Phenotypic Characterization of Skin-Infiltrating T Cells in Cutaneous T-Cell Lymphoma: Comparison With Benign Cutaneous T-Cell Infiltrates

By Barton F. Haynes, Lucinda L. Hensley, and Brian V. Jegasothy

Using a panel of monoclonal antibodies, we have studied cell surface antigens of infiltrating mononuclear cells in skin biopsies from patients with cutaneous T-cell lymphoma (CTCL) and compared them with the T-cell surface phenotype seen in benign cutaneous T-cell infiltrations (e.g., contact dermatitis, delayed hypersensitivity skin tests, granuloma annulare) and in dermal infiltrates of lymphomatoid granulomatosis patients. We found that unlike circulating CTCL (Sézary) cells, CTCL cells infiltrating skin epidermis frequently expressed the T-cell antigen 3A1. Cutaneous infiltrates in 10 patients with mycosis fungoides (MF) and 1 patient with Sézary syndrome were presented with the clinical syndrome of either mycosis fungoides or Sézary syndrome, the circulating malignant T cells had a mature T-cell phenotype and were 3A1 - OKT4+ OKT8- and OKT8+. In contrast, study of T acute lymphoblastic leukemia malignant cells with the same panel of reagents demonstrated a marked heterogeneity of T-cell phenotype; however, all T acute lymphoblastic leukemia cells studied were 3A1+. These observations provided hope that the unusual T-cell phenotype of circulating CTCL (Sézary) cells (3A1+ OKT4+, OKT8+) might prove diagnostically useful in the clinical setting where diagnosis of CTCL is so difficult, i.e., when presentation is by skin involvement.

Thus, the present study was undertaken to study the phenotype of infiltrating malignant T cells in patients with CTCL and to compare the CTCL cell phenotype with that of infiltrating T cells in classic delayed hypersensitivity skin reactions and with the skin T-cell phenotype in chronic dermatoses.

MATERIALS AND METHODS

Patients Studied

All patients with forms of cutaneous T-cell lymphoma (CTCL) were seen at Duke Hospital and the Durham Veterans Administration.

From the Department of Medicine, Division of Rheumatic and Genetic Diseases and Dermatology, Duke University School of Medicine, Durham, N.C.

Supported in part by Grant CA28936 from the National Institutes of Health and the Medical Research Service of the Veterans Administration.

Submitted August 19, 1981; accepted April 5, 1982.

Address reprint requests to Dr. Barton F. Haynes, Box 3258, Duke University School of Medicine, Durham, N.C. 27710.

© 1982 by Grune & Stratton, Inc.
Table 1. Clinical Characteristics of Cutaneous T-Cell Lymphoma Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>WBC* (Sézary Morphology/µm²)</th>
<th>Cutaneous Lesion Type</th>
<th>Clinical Stage†</th>
<th>Therapy‡ Before Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sézary syndrome</td>
<td>59</td>
<td>F</td>
<td>10,600 (6,572)</td>
<td>Exfoliative erythroderma</td>
<td>III</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Mycosis fungoides</td>
<td>64</td>
<td>F</td>
<td>5,300 (0)</td>
<td>Erythematous plaques</td>
<td>IB</td>
<td>Topical N₂ mustard</td>
</tr>
<tr>
<td>3</td>
<td>Mycosis fungoides</td>
<td>53</td>
<td>M</td>
<td>8,700</td>
<td>Small scaley papules</td>
<td>IB</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Mycosis fungoides</td>
<td>57</td>
<td>M</td>
<td>6,300 (0)</td>
<td>Small erythematous plaques</td>
<td>IB</td>
<td>Topical steroids</td>
</tr>
<tr>
<td>5</td>
<td>Mycosis fungoides</td>
<td>56</td>
<td>M</td>
<td>7,300 (0)</td>
<td>Erythematous plaques and tumors</td>
<td>IIB</td>
<td>Topical steroids</td>
</tr>
<tr>
<td>6</td>
<td>Mycosis fungoides</td>
<td>68</td>
<td>F</td>
<td>6,800 (0)</td>
<td>Small scaley papules</td>
<td>IB</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>Mycosis fungoides</td>
<td>54</td>
<td>M</td>
<td>7,400 (0)</td>
<td>Small erythematous plaques</td>
<td>IB</td>
<td>Topical N₂ mustard</td>
</tr>
<tr>
<td>8</td>
<td>Mycosis fungoides</td>
<td>66</td>
<td>F</td>
<td>9,200 (0)</td>
<td>Tumors, plaques</td>
<td>IVB</td>
<td>Topical N₂ mustaard</td>
</tr>
<tr>
<td>9</td>
<td>Mycosis fungoides</td>
<td>59</td>
<td>F</td>
<td>6,000 (0)</td>
<td>Erythematous plaques and tumors</td>
<td>IIB</td>
<td>Topical steroids</td>
</tr>
<tr>
<td>10</td>
<td>Mycosis fungoides</td>
<td>55</td>
<td>M</td>
<td>9,700 (970)</td>
<td>Large erythematous plaques</td>
<td>IIA</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>Mycosis fungoides</td>
<td>52</td>
<td>F</td>
<td>4,200 (42)</td>
<td>Tumors</td>
<td>IIIB</td>
<td>None</td>
</tr>
</tbody>
</table>

