Imbalance in Phenotypic Expression of T Cell Subpopulations during Different Evolutional Stages of Lichen planus Lesions

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Immunoenzymatic (in light and in electron microscopy) and immunofluorescence techniques were performed, using monoclonal antibodies, on tissue sections of early lichen planus (LP) lesions versus late LP lesions from 20 patients. Control procedures were carried out in peripheral blood T cells from the same patients and from healthy donors. The OKT4-Leu3A/OKT8-Leu2A ratio in peripheral blood from LP patients and from donors was lower than in dermal infiltrate of early LP lesions, but higher than in dermal infiltrate of late LP lesions. It is conceivable that in early LP lesions OKT4-Leu3A-positive cells may be antigen-specifically 'educated' by immunostimulatory cells. In late LP lesions, OKT8-Leu2A-positive cells could be cytotoxic to keratinocytes; it is likely, however, that this latter population may moreover have immunoregulatory, resolutional functions. Key words: Cell infiltrates; Monoclonal antibodies; Immunocytochemistry; Immunoelectron-microscopy. (Received December 29, 1982.)

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Histopathological features of lichen planus (LP) are different during evolutional stages of the disease. In a previous investigation, we observed a lower lymphocyte/macrophage ratio in dermal infiltrate of LP in early (1-3 weeks old) lesions as compared with that occurring in late (1-12 month old) lesions (1). Moreover, Ragaz & Ackerman (2) noticed an increased number of Langerhans cells in an early phase of the lesions, followed by the appearance of lymphocytes.

A hypothesis on the immunological nature of LP was worked out on the basis of morphological evidence. In a first phase, T lymphocytes in LP lesions interact with macrophages and Langerhans cells (2, 3); in a second phase, T cells presumably attack and destroy keratinocytes (2, 3, 4). In our opinion, this hypothesis is supported by the above-mentioned macrophage/lymphocyte ratio and Langerhans cells/lymphocyte ratio. The occurrence of different reciprocal proportions, in evolutional stages of LP, between T lymphocyte subpopulations having different functions, could further support the above hypothesis; the present study was therefore performed to assess quantitative data on T cell subsets in early versus late LP, and to verify some apparently controversial literature reports (5, 6, 7).

MATERIALS AND METHODS

Elliptical biopsies were obtained from papules of the forearm in 20 patients. The duration of the lesions ranged from 2-3 days to 3 weeks (early LP lesions) in nine patients, and from 1 month to 1 year (late LP lesions) in 11 patients; the patients had been suffering from clinically typical LP for the same periods.

The following “OK” (Ortho) and “Leu” (Becton Dickinson) monoclonal antibodies were used:
Table I. Percentage ranges of positive cells of T-subsets, referred to 100 OKT3-Leu1-positive lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Early LP (9 cases)</th>
<th>Late LP (11 cases)</th>
<th>Controls (8 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP lesions</td>
<td>OKT4-Leu3 A</td>
<td>70-100</td>
<td>30-80</td>
</tr>
<tr>
<td></td>
<td>OKT8-Leu2 A</td>
<td>0-30</td>
<td>20-70</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>OKT4-Leu3 A</td>
<td>55-65</td>
<td>55-65</td>
</tr>
<tr>
<td></td>
<td>OKT8-Leu2 A</td>
<td>35-40</td>
<td>35-40</td>
</tr>
</tbody>
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OKT3, Leu1, detecting T cells; OKT4, Leu3A, reactive with "helper-inducer" T cell subset; OKT8, Leu2A, reactive with "suppressor-cytotoxic" T cell subset. The following conjugated antisera were employed: fluorescein-conjugated goat anti-mouse lgG (Meloy); peroxidase-conjugated F (ab')2 fragment goat anti-mouse IgG (Cappel). Control serum: mouse purified lgG fraction (Pel-Freez). All antibodies and sera were diluted 1:10 with PBS.

For double-step immunofluorescence, 4 µm unfixed frozen serial sections were first incubated with monoclonal antibodies (30 min) at room temperature, then washed in PBS (30 min), subsequently incubated with the fluorescein conjugate (30 min), and, after washing, examined with a Zeiss fluorescence microscope having epi-illumination.

