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CLASSIFICATION OF IMMUNOGLOBULINS IN THE DERMO-EPIDERMAL JUNCTION IN LUPUS ERYTHEMATOSUS

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SUMMARY

Immunofluorescence studies of skin lesions of discoid and systemic lupus erythematosus and also of the unaffected skin in systemic lupus erythematosus, revealed γ-globulin (IgG) apparently always present in the region of the dermo-epidermal junction; macroglobulin (IgM) and IgA were coincidentally bound to this region.

In all the sections examined, L-chains type κ and λ proteins could, with the aid of the 'double layer' method, be demonstrated in the dermo-epidermal junction.

The impression was gained that the immunoglobulins IgM and IgA are mainly located in the adjacent sites of the dermo-epidermal junction, whereas IgG appears to be localized on the junction itself.

The heterogeneity in respect to the occurrence of these classes of immunoglobulins is similar to that already known for antinuclear factors.

In previous papers (Cormane, 1964; Kalsbeek & Cormane, 1964) we reported on the occurrence of immunoglobulins and complement concomitantly bound to the region of the dermo-epidermal junction in skin lesions of discoid and systemic lupus erythematosus and in unaffected skin in the latter. These observations led to the present study, with the aid of the fluorescent antibody technique, of the classes of immunoglobulins involved.

Three major classes of immunoglobulins are distinguished: IgG, IgA and IgM. Recently, a new class of immunoglobulin has been detected (Rowe & Fahey, 1965), which is, however, not considered in this paper.

The differences between these immunoglobulins are determined by the structure of the

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H(heavy)-chains, which also confer immunological specificity. All classes of immunoglobulins have structural units in common: L(light)-chains. Based on the similarity to the corresponding types of Bence-Jones proteins, the L-chains are classified as belonging to either of two immunological types, κ or λ.

For this study antisera specific for each class of immunoglobulins, as well as antisera specific for each type of L-chain, were used to study the presence of these immunoglobulins in skin lesions from patients with discoid and with systemic lupus erythematosus and also in unaffected skin from patients with the disease in the systemic form.

MATERIALS AND METHODS

For this study, biopsy samples were taken from skin lesions of twelve patients with discoid and four with systemic lupus erythematosus. These patients had not been treated. Biopsies taken from unaffected skin were obtained from two patients with the systemic form of this disease. Control biopsies were obtained from five healthy individuals.

The methods of preparation of the sections have been previously described (Cormane, 1964).

From each biopsy, sections were exposed to unlabelled antisera I–IV (*vide infra*) or to fluorescein-labelled antisera V–X (*vide infra*).

The sections stained with the conjugates were directly mounted in FTA-mounting fluid (Difco) whereas the sections exposed to the unlabelled anti-human immunoglobulins were subsequently stained with fluorescein-labelled sheep anti-rabbit globulin XI.

The mounted sections were examined with the standard universal fluorescence and photomicroscope of Zeiss, using as 'exciting' filter UG I (2 mm) and as 'barrier' filters —65/41.

Antisera

The following antisera were used:

I. Rabbit anti-human IgM;
II. Rabbit anti-human IgA;
III. Rabbit antiserum specific for human κ-chain (type I L-chain);
IV. Rabbit antiserum specific for human λ-chain (type II L-chain).

The anti-IgM antiserum was obtained by immunizing rabbits with a purified pathological M-immunoglobulin from a patient with macroglobulinaemia of Waldenström.

The anti-IgA antiserum was obtained by immunizing rabbits with a purified pathological 10 S–IgA fraction from a patient with multiple myeloma.

The immunoglobulin fractions were isolated from the antiserum with Rivanol followed by precipitation with ammonium sulphate. The precipitates were dialysed against distilled water and freeze-dried. From the freeze-dried rabbit immunoglobulins a 2% solution in saline was prepared. These solutions were absorbed with human IgG to render these antisera specific for IgM and IgG respectively.

