Immunohistochemical study of lichen planus

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SUMMARY The distribution of IgM and IgG in 20 cases of lichen planus and five cases of non-specific inflammation were studied by the unlabelled antibody-enzyme (PAP) technique. Routine paraffin sections were used. In lichen planus deposits of immunoglobulins were seen in and around epithelial cells, in colloid bodies, at epidermo-dermal junctions, and in some inflammatory cells. All cases examined for IgM and eight out of 13 cases examined for IgG were positive. The peripheral migrating epidermal cells were mostly negative. The application of the PAP technique to skin biopsy and the significance of the findings in lichen planus are discussed.

Various immunoglobulins and complement fractions have been demonstrated in lichen planus (LP) by immunofluorescent techniques (Baart de la Faille-Kuyper and Baart de la Faille, 1974; British Medical Journal, 1974; Abell *et al.*, 1975). The recently introduced immunoperoxidase technique may also be used to detect them (Burns, 1975). The test is done on ordinary paraffin-processed histological sections and provides permanent staining and exact localisation of the components. We report here the results of a study by this technique of the distribution of IgM and IgG in LP. We try to explain the findings and relate them to the theories on the pathogenesis of the disease. We also comment on the application of the immunoperoxidase technique to skin biopsy.

Material and methods

Routine paraffin sections were prepared from tissue taken from 25 patients suspected clinically of having LP. The specimens for biopsy were from the skin in 23 cases and from the mouth in two cases. They were removed in the Departments of Dermatology and Oral Surgery of the Royal Free Hospital during the last three years. The sections were deparaffinised in xylene and stained for IgM or IgG by the unlabelled antibody-enzyme (PAP) method described by Burns (1975). Fifteen cases were examined for IgM and 15 for IgG, five cases being examined for both. Sections

stained with haematoxylin and eosin were studied to confirm the diagnosis.

Results

The diagnosis of LP was confirmed in 20 of the 23 biopsies of skin (group A, Table). The criteria used for diagnosis were the presence of irregular acanthosis with liquefaction degeneration of the basal layer and a band-like inflammatory mononuclear cellular infiltrate in the upper dermis (Fig. 1). The 'migrating' spindle-shaped basophilic cells described by Marks *et al.* (1973) were seen at the margins of most lesions. Five cases showed subepidermal bullae. Group A included 11 females and 9 males. Their ages ranged from 9 to 76 years with an average of 46.

The remaining five biopsies, including the two oral biopsies (group B, Table), showed non-specific histological features with varying combinations of subepithelial inflammatory cellular reaction, regular acanthosis, and an increase in melanin-containing cells. No degenerating basal cells and no 'migrating' cells were seen in any of these cases. This group included three females and two males. Their ages ranged from 35 to 49 years with an average of 43.

The immunological deposits were detected as homogenous dark brown material in and around epithelial cells (Figs. 2, 3), in inflammatory cells (Figs. 3, 4), and at the epidermo-dermal junctions (Fig. 4). The number of stained cells and the intensity of the stain varied from case to case. Accordingly a semiquantitative method was used to indicate the degree of positivity (Table). A similar method was used to quantify mononuclear inflammatory cells and melanin-containing cells.

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Table Findings in 20 cases of lichen planus (group A) and non-specific inflammation (group B)

Case No.	Sex and age (years)	Inflammatory cellular infiltrate	Dermal melanin-containing cells	IgM			IgG		
				Intra- and periepithelial	Epidermo-dermal junction	Inflammatory cells	Intra- and periepithelial	Epidermo-dermal junction	Inflammatory cells
Group A									
1	M 65*	++ +	—	++ +	+	+			
2	F 45*	++ +	—	++ +	++	++			
3	F 61	++ +	—	++ +	—	—			
4	M 49	++ +	++ +	—	++	—			
5	M 49	++ +	—	—	++	—			
6	M 24	++ +	++ +	—	—	—			
7	F 43	++ +	++ +	—	—	—			
8	F 9	++ +	++ +	++ +	++	—	++ +	—	—
9	F 71	++ +	—	++ +	++	—	++ +	—	—
10	M 25	++ +	+	++ +	—	—	++ +	—	—
11	F 68*	++ +	—	—	—	—	++ +	++	+
12	M 54	++ +	++ +	—	—	—	++ +	++	++
13	M 20*	++ +	++ +	—	—	—	++ +	++	++
14	M 45	++ +	++ +	—	—	—	++ +	++	++
15	F 73*	++ +	+	—	—	—	++ +	++	++
16	F 21	++ +	++ +	—	—	—	++ +	++	++
17	F 21	++ +	++ +	—	—	—	++ +	++	++
18	F 65	++ +	+	—	—	—	++ +	++	++
19	M 28	++ +	—	—	—	—	++ +	++	++
20	F 76	++ +	++ +	—	—	—	++ +	++	++
Group B									
21	M 45+	++ +	++ +	—	—	—	++ +	++	—
22	F 49†	++ +	++ +	++	—	—	++ +	++	—
23	F 35	++ +	++ +	—	—	—	—	—	—
24	M 49	++ +	—	—	—	—	—	—	—
25	F 35	++	++	—	—	—	—	—	—