A double-layer immunocytochemical procedure was moreover performed, again on serial, unfixed, 4 µm-thick cryostat sections. After blocking of endogenous peroxidases (8), monoclonal antibodies at room temperature were stratified on the sections for 60 min and subsequently washed in PBS (60 min); then incubated with the peroxidase conjugate (60 min), and washed again in PBS (60 min) and in Tris-HCl buffer, pH 7.6 (15 min). The enzymatic activity was finally determined (9). A number of sections were counterstained with toluidine blue, to allow better quantitative examination. Exhaustive data about the entire procedure are reported in previous publications (10, 11).

For immunoelectron microscopy, 40-50 µm frozen sections were fixed for 2 hours in 3% paraformaldehyde, incubated 12 hours with monoclonal antibodies at 4°C, washed 12 hours in PBS.

Fig. 1. Leu1 staining pattern. Many and many labelled cells in papillary dermis. In situ double-layer immunofluorescence technique (x350).
Fig. 2. OKT4 staining pattern. (A) Early lesion: many labelled cells in superficial dermis (×280). (B) Late lesion: occasional labelled cells in middle dermis (×280). In situ double-layer immunoenzymatic technique in light microscopy.

incubated 12 hours with the peroxidase conjugate, again washed for 12 hours in PBS, and finally fixed for 1 hour in 3% glutaraldehyde. After disclosure of peroxidase activity (9) and washing in distilled water, the specimens were postfixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Araldite. Ultrathin sections were obtained with an LKB ultramicrotome. Counterstaining of one-half of the sections was done with uranyl acetate and lead citrate, whilst the remainder were not counterstained, to distinguish better labelled from unlabelled cells. Examination was finally made in a Siemens "Elmiskop 1" electron microscope. Further details of the immunoelectronmicroscopy procedure are described elsewhere (12).

Fig. 3. OKT8 staining pattern. (A) Early lesion: no labelled cells are visible in papillary dermis (×400). (B) Late lesion: scattered labelled cells in middle dermis (×400). In situ double-layer immunoenzymatic technique in light microscopy.
Monoclonal antibodies were tested on peripheral blood T cells from LP patients and from healthy donors. E-rosette-forming cells (13) from subjects of both groups were isolated on Ficoll-metrizoate, and the pelleted T lymphocytes were purified by means of ammonium chloride. The T-enriched population was then tested with the monoclonal antibodies, in a microlymphocytotoxicity assay (14). For control purposes, the monoclonal antibodies were omitted, or replaced by the mouse IgG fraction.

For quantification purposes, the proportions of T subsets in the infiltrate were calculated at 400×, using a 0.01 mm² reticle, each square area containing about 100–125 cells; five fields per section were counted by two of us and the average estimated. The number of OKT3-Leu1-positive cells was postulated to represent the T population. The number of OKT4-Leu3A and of OKT8-Leu2A-positive cells was compared with the number of OKT3-Leu1-positive cells, and recorded as a percentage of this latter population (Table I).

RESULTS
Results are summarized in Table I.

Immunofluorescent-positive cells were identified by the intense labelling of the cell membrane (Fig. 1). Light (Figs. 2, 3, 4) and electron (Figs. 5, 6) microscopy visualized the peroxidase-labelled lymphocytes and the counterstained unlabelled cells.

Brown deposits on the cell membrane characterized the positive cells in light microsc-
Infiltrating T cells labelled with OKT\(^4\)-Leu\(^3\)A antibodies ranged from 0 to 30\% in early lesions (Fig. 3 A) and from 20 to 70\% in late lesions (Fig. 3 B). Single, isolated cells infiltrating the lower layers of the epidermis, particularly detectable in late lesions, reacted almost constantly with OKT\(^8\)-Leu\(^2\)A (Fig. 4).