*WBC, peripheral white blood cell count on the day of study.†Staging classification of National Workshop of Cutaneous T-Cell Lymphomas.‡In each patient studied, except patient 7, therapy had been discontinued at least 2 wk prior to skin biopsy.

Hospital from January 1981 to May 1981. These included 10 patients with various stages (IIB to IVB) of mycosis fungoides7 (Table 1). Time of onset of CTCL prior to study ranged from 6 mo to 6 yr. Four patients with a variety of other types of cutaneous T-cell infiltrations were also studied. These included two patients with lymphomatoid granulomatosis,9 and one patient each with either contact dermatitis or granuloma annulare19 (Table 2). As controls, skin biopsies were studied from 3 normal subjects (all males, ages 20-45 yr), one person each with a positive chromium patch test, positive intradermal strength protein-purified derivative (PPD) test or positive intradermal histoplasmin test, and one patient with a cutaneous B-cell lymphoma.

Monoclonal Antibodies

 Twelve monoclonal antibodies were used. 7 were T-cell-specific, while 5 were specific for non T-cell-associated cell surface antigens.9 For ease in the evaluation of data, the T-cell-specific monoclonal reagents were grouped as follows: group I antibody (NA1/34) defines a thymocyte-specific antigen, human thymocyte antigen-I (HTA-I), absent on peripheral T cells, which is the same antigen defined by another monoclonal antibody (OKT6).20,21 Group II antibodies (10.2 and 9.6) define antigens present on all thymocytes and all peripheral T cells.22,23 Group III antibodies (3A1, OKT4, OKT8) define antigens present on most thymocytes but only on a subset of peripheral T cells,11,14 while group IV antibody (OKT3) defines an antigen detected on a subset of thymocytes but on all peripheral T cells.14 Of the non-T-cell-specific antigens, antibody 3F10 binds to a nonpolymorphic determinant of the 44,000 mol wt HLA-A, B, and C antigen (hereafter designated HLA).24 L-243 binds to a nonpolymorphic determinant on human la-like antigen (hereafter designated anti-la).25 Antibodies 5E9 and OKT9 bind to a 90,000 mol wt antigen found on activated human cells (the transferin receptor).26,27 and 4F2 binds to a 120,000 dalton non-la antigen shared by human monocytes and activated lymphocytes.28

Table 2. Clinical Characteristics of Patients With Cutaneous Inflammatory Diseases

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>WBC*</th>
<th>Cutaneous Lesion Type</th>
<th>Therapy‡ Before Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Contact dermatitis</td>
<td>49</td>
<td>M</td>
<td>8,000</td>
<td>Lichenified macules, papules</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Granuloma annulare</td>
<td>43</td>
<td>F</td>
<td>6,200</td>
<td>Nodules</td>
<td>Topical steroids</td>
</tr>
<tr>
<td>3</td>
<td>Lymphomatoid granulomatosis</td>
<td>60</td>
<td>M</td>
<td>4,500</td>
<td>Ulcerated nodule</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Lymphomatoid granulomatosis</td>
<td>43</td>
<td>M</td>
<td>16,500</td>
<td>Ulcerated nodule</td>
<td>Systemic steroids§</td>
</tr>
</tbody>
</table>

