basement zone. Blood vessels show homogeneously staining walls (arrows). C3d is also deposited along elastic fibres. E=epidermis. Oil immersion, $\times 22$.

tion of IgG and albumin is at least partly the result of the subendothelial accumulation of blood-borne material, which is awaiting subsequent sieving through the basement membrane. An exaggerated form of this type of staining has been frequently found in lupus erythematosus and porphyrias (1, 2) in which alterations in vascular permeability are not unlikely.

We are in the dark about the origin of C components in the healthy skin. It is possible that at least some of these components are synthesized in the skin. Support for this possibility comes from the observations of Lai a Fat and co-workers (10), who

demonstrated synthesis of C3 by explants of healthy skin.

Another possibility is, of course, that the C components in the skin are derived from the circulation.

The occurrence in the EBZ of C3 fragments, in the absence of C1q and C4, may indicate that C3 has been activated at this site via pathways other than the classical one (5, 6). Further studies are needed to clarify the biochemical background of activation in the healthy skin.

Another enigma concerns the absence of the recognized consequences of C3 activation, which

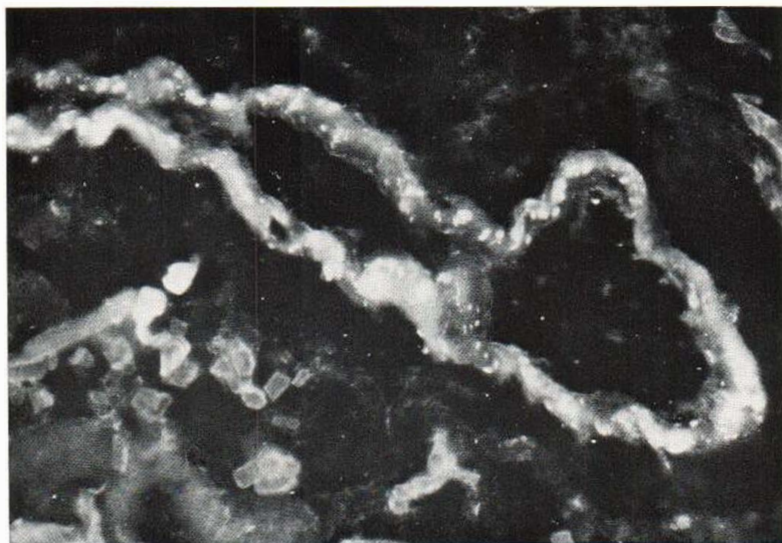


Fig. 4. Blood vessel of the sub-papillary plexus with globules of C3d in its wall. Elastic fibres also show some staining. Oil immersion, $\times 54$.

include anaphylatoxin production (C3a), and neutrophil chemotaxis (C3b).

This may imply that C3-cleavage in the healthy skin somehow does not result in the formation of biologically active fragments. Another plausible explanation would be that some control mechanism is involved which initiates the rapid inactivation of C3a and C3b.

The presence of granular deposits of IgM and C (especially those in the vessel walls) may be explained by assuming that these proteins form part of immune complexes. Such complexes may represent residual products of the activities of the "daily" immunological defence, which become secondarily entrapped in the walls of peripheral capillaries. It has been demonstrated that only complexes or proteins larger than 19S in size become deposited at such sites (3). The preferential occurrence of IgM could be explained along this line of thought.

The possibility is also admissible that the IgM represents immunoconglutinin bound to fixed C3, or C4 (9).

Another question which remains to be answered concerns the biological significance of the C components and fragments in the healthy state. Wolters (13) has demonstrated that C3d has permeability-increasing activity of short duration (about 10 minutes), when injected into guinea pig skin. Only the first fraction of C3d formed after C3b-cleavage appeared to have the permeability-increasing capacities. The author has suggested that C3, probably by enzymic activity, may directly or indirectly split tissue kinogens into kinins, which in turn would cause an acute increase of vascular permeability. On the basis of these findings, it is tempting to speculate that C activation in the healthy skin, leading to the generation of C3d, may play some role in permeability processes.

Our findings raise more questions than they can answer. We hope, however, that they will draw attention to the possibility that the C system, or parts thereof, may be operative in physiological mechanisms. They do at least serve as a warning against assuming that the concomitant occurrence of immunoglobulins and C in tissues is evidence *per se* of a pathological condition.

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