Immunoelectronmicroscopy confirmed the results of light microscopy. Peroxidase deposits were visible along the cell membrane of positive cells, especially evident in unstained sections (Fig. 5); however, cell details were more distinct after counterstaining (Fig. 6). Moreover, OKT\(^4\)-Leu\(^3\)A-positive cells often demonstrated a high N/P ratio (Fig. 6), whilst OKT\(^4\)-Leu\(^3\)A-negative cells were often filled with cytoplasmic organelles (Fig. 6), this latter appearance often being shared by OKT\(^8\)-Leu\(^2\)A-positive cells (Fig. 5).

The E-rosette-forming cells of peripheral blood almost always reacted with OKT-
3-Leu1: 55-65% of T cells expressed OKT4-Leu3A phenotype, whilst 35-40% were detected by OKT8-Leu2A (Table 1). There was no difference between the ratios of OKT4-Leu3A/OKT8-Leu2A cells in the peripheral blood of LP patients and controls, respectively.

DISCUSSION
The present investigation confirmed that the entire population of lymphocytes infiltrating LP lesions is OKT3 and Leu1 positive (5, 6, 7, 15).

We found the ratio OKT4-Leu3A/OKT8-Leu2A-positive cells higher in early stages of LP lesions than in late LP lesions. We are inclined to believe that these different proportions in helper-inducer/suppressor-cytotoxic subclasses in different stages of LP lesions can be referred to different evolutionary phases of LP. In an early phase inducer cells may receive an antigen-specific ‘education’ from immunostimulatory cells such as macrophages and Langerhans cells. In a late phase suppressor-cytotoxic cells presumably play an effector role against keratinocytes, and/or an immunoregulatory, suppressive role.

In early phases of LP lesions, macrophages (1) and Langerhans cells (2) are largely represented. Within the LP dermal infiltrate the above cells were frequently observed in close connections, often in rosette-like patterns, with lymphocytes (16) of the T type (3). Moreover, in normal (17) and in pathological (18) conditions the lymphocytes observed in close contact with large, non-lymphoid cells were identified as helper-inducer T cells. It is therefore presumable that in early LP a type of cell cooperation could exist, prominently mediated by macrophages and Langerhans cells, interacting on an inducer T subpopulation.

In late phases of LP lesions, it is presumable that an OKT8-Leu2A-positive subpopulation may have two different functions: cytotoxic, and suppressive.

Cells bearing the OKT8-Leu2A phenotype may act as cytotoxic agents against keratinocytes. In fact, there is firm evidence that activated T cells, which are well represented in LP lesions (4, 15, 19), attach to and kill basal epidermal cells by apoptosis (4). Using ultrastructural and immunocytochemical methods, we were able to confirm that T lymphocytes in LP are frequently juxtaposed in the epidermis to degenerated keratinocytes (3). The disruption of keratinocytes is not detectable in the very early stages of LP. when lymphocytes are lacking; the appearance on the scene of lymphocytes is followed by the disruption of keratinocytes (2). In the present study we found that intra-epidermal lymphocytes almost constantly express the OKT8-Leu2A phenotype: the degenerative changes in keratinocytes could therefore be evoked by their cytotoxic potential.

It may be suggested, however, that some of the OKT8-Leu2A-positive cells may have merely suppressive (18, 20, 21, 22), rather than cytotoxic, functions. In contrast to more recent lesions, in one presumably resolving LP lesion, OKT8-positive cells formed an appreciable proportion of the cellular infiltrate (5). We are inclined to believe that the expression of suppressive functions by an OKT8-Leu2A subpopulation may act, in late stages of LP, as a factor possibly inducing LP lesions to resolve.

Presumably, both cytotoxic and suppressor functions are expressed by an OKT8-Leu2A-positive subpopulation: the relative proportions and the functional importance of these two subclasses, possibly bearing different antigens, will be explained when antibodies capable of distinguishing suppressor T subset from cytotoxic T subset become available.

It is our opinion that the controversial data about T cell subsets in LP (5, 6, 7) are only apparent. The imbalance in phenotypic expression of T subpopulations in different cases of LP is understandable when one considers that this disease is a result of gradually
evolving stages. The immunological hypothesis regarding LP is in our opinion supported by the fact that in LP lesions a continuous progressive change in the proportions among infiltrating subpopulations occurs, in a constantly evolutional dynamic process.

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REFERENCES