Different In Situ Distribution Patterns of Dendritic Cells Having Langerhans (T6+) and Interdigitating (RFD1+) Cell Immunophenotype in Psoriasis, Atopic Dermatitis, and Other Inflammatory Dermatoses

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Dendritic cells bearing Langerhans cell (OKT6+) or interdigitating cell (RFD1+) immunophenotype may be regularly detected within the dermis of chronic skin diseases characterized by a lymphohistiocytic (lymphoreticular) infiltrate. These 2 subsets of antigen-presenting cells within the dermis of lesions of exacerbating chronic plaque psoriasis, exacerbating nummular dermatitis (discoid eczema), atopic dermatitis, allergic contact dermatitis, pityriasis rosea, lichen ruber planus, and cutaneous lupus erythematosus were quantified using computer-assisted morphometry.

The mean dendrite length per dermal dendritic cell was significantly higher for RFD1 than for OKT6 (74.4 ± 0.98 μm vs 70.0 ± 1.26 μm; p = 0.0023). The mean dendrite length per dermal dendritic cell was remarkably constant for each marker in the various diagnostic categories studied.

Disease-specific patterns of total dendrite length and number (expressed per 100 infiltrating mononuclear cells) of these 2 dendritic cell types within the subepidermal infiltrates were obtained. Pityriasis rosea was characterized by its unique high percentage of OKT6+ Langerhans cells. Atopic dermatitis and psoriasis had relatively high percentages of both RFD1+ interdigitating cells and OKT6+ Langerhans cells. Nummular dermatitis had an intermediate number and total dendrite length for OKT6, but was relatively low in RFD1+ cells. Allergic contact dermatitis, lichen planus, and lupus erythematosus had low numbers and dendrite lengths for both dendritic cell subsets.

It is suggested that pityriasis rosea is characterized by an abnormal migration pattern of Langerhans cells. Psoriasis and atopic dermatitis may be examples of diseases in which skin-localized antigen-presenting and T-cell-inducing events are continuously taking place. The other diseases may reflect inflammatory processes in which local antigen presentation is less relevant to the tissue reaction. J Invest Dermatol 87:358–361, 1986

In situ studies of the distribution and immunophenotype of inflammatory cells in a variety of skin diseases using monoclonal antibodies (McAb) defining leukocyte subsets (cluster of differentiation, CD [1]) have resulted in noteworthy and sometimes disease-characteristic data. When chronic granulomatous and acute granulocytic diseases are excluded, the subepidermal infiltrates of most inflammatory dermatoses are characterized by an almost ubiquitous preponderance of the T4 (CD4) positive helper/inducer T-cell subset. Among others, these results have been obtained in the now further studied lymphohistiocytic (lymphoreticular) skin diseases such as psoriasis [2–5], cutaneous lupus erythematosus (LE) [6,7], lichen planus [8–10], allergic contact dermatitis [11–13], atopic dermatitis [14–16], nummular dermatitis* and pityriasis rosea [17]. Preponderance of the T8+ (CD8+) subset is exceptional but has been described in the skin of graft-versus-host disease [9,18], erythema multiforme [19], and pityriasis lichenoides acuta [20].

In many dermatoses these T lymphocytes are admixed with significant proportions of antigen-presenting cells (APCs) that are not thought to internalize and process complex proteins. The family of dendritic APCs in humans encompasses Langerhans cells (LCs), indeterminate cells, veiled cells, interdigitating (reticulum) cells (ID), and dendritic reticulum cells. APCs within the subepidermal T-lymphocytic inflammatory skin infiltrates tend to have the immunophenotype of T6+ (CD1+), LCs, and RFD1+ ID cells [21].