*WBC, white blood cell count at the time of study.†Unless otherwise stated, therapy was discontinued at least 2 wk prior to skin biopsy.‡WBC performed 14 hr following an oral dose of 4 mg dexamethasone.§Skin biopsy performed 4 days after institution of high-dose dexamethasone therapy (4 mg daily).
Acetone-fixed skin tissue slides were incubated for 30 min in a moist chamber with a saturating amount of monoclonal antibody. Following 3 rinses with cold phosphate-buffered saline (PBS), a saturating amount (1:100 final dilution) of affinity-pure FITC-conjugated (F/P-6.0) goat anti-mouse IgG (TAGO, Inc., Burlingame, Calif.) was layered on the slide. Following 3 rinses in PBS, the slides were briefly dipped in distilled water, allowed to air dry, overlayed with 30% glycerol in PBS, coverslipped, and then read on a

**Preparation of Skin Tissue Sections**

Fresh skin tissue was obtained by 4-mm punch biopsy at the time of diagnostic workup for infiltrative skin disease of unknown etiology. The skin was placed in RPMI 1640 medium and a portion was frozen in an ethanol dry ice slurry. Following embedding in OCT Embedding Compound (American Scientific Products), 4-μm sections were cut (at −20°C), fixed 10 min in cold acetone, and stored at −110°C until use. Other 4-μm skin sections cut at the same time as the acetone-fixed sections were fixed in formaldehyde and stained with hematoxylin and eosin.

**Surface Marker Analysis of Lymphocytes in Skin Tissue Sections Using Monoclonal Antibodies**

Acetone-fixed skin tissue slides were incubated for 30 min in a moist chamber with a saturating amount of monoclonal antibody. Following 3 rinses with cold phosphate-buffered saline (PBS), a saturating amount (1:100 final dilution) of affinity-pure FITC-conjugated (F/P-6.0) goat anti-mouse IgG (TAGO, Inc., Burlingame, Calif.) was layered on the slide. Following 3 rinses in PBS, the slides were briefly dipped in distilled water, allowed to air dry, overlayed with 30% glycerol in PBS, coverslipped, and then read on a

---

**Fig. 1.** Expression of antigen 3A1 by skin-infiltrating T cells in CTCL. Frozen sections of skin (CTCL patient 1) were incubated with P3 × 63 ascitic fluid and FITC goat anti-mouse IgG (for control background fluorescence) (A) stained with hematoxylin and eosin (B) for morphology or incubated with 3A1 antibody and FITC goat anti-mouse IgG (C). B and C show typical invading intraepidermal Sézary cells in epidermis with Pautrier’s abscess formation (arrow). Many of the cells in the Pautrier’s abscess were 3A1+ (C) (arrow). (D) Dermal-infiltrating T cells in skin from CTCL patient 2 were also strongly 3A1+ (×400).
Nikon Optiphot fluorescent microscope. In each experiment, background staining with comparable dilutions of P3 x 63 ascites fluid and FITC-conjugated goat anti-mouse IgG was low, with skin lymphoid elements and epithelial elements nonfluorescent (Fig. 1A). Each patient’s biopsy was phenotyped using 4–5 sections a minimum of 2 times, and most subjects were studied 3 or more times. Several patients had biopsies taken from different sites on the body: patients had biopsies taken from different sites. In some instances (in the Results and Discussion sections), the phenotype of dermal infiltrating T cells in the various disease states are described as well.