Note: +++, ++, and + = Pronounced, moderate, and mild dark brown deposits respectively. ± = Diffuse intercellular pale brown staining. - = Negative.

*Cases with subepidermal bullae. †Oral biopsy.

A homogenous pale brown interepithelial staining was obtained in a few LP and control cases (shown ± in Table). The stain was diffuse, affecting every part of the epidermis, in and outside the lesion, and was thus considered non-specific.

Melanin granules appeared to cross-react with the stain and develop a brownish coloration. Yet melanin as such could easily be identified by its distinct granular appearance and slightly darker colour.

LICHEN PLANUS

All the 11 cases stained for IgM and eight out of the 13 cases stained for IgG were positive.

IgM The positivity was strong in four cases, moderate in two, and mild in five. All the cases showed intra- and periepithelial deposits. Only seven cases had deposits at the epidermo-dermal junction. The cellular infiltrate included recognisable IgM-containing cells in only four cases. Seven cases had dermal melanin-containing cells.

IgG Three cases were strongly, one moderately, and four mildly positive. Three of the remaining five cases showed a weak diffuse intercellular staining. Two cases were completely negative. The epidermo-

dermal junction showed positive deposits in only four cases, of which three had epithelial deposits and one diffuse intercellular staining. The cellular infiltrate included IgG-positive cells in only two cases. Dermal melanin-containing cells were absent in two cases.

CORRELATIONS

The four cases examined for both immunoglobulins showed intraepithelial deposits of similar degree of positivity. The reaction at the epidermo-dermal junction was similar in two cases and different in the other two. No correlation could be detected between the immunoglobulin deposits and the severity of the inflammatory cellular reaction or the number of melanin-containing cells. All the five cases with bullae were positive for all the immunoglobulins tested. The cellular infiltrate in four of these included immunoglobulin-containing cells.

COLLOID BODIES AND MIGRATING CELLS

The colloid bodies always contained heavy immunoglobulin deposits. Almost all the 'migrating' cells were negative. However, a positive cell was occasionally seen.

Fig. 1 *Lichen planus: liquefactive degeneration of basal cells, and invasion of lower part of the epidermis with mononuclear inflammatory cells. Note 'colloid' body with lymphocytes applied to border (arrow) (haematoxylin and eosin $\times 360$).*

Fig. 2 *Lichen planus: dark intra- and periepithelial deposits of IgG in suprabasal and prickly-cell layers. Unlabelled antibody-enzyme (PAP) technique counterstained with celectine blue ($\times 88$).*

Fig. 3 *Lichen planus: intra- and periepithelial deposits of IgM in suprabasal and prickle-cell layers. Note negative degenerating basal cells (B) and migrating spindle-shaped peripheral cells (M) ($\times 370$).*

Fig. 4 *Lichen planus: 'perilesional' epidermo-dermal deposits of IgM (J). Basal layer, basement membrane, and deposits are interrupted at site of lesion and invaded by mononuclear inflammatory cells. Two small vesicles (V) contain degenerated IgM-positive epithelial and inflammatory cells ($\times 140$).*

CONTROL GROUP

None of the five cases showed any detectable epithelial deposits. However, one case (from oral mucosa) showed a weak diffuse intercellular staining for both IgM and IgG, and another case showed positive IgM epidermo-dermal deposits.