The anti-κ serum was prepared by immunizing rabbits with the S-fragment from a type I G-myeloma protein and was used as such.*

* Gift from Dr G. M. Bernier, Department of Biochemistry, University of Florida, Gainesville, Florida.

The anti-λ serum was prepared by immunizing rabbits with a purified type II Bence-Jones protein.

These anti L-chain antisera were specific for the corresponding type of L-chain as judged by immunodiffusion experiments.

Fluorescent antisera

The following antisera conjugated with fluorescein isothiocyanate were used:

V. Rabbit anti-human IgM;
VI. Rabbit anti-human IgA;
VII. Rabbit anti-human L-chain, type κ;
VIII. Rabbit anti-human L-chain, type λ;
IX. Rabbit anti-human IgG;*
X. Horse anti-human globulin;†
XI. Sheep anti-rabbit globulin.†

RESULTS

In all biopsies examined the exposure of the corresponding sections to fluorescein-labelled horse anti-human globulin (conjugate X) and to rabbit anti-human IgG (conjugate IX), resulted in staining in the region of the dermo-epidermal junction.

Inconstant results, however, were obtained after exposure of the sections to fluorescein-labelled rabbit anti-human IgM (conjugate V) ('single layer' method). Two biopsies obtained from skin lesions of patients with systemic lupus erythematosus and also two of the twelve biopsies from the cases with discoid lupus erythematosus, showed staining of the dermo-epidermal junction. The same results were obtained after exposure of the sections concerned to non-labelled rabbit anti-human IgM serum and subsequent exposure to sheep anti-rabbit globulin (conjugate XI): in the 'double layer' method.

In view of the fact that IgG could be located in the region of the dermo-epidermal junction of the sections of all biopsies examined, the occurrence of either or both κ and λ immunoglobulins could be expected in the same region.

Negative staining results were, however, obtained with the single layer method; that is after exposure of the sections to fluorescein-labelled rabbit anti-human L-chain, type κ and λ (conjugates VII and VIII).

Positive results were obtained after exposure of the sections to non-labelled antibodies directed against L-chains (type κ and λ respectively) and subsequent staining with fluorescein-labelled sheep anti-rabbit globulin (conjugate XI).

In only one case of discoid lupus erythematosus was IgA located in the region of the dermo-epidermal junction with the aid of the single layer and double layer methods. The procedures were in accordance with those mentioned above, using conjugate VI for the single layer method and antisera II in combination with conjugate XI for the double layer method.

* Obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.

† Progressive Laboratories, Inc., Distributed by Roboz Surgical Instrument Co. Inc., 810–18th Street, N.W. Washington 6, D.C.

It is noteworthy, in this respect, that of the four biopsies in which IgM could be located in the region of the dermo-epidermal junction, one discoid lupus erythematosus case and one systemic lupus erythematosus case showed at high magnification ($\times 400$ and oil immersion) a very small clear zone coinciding with the dermo-epidermal junction, as identified by simultaneous phase-contrast examination. The macroglobulin appears to be mainly localized