<p>| <strong>Table 3. Phenotypic Characterization of Lymphocytic Epidermal Infiltrates of Patients With Cutaneous T-Cell Lymphoma</strong> |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Patient Number/Diagnosis</th>
<th>Sezary</th>
<th>MF</th>
<th>MF</th>
<th>MF</th>
<th>MF</th>
<th>MF</th>
<th>MF</th>
<th>MF</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.6</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.2</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OKT3</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HTA-1*</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3A1</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OKT4</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OKT8</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA†</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>la‡</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>la‡</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>la‡</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*HTA-1 was on Langerhan’s cells of the epidermis while absent on infiltrating Sézary cells. Frequently, Langerhan’s cells constituted a significant proportion of the cells in Pautrier’s abscess and dermal infiltrates.
†HLA, nonpolymorphic determinant of HLA-A, B, and C molecule, as defined by monoclonal antibody 3F 10.
‡la, nonpolymorphic determinant of human la-like molecule as defined by monoclonal antibody L-243.

RESULTS

**Phenotype of Skin-Infiltrating T Cells in Cutaneous T-Cell Lymphoma**

One Sézary leukemia patient was seen at Duke Hospital during the study period of January–May 1981 (patient 1, Tables 2 and 5). She had abnormal circulating lymphocytes and skin lesions containing Pautrier’s abscesses. Her circulating CTCL cells were unusual in that they did not rosette with sheep erythrocytes and contained nuclear terminal deoxynucleotidyl transferase activity. The T-cell phenotype of patient 1 CTCL cells in both the peripheral blood and the skin was similar; they were 10.2*, OKT3*, OKT4*, and 3A1*, while being OKT8, 9.6. Of note, the circulat-
ing CTCL cells were Ia⁺, while in the skin they were Ia⁻ (Table 3). The epidermal infiltrating CTCL cells in the skin of 6 of 9 MF patients were uniformly 3A1⁻ (Fig. 1 B–D). However, in the remaining four patients (patients 3, 6, 9, 11, Tables 1 and 5), 3A1 antigen was absent or expressed only on a subpopulation of infiltrating epidermal and dermal T cells (Fig. 2, A and B), while the phenotypic expression of the other T-cell antigens by epidermal and dermal infiltrating T cells was uniform. Six MF patients had phenotypes of infiltrating T cells that were uniformly OKT4⁺, OKT8⁻ (patients 2–7, Tables 1 and 5). All infiltrating T cells except those from patients 2, 8, and 10 appeared Ia⁻ (Fig. 3). MF patients 8 and 9 had skin histologic staining patterns similar to the other MF patients, but their epidermal infiltrating T cells lacked both OKT4 and OKT8 antigens.

Patient 10 had classic generalized plaque stage MF (Table 5), yet had a skin T-cell phenotype unlike any other MF patient and was OKT4⁻ and OKT8⁻. Antigens 5E9 (transferrin receptor) and 4F2 were expressed by variable percentages of infiltrating skin T cells in all CTCL patients (data not shown). One patient with B-cell lymphoma metastatic to skin was studied; the dermal infiltrating B cells lacked all T-cell antigens in our panel and were all Ia⁻. To rule out the possibility that acetone fixation might change cell surface reactivity with various monoclonal reagents as compared to viable T cells in suspension, cytocentrifuge preparations of two 3A1⁻, Ia⁻, T4⁻ Sézary peripheral blood cell suspensions were snap-frozen, fixed, and reactivity with anti-Ia, OKT4, and 3A1 antibodies tested. We found that the phenotypes of both patients' peripheral blood Sézary cells following acetone fixation were identical to their phenotype as viable cells in suspension. However, we cannot rule out an effect of OCT embedding compound as a source of artifact in comparison of skin and peripheral T-cell phenotypes.

In summary, the majority of infiltrating epidermal

![Figure 2](https://example.com/figure2.jpg)  
**Fig. 2.** Variable expression of antigen 3A1 by skin-infiltrating T cells of some CTCL patients. (A) Dermal-epidermal junction from skin biopsy of CTCL patient 3. In this patient, while most dermal infiltrating cells reacted strongly with 10.2 (pan-T antibody) (arrow), only a percentage of T cells reacted with antibody 3A1 (B) (arrow). Linear fluorescence in B is collagen autofluorescence (x 400).