Discussion

Our results confirm previous studies of LP using immunofluorescence techniques (Baart de la Faille-Kuyper and Baart de la Faille, 1974; Abell *et al.*, 1975) and establish the immunoperoxidase method as a new and useful means of investigating skin diseases. By being able to do the test on old, stored paraffin sections and by providing a permanent record of the exact location of the positive areas within easily identifiable counter-stained surroundings the method has great potentialities in advancing the use of immunological studies in the diagnosis and understanding of the pathogenesis of skin diseases and in comparing the results with those of other conventional methods used in studying skin. The positive reaction was always easy to identify as dark brown deposits, quite distinct from the reaction obtained with melanin granules.

The possibility of the epithelial stained material being something other than immunoglobulins—namely, keratohyalin or products of melanin—was excluded because: (1) the positivity was always limited to the diseased areas, (2) cases in group B with non-specific inflammation associated with acanthosis and hyperkeratosis were negative, (3) there was no correlation between the number of dermal or epidermal melanin-containing cells and the results of the stain, and (4) the deposits demonstrated by immunofluorescence in LP (Baart de la Faille-Kuyper and Baart de la Faille, 1974; Abell *et al.*, 1975) occupied similar sites.

The identification of IgM in all LP cases examined and of IgG in only some accords with the findings of the previous authors. However, in our cases the deposits appeared in more epidermal cells and in fewer epidermo-dermal junctions. This might be due to severe degeneration and inflammation in our cases. Colloid bodies are always strongly positive and the degree of positivity might be related to the degree of degeneration. The epidermo-dermal junction is usually only positive in the normal looking areas next to the lesions (Fig. 4). Such normal looking areas were absent in most of our cases with negative dermo-epidermal junction.

The diagnostic significance of immunoglobulin deposits in LP was questioned by Abell *et al.* (1975), but it seems from our results that they might be of

help in differentiating LP from other clinically and histologically related conditions. Further studies on other skin diseases should clarify this point.

The deposits can, however, be of great help in understanding the pathogenesis of the disease. The small areas of basal cell degeneration without inflammatory cells that were sometimes seen appeared to be always associated with heavy dermo-epidermal immunoglobulin deposits. Possibly the first change that occurs after the change in the antigenic character of basal cells (Sarkany and Gaylarde, 1971) or their basement membranes (Abell and Ramnarain, 1975) is the appearance of immunoglobulins at these sites even before the arrival of any inflammatory cells. This might be achieved by diffusion from blood vessels. Such diffusion was demonstrated in eczema by immunofluorescence (Welbourn *et al.*, 1976). The diffused globulins spread in all directions but become especially concentrated at the epidermo-dermal junction and on collagen fibres. The presence of an intact basal cell layer seems to hinder the upward diffusion of the globulins. When the basal layer is much destroyed the immunoglobulin diffuses freely upwards and the epidermo-dermal immunoglobulin condensation disappears from these areas.

The distribution of the intact epidermal immunoglobulin-containing cells corresponds with the distribution of the large eosinophilic cells found by Black and Wilson-Jones (1972) to have a much depressed respiratory enzyme activity. These cells constitute the main bulk of the acanthotic areas overlying the basal erosion and inflammation. A relation between the presence of immunoglobulins, depression of respiratory enzymatic activity, and the decreased epidermal cell turnover suggested by several authors (Black, 1972) is highly probable.

The absence of any immunoglobulin deposits in almost all the 'migrating' spindle-shaped cells seen at the margins of some lesions supports the idea that these are physiologically and immunologically normal cells derived from the adjacent unaffected cells in an attempt at replacing the destroyed ones and limiting the spread of the disease reaction (Marks *et al.*, 1973).

The presence of heavy deposits of immunoglobulins in colloid bodies confirms the results of Ueki (1969) and Baart de la Faille-Kuyper and Baart de la Faille (1974). They were mostly seen in areas with heavy lymphocytic infiltration and sometimes in close contact with them (Fig. 1). Probably lymphocytes play a role either in the formation or the final disposal of these bodies. The presence of detectable immunoglobulins in only a minority of the inflammatory cells accords with the findings of Walker (1976) that most of the inflammatory cells in

LP are T-lymphocytes, with only a few B-lymphocytes and macrophages.

In conclusion, immunological reactions involving immunoglobulins and lymphocytes seem to play an important role in the sequence of events that lead to the development of lichen planus lesions. These reactions can be demonstrated on ordinary paraffin sections by the PAP technique and could be of help in the diagnosis of clinically and histologically doubtful lesions.

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