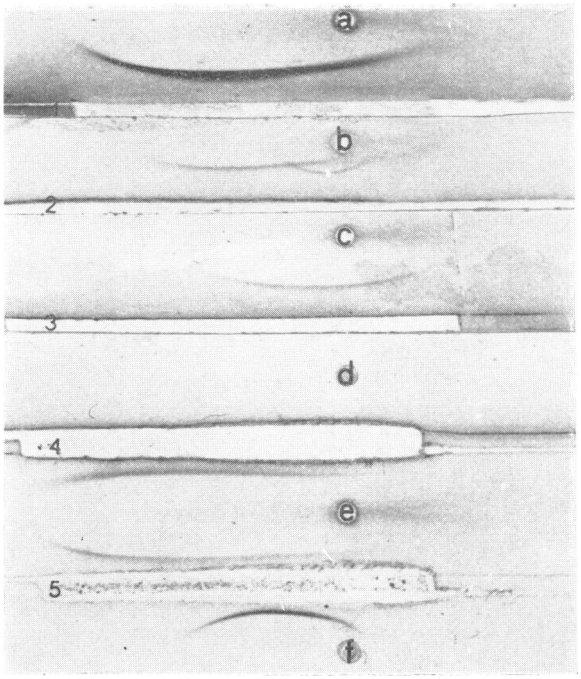


FIG. 1. Immunoelectrophoresis on agar-gel. Anode at the right in each figure. Antigen in a, b, c and e is normal human serum; antigen in d and f is Bence-Jones protein of type λ. The antisera used were (1) anti-IgG, (2) anti-IgM, (3) anti-IgA, (4) anti-κ, and (5) anti-λ.

in the upper dermal region adjacent to the membrane. The occurrence of this class of immunoglobulin in the basal parts of the basal layer was much less definite.

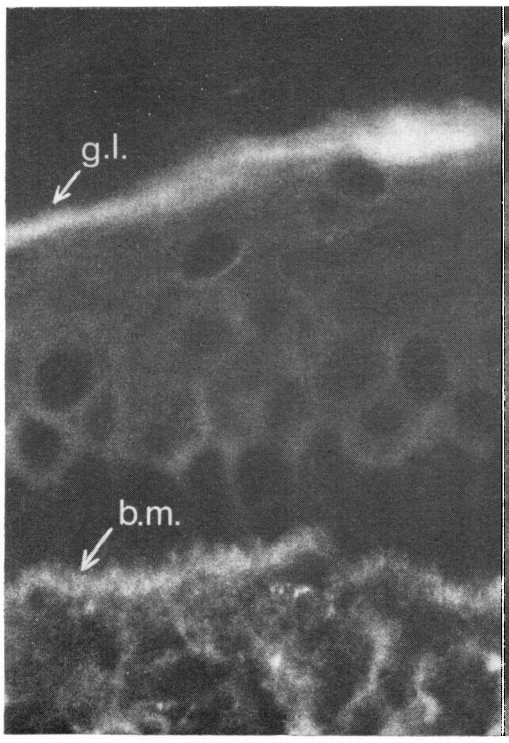
The same phenomenon could be observed in most of the sections using the anti-κ and anti-λ sera and in some of the DLE biopsy sections in which the occurrence of IgA could be demonstrated with the aid of the double layer method.

Immunological specificity

After exposure of the sections obtained from five healthy individuals to the non-labelled antisera and the fluorescein-labelled antisera, using the single layer and double layer method respectively, no staining of the dermo-epidermal junction was seen. Moreover, the fluorescein-labelled sheep anti-rabbit globulin failed to stain the region of the dermo-epidermal junction of the sections of the biopsies used for this study.

In view of the fact that negative staining results were obtained with the fluorescein-labelled anti-κ and anti-λ antisera, these antisera were also used before conjugation for the

double layer method. Only the sections showing strong staining of the region of the dermo-epidermal junction showed a positive staining of this region after exposure to both of the anti-L-chain sera followed by labelled sheep anti-rabbit globulin (conjugate XI). Presumably, the antibody titres of these sera were lower than those of the antisera III and IV.