In this study, dermal APCs having the immunophenotype of LCs or ID cells were quantified. Using computer-assisted mor-


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phometry, the total number and cytoplasmic (dendrite) membrane length in relation to the number of infiltrating mononuclear cells of both major APC subsets were quantitated in psoriasis, atopic dermatitis, and a variety of other benign inflammatory skin diseases. Dendrite membrane length was measured as it might give an indication of the amount of direct dendritic cell–T cell contact within the subepidermal infiltrates.

Results indicate significant differences in the dendritic cell (DC) composition of the dermal lymphohistioctytic infiltrates studied. Pityriasis rosea stands alone in its excessively increased number of dermal LCs. Psoriasis and atopic dermatitis symptomatic skin contain significantly increased dermal RFDI+ ID cells as well as dermal OKT6+ LCs.

PATIENTS AND METHODS

A total of 42 biopsies obtained from 42 patients with different dermatoses were studied. Psoriasis biopsies (7 biopsies) were always taken from the edge of a plaque, mostly from the elbow or the back, in exacerating chronic plaque psoriasis. Biopsies from patients with nummular dermatitis (7 biopsies) were taken from the edge of an exacerbating nummular lesion, in most cases from an extremity. Biopsies from involved skin in atopic dermatitis (7 biopsies) were taken from the elbow fold, all cases having subacute eczema. Allergic contact dermatitis lesion biopsies (6 biopsies) were taken from 72-h elicitation reactions on the back. Pityriasis rosea lesion biopsies (7 biopsies) included in this study were all peripheral. Biopsies of lichen ruber planus lesions (3 biopsies) were taken from various sites and LE lesions (5 biopsies) were taken from 2 patients having subacute LE, the 3 others having discoid LE. All patients were untreated for a period of at least 3 weeks prior to biopsy; in most cases they had not received any treatment at all.

Immunoperoxidase Technique A 2-stage monoclonal antibody peroxidase-conjugated rabbit antimouse Ig staining system with 3-amino-9-ethyl-carbazol (AEC) as staining reagent was employed as the principal immunophenotyping procedure. Processing of tissue specimens, identification of cell types, immunoperoxidase technique, and the controls used were essentially the same as described previously [3,17]. In short, fresh frozen (−196°C) skin specimens were sectioned (6 μm), fixed in fresh pure acetone, and incubated with the McAb for 60 min in a humidified atmosphere. Next, sections were incubated with peroxidase-conjugated rabbit antimouse Ig for 30 min in a humidified atmosphere. Staining was achieved by incubation of sections in a freshly prepared acetate buffer solution (pH 4.9) containing AEC, dimethylformamide, and hydrogen peroxide. The staining reaction was visually controlled and stopped by washing in acetate buffer. Slides were fixed in formaline (4%) and counterstaining was achieved by passage through Mayer’s hematoxylin for 2 min, to give a slight nuclear visualization. Finally, sections were mounted with Aquamount.

McAb employed in this study were LC marker T6 (OKT-6: Orthoclone, Sunnyvale, California) and ID–cell marker RFDI (L. W. Poulter, Department of Immunology, Royal Free Hospital School of Medicine). OKT-6 stains corticothymocytes and LC [22,23] while RFD1 reacts with ID cells and a small subpopulation of B cells [24]. RFD1 appears to recognize an epitope of class II major histocompatibility complex (MHC) molecules. High concentrations of the epitope on ID cells make this reagent a specific marker for these cells at appropriate dilution.

Morphometry All stained sections were microphotographed under standard magnification conditions. Resulting color slides were projected under standard conditions on a graphic tablet connected with a microcomputer (Apple IIe). Using a graphic pen and appropriate software, dermal DL (total cytoplasmic membrane staining) of each mononuclear specificity could be determined for each specimen under study. Also, total numbers of McAb-positive, dermal nucleus containing cells were determined. Dendrite length was expressed in micrometers per 100 infiltrating mononuclear cells. Total number of stained cell bodies was expressed as a percentage of the total number of infiltrating mononuclear cells. Different observers (IDvG, SRK) gave almost exactly identical numbers when judging the same biopsies, differences staying under 1%.