![Figure 3](https://example.com/figure3.jpg)  
**Fig. 3.** Expression of Ia-like antigens by skin-infiltrating CTCL cells. Dermis of skin biopsy from CTCL patient 5. Most infiltrating small lymphocytes stain brightly with anti-Ia (arrow) (x 400).
T cells in patients with CTCL bear most of the pan T-cell antigens and the T-cell subset antigen OKT4, with only an occasional exception. In addition, unlike many CTCL patients with the leukemic stage of the disease, 4'9 80% of the infiltrating epidermal T cells in 7 patients studied expressed antigen 3A1.

Reactivity Patterns of Monoclonal Reagents With Normal Skin

Normal skin contained only rare T cells. Some were OKT4+, while others were OKT8+. All were 3A1+, 10.2+, and 9.6+. Only a rare intraepidermal T cell was seen. A monoclonal antibody that selectively binds to peripheral monocytes (4F2) did not bind to Langerhan's cells, but did selectively label the basal layer of the epidermis (Fig. 4A). Antigen 4F2 is an antigen of cell activation and is found on mitogen and alloantigen-activated lymphocytes and most rapidly dividing cells.28,31 Anti-la (L-243) in addition to binding to Langerhan's cells, also bound to dermal vessels and to some perivascular cells. In our study, normal human intraepidermal Langerhan's cells reacted only with anti-HTA-1, anti-la, and anti-HLA reagents (Fig. 4B).

T-Cell Phenotypes in Cutaneous Delayed Hypersensitivity Reactions

An indurated area of skin was biopsied in a normal subject following the intradermal placement of either a histoplasmin or intermediate strength PPD skin test. In addition, the indurated skin area from a positive chromium patch in a patient with contact dermatitis (subject 1) was biopsied and studied. The infiltrating cells in the histoplasmin and chromium patch tests were T cells and expressed pan-T-cell antigens 9.6 and 10.2. Reactivity with OKT3 was variable. Most of the infiltrating T cells were 3A1+, OKT4+, and OKT8+. The pattern of the infiltrate seen in the skin infiltrate of the intermediate strength PPD was more complex, however, with the infiltrate a mixture of OKT4, OKT8, and B cells (Fig. 5, A-D). In all 3 cases of classic delayed hypersensitivity reactions, the infiltrating T cells appeared HLA+ and la+. The infiltrating T cells appeared HLA+ and la+ (Table 4).

T-Cell Phenotypes in Cutaneous Inflammatory Diseases

Contact dermatitis. The infiltrating T-cell phenotype in the skin of patient 1 with contact dermatitis was identical to that in his patch test biopsy infiltrate taken the same day, i.e., HTA-1+, 9.6+, 10.2+, 3A1+, OKT4+, OKT8+, la+, and HLA+ (Table 4).

Granuloma annulare. Granuloma annulare is a chronic cutaneous disease characterized by dermal...
collagen necrobiosis in patches with surrounding lymphocytic and giant cell infiltration. Vasculitis is rarely seen. The primary type of infiltrating lymphocyte is thought to be T cell. Indeed, in our case studied (patient 2, Table 5), the infiltrate was homogeneously T cells (10.2, 9.6) and 3Al, OKT4, OKT8. Unlike the T-cell infiltrates in classic delayed hypersensitivity reactions, the infiltrating T cells in granuloma annulare were la.

**Lymphomatoid granulomatosis.** Lymphomatoid granulomatosis is an unusual form of granulomatous vasculitis that has a propensity for skin involvement and is characterized by invasion of various tissues with a lymphoreticular infiltrate. Preliminary observations have indicated that the primary infiltrating cell in LyG is thymus-derived. In our study, one patient was untreated (patient 3, Table 5), while a second patient (patient 4, Table 5) had been treated with high-dose steroids for 3 days prior to study. Both patients' dermal infiltrating cells were primarily mature T cells (9.6, OKT4, HTA-1), but some differences in their T-cell phenotypes was present. Patient 7 was 10.2, 3Al, while the infiltrating cells of patient 8 were uniformly 10.2 and 3Al. Both were HLA, Ia. The primary infiltrating cell in vessel walls in both patients 7 and 8 was 9.6, OKT4, OKT8 (Fig. 6).