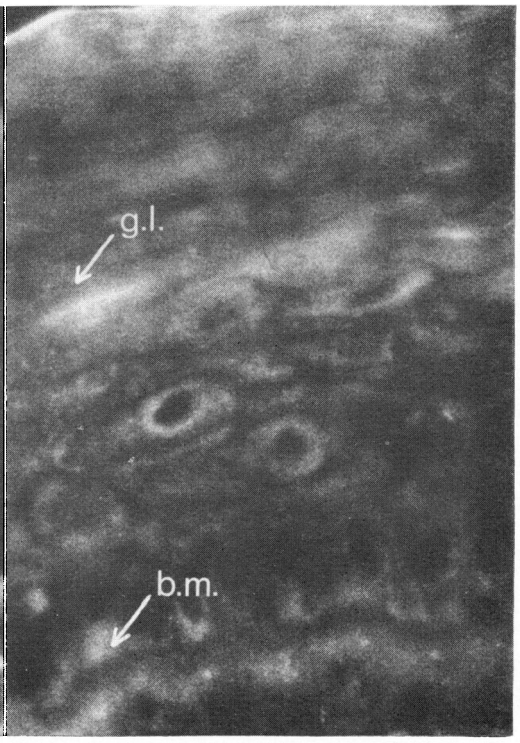


FIG. 2 FIG. 3

FIG. 2. Chronic discoid lupus erythematosus, 'diseased' skin. Note the specific staining of the region of the dermo-epidermal junction (b.m.) after exposure of the section to conjugated anti-IgG (conjugate IX). The staining of the granular layer (g.l.) is non-specific.

FIG. 3. Chronic discoid lupus erythematosus: 'diseased' skin. Note the specific staining of the region of the demo-epidermal junction (b.m.) and the small clear zone in this region coinciding with the dermo-epidermal junction, after exposure of the section to rabbit antiserum specific for human λ chain (type II L-chain) and subsequent staining with conjugated sheep anti-rabbit antiserum (conjugate VI).

An appropriate specificity test is the 'blocking test' (Nairn, 1964). This test is, however, only of value if a staining of the specific sites can also be obtained with the fluorescein-labelled antisera by the single layer method. A previous exposure of sections to the same unconjugated antisera will inhibit staining owing to blocking of the specific sites.

As we could not obtain a positive staining after exposure of the sections to the fluorescein-labelled anti-IgA (anti-κ and anti-λ) sera (conjugates VII and VIII respectively), this blocking test could not be applied.

DISCUSSION

The anti-heavy and the anti-light chain sera used in this study have been shown to be capable of detecting the different major classes and types of immunoglobulins in the dermo-epidermal junction in discoid and systemic lupus erythematosus.

In view of the fact that lupus erythematosus is generally considered to be an autoimmune disease, the occurrence of immunoglobulins in the skin of patients suffering from various forms of this disease may be envisaged as playing a role in the pathogenesis of the skin lesions. However, it is certainly not the only mechanism because IgG was also observed in the clinically unaffected skin of the forearm in the case of systemic lupus erythematosus. The conspicuousness of the fluorescent staining in the sections of these biopsies depended to a large extent on the optical qualities of the fluorescence microscope used. Reliable interpretations could only be made with the aid of an oil immersion objective, and in order to obtain maximum fluorescence emission we preferred incident illumination.

In this study, heterogeneity in respect to L-chains, in addition to heterogeneity in respect to immunoglobulin class, could be demonstrated for factors directed against substances in the region of the dermo-epidermal junction in lupus erythematosus. Essentially the same results were obtained in studies on antinuclear factors (Barnett *et al.*, 1965). This supports the assumption already suggested by Barnett *et al.* (1965), that the antibodies detected are produced by a rather unrestricted, or heterogeneous population of plasma cells.

It is a matter of more than academic interest to know whether antibodies are IgG, IgA, or IgM globulins, since they differ in some important biological respects.

From experimental data it is known that very early in immunization, macroglobulin (IgM) antibodies are commonly found, but later on may become relatively unimportant. IgA should be able to sensitize the skin so as to give immediate type reactions, whereas antibodies against the same antigen with properties of IgG should not.

In this respect the occurrence of IgA in one case of the rather small number of selected cases for this study is of interest. It was also found in uninvolved skin of a case of systemic lupus erythematosus not mentioned here.

IgG appeared to be consistently located on the dermo-epidermal junction, whereas the other immunoglobulins were often localized in the adjacent sites—mainly on the dermal side—of this junction.

The occurrence of the different immunoglobulins in the region of the dermo-epidermal junction in lupus erythematosus needs further investigation. These results may provide a better understanding of the significance of this phenomenon.

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