Statistical Methods For convenience, statistical methods are described in appropriate parts of Results.

RESULTS

For each diagnostic category, the number of biopsies, dendrite length (DL) per 100 mononuclear cells (median and range), and percentage of infiltrating DCs (median and range) of results obtained with OKT6 may be found in Table I. Concordant findings obtained with RFD1 may be found in Table II. Values and means of OKT6+ and RFD1+ DL per 100 mononuclear dermis infiltrating cells in each diagnostic category are also depicted in Fig 1.

Wilcoxon’s signed rank test was used to calculate p values for differences between OKT6 and RFD1 staining within each diagnostic category. Total dermal DL and percentage of OKT6+ cells were found to be significantly higher than RFD1+ DL and cell percentages in pityriasis rosea (p = 0.02) and nummular dermatitis (p = 0.02). In all other diagnostic categories, no significant differences between the 2 subsets could be substantiated.

Wilcoxon’s test was used to calculate p values for differences in OKT6 and RFD1 staining patterns between the various diagnostic categories. It was evident that pityriasis rosea stands alone in its highly increased dermal number and total DL of OKT6+ cells (p ≤ 0.005). Psoriasis, atopic dermatitis, lichen planus, and nummular dermatitis had significantly higher values of dermal OKT6+ cells when compared with contact dermatitis (p ≤ 0.05) and LE (p ≤ 0.05). They did not differ from each other in this respect.

Psoriasis and atopic dermatitis had significantly increased percentages of dermal RFD1+ DCs as compared with all other diagnostic categories (p ≤ 0.05), especially when compared with all other diagnostic categories (p ≤ 0.05), especially when compared with RFD1 staining patterns of nummular dermatitis and pityriasis rosea (p ≤ 0.005). RFD1 staining patterns of psoriasis

<table>
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<th>Table I. OKT6</th>
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<td><strong>Dermatosis</strong></td>
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<td></td>
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<tr>
<td>Psoriasis</td>
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<tr>
<td>Nummular dermatitis</td>
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<tr>
<td>Atopic dermatitis</td>
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<tr>
<td>Allergic contact dermatitis</td>
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<tr>
<td>Pityriasis rosea</td>
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<tr>
<td>Lichen planus</td>
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<td>Lupus erythematosus</td>
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and atopic dermatitis did not differ from each other, nor did RFD1 staining patterns of the other diseases studied.

Dendrite length per dermis infiltrating DC was analyzed by linear regression analysis, using the formula \( y = \beta x \). The independent variable \( x \) is the percentage of dendritic cells per 100 infiltrating mononuclear cells. The dependent variable \( y \) is the DL per 100 infiltrating mononuclear cells. The coefficient of regression \( \beta \) is thus an estimation of DL per DC. The estimated coefficient for OKT6 was 70.0 ± 1.26 (\( \mu \)m). By analysis of variance, no difference in \( \beta \) between the various diagnostic categories for OKT6 could be demonstrated. The estimated coefficient for RFD1 was 74.4 ± 0.98 (\( \mu \)m). Again, no difference in \( \beta \) between the various diagnostic categories for RFD1 could be demonstrated. Analysis of variance did, however, indicate a clear difference between the 2 regression coefficients of OKT6 and RFD1. These results show that the morphology of OKT6+ cells within the subepidermal infiltrates did not differ among the diagnostic categories studied. Also, such a statement could be made of the morphology of RFD1+ DC: no difference in their DL per infiltrating DC. However, morphology of OKT6+ and RFD1+ cells in general did differ in this series. The calculated DL per cell was slightly but significantly larger for RFD1 as compared with OKT6.

**DISCUSSION**

Our results as to the distribution of dermal dendritic APCs indicate significant and sometimes disease-specific quantitative differences among the dermatoses studied. In pityriasis rosea and nummular dermatitis, the total DL and number of OKT6+ cells per 100 dermis infiltrating mononuclear cells outnumbers corresponding values for RFD1+ cells.

A previous semiquantitative investigation [17] in pityriasis rosea in which abnormally high numbers of OKT6+ cells were described within involved skin could thus be confirmed quantitatively. This dermal LC accumulation may indicate abnormal LC migration processes during pityriasis rosea pathogenesis. Such a pattern was not observed in any other dermatosis included in this study.

The presence of relatively increased numbers of RFD1+ DC was not, as was previously suggested [25], psoriasis specific. Both psoriasis and atopic dermatitis symptomatic dermis contained significantly increased numbers of ID cells when compared with the other dermatoses.

Dendrite length per infiltrating DC was almost constant (70.0 ± 1.26 \( \mu \)m for OKT6, 74.4 ± 0.98 \( \mu \)m for RFD1) in all the different diseases studied. Counts were thus not influenced by a theoretical presence of another class of RFD1+ or OKT6+ monocyte/macrophages in a particular disease, as this would immediately lead to differences in DL per DC in that disease.

The constant DL/DC ratio for both markers indicates a constant morphology of DCs within the subepidermal infiltrates of the dermatoses studied. Our results exclude the possibility that DCs differ in their morphology when different diseases are studied. Putative differences in functional capacity of these DCs (i.e., antigen presenting) could not be detected by their differences in morphology.

The exact interrelationship between the various subtypes of dendritic APCs is as yet unknown. Some hypothesize that epidermal LCs are derived from circulating monocyte precursors (with indeterminate cells as the intermediate subtype). Upon leaving the epidermis, these LCs enter the lymphatics as vascular cells, but gradually obtain ID cell characteristics while losing their Birbeck granules. Within the T-cell areas of draining lymph nodes, both ID cell and LC subsets may then be encountered.

It is interesting to note that in LE, the presence of dendritic APCs of both immunophenotypes was relatively low. Neither of the 2 major hypotheses existing for cutaneous LE pathogenesis requires these cells at the site of damage. Neither the antibody-dependent cellular cytotoxicity hypothesis of Norris [26] nor the membrane attack hypothesis of Biesecker et al [27] makes the presence of large accumulations of these specialized cells necessary.

On the other hand, it thus seems justified to conclude that local antigen processing by nonphagocytosing cells of the LC or ID-cell immunophenotypes is important and operative in psoriasis and atopic dermatitis pathogenesis.

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**REFERENCES**

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**Table II. RFD1**

<table>
<thead>
<tr>
<th>Dermatosis</th>
<th>No. of Biopsies (No. Without Any Staining)</th>
<th>Dendritic Length (( \mu )m) per 100 Mononuclear Cells</th>
<th>Dendritic Cells (%) per 100 Mononuclear Cells</th>
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<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>7 (0)</td>
<td>247</td>
<td>82–301</td>
</tr>
<tr>
<td>Nummular dermatitis</td>
<td>7 (2)</td>
<td>24</td>
<td>0–66</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>7 (0)</td>
<td>315</td>
<td>164–390</td>
</tr>
<tr>
<td>Allergic contact dermatitis</td>
<td>6 (2)</td>
<td>86</td>
<td>0–240</td>
</tr>
<tr>
<td>Pityriasis rosea</td>
<td>7 (1)</td>
<td>75</td>
<td>0–103</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>3 (0)</td>
<td>43</td>
<td>25–82</td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>5 (0)</td>
<td>66</td>
<td>11–240</td>
</tr>
</tbody>
</table>

**Figure 1.** Individual values and means of OKT6+ (solid circle) and RFD1+ (open circle) DL (\( \mu \)m) per 100 mononuclear dermis-infiltrating cells in psoriasis (PS), nummular dermatitis (ND), atopic dermatitis (AD), allergic contact dermatitis (ACD), pityriasis rosea (PR), lichen planus (LP), and cutaneous lupus erythematosus (LE).