**The Epidermal Langerhan's Cell in CTCL and Benign Inflammatory Dermatoses**

In both benign and malignant cutaneous T-cell infiltrations, there were large numbers of epidermal Langerhan's cells (Fig. 4B). Moreover, in all CTCL patients and in half of the patients with benign dermatoses, there was a prominent Langherhan's cell component to the dermal infiltrates as well (Fig. 7).

Langerhan's cells in dermal infiltrations were seen in benign or malignant T-cell infiltrations but were not seen in the B-cell infiltrate of cutaneous B-cell lymphoma (data not shown). Finally, in all 11 CTCL patients, many of the cells surrounding and in Pautrier's abscesses were HTA-1 (Fig. 7).

**Distribution of Major Histocompatibility Antigens (HLA, Ia) in Benign and Malignant Cutaneous T-Cell Infiltrates**

While most benign and malignant infiltrating T cells expressed HLA and Ia antigens as determined by rim fluorescence in skin frozen sections (Fig. 3), a contrast in the dermal distribution of HLA and Ia antigens was noted in the comparison of normal skin with either benign or malignant dermatoses. In normal skin, the dermal connective tissue was negative for HLA and Ia (with the exception of blood vessels), while the dermal stroma around cells in benign or malignant T-cell infiltrates was strongly positive for HLA and Ia. Similar staining of the dermal stroma in CTCL infiltrates with anti-HLA and anti-Ia reagents was seen as well (data not shown).
A major goal of this study was to compare the T-cell phenotype of infiltrating CTCL cells with that of infiltrating T cells in benign cutaneous inflammatory diseases. We wanted to determine if the previously reported phenotype of circulating CTCL (Sézary) cells (3A1, OKT4\(^+\), OKT8\(^-\)) could be used diagnostically to differentiate early CTCL skin lesions from benign chronic dermatoses.\(^{4,16\text{--}18}\) We found that the T-cell phenotype per se as defined by our marker panel gave no specific cell surface marker profile that was completely diagnostic of CTCL. The infiltrates in patients with various types of CTCL were similar to that seen in contact dermatitis, granuloma annulare, and lymphomatoid granulomatosis. Moreover, within the benign and malignant groups of dermatoses, there was T-cell phenotype heterogeneity.

Antigen 3A1 is found on most peripheral E-rosette-positive T cells and on normal thymocytes. Though a functional role for this cell surface molecule has yet to be established, from studies on cell lines and a number of normal tissues, the antigen appears to be T-cell-specific.\(^{16}\) In all the leukemic CTCL patients studied in two previous reports,\(^{4,16}\) most circulating leukemic cells were 3A1\(^+\). Fox et al. have also found that Sézary leukemia cells were 3A1\(^+\).\(^{34}\) In the present study, the intraepidermal CTCL cells in skin were uniformly 3A1\(^+\) in 7 of 11 patients. In addition, one patient with exfoliative erythroderma (patient 1, Tables 1 and 5) had 3A1\(^-\) T cells in Pautrier's abscess and had a high percentage of circulating 3A1\(^+\) Sézary cells. This patient was unusual in that while her T-cell phenotype was that of a mature T cell (10.2\(^+\), OKT4\(^+\), OKT8\(^-\), 3A1\(^+\), HTA-1\(^+\)) the cells did not form E-rosettes at 37\(^\circ\) or 4\(^\circ\)C, did not react with the pan-T-cell reagent 9.6, and contained nuclear terminal deoxynucleotidyl transferase activity. This T-cell phenotype is similar to that reported for some T-ALL patients.\(^{16}\) Whether 3A1 positivity in this case is in any way predictive of a particular clinical course remains to be determined.

We have not as yet had the opportunity to study the skin lesions of a patient with a 3A1\(^+\) Sézary leukemia. However, in the two patients thus far studied with skin involvement and circulating CTCL cells, the T-cell phenotype in the skin and periphery have been the same.

There are several possible explanations for the variable expression of the T-cell antigens, and 3A1 antigen in particular, by CTCL (Sézary) cells. First, as monoclonal antibodies recognize only one particular determinant of complex cell surface antigens, small changes in cell surface antigen structure during progression of CTCL could alter the reactivity of monoclonal antibodies with CTCL cells, though the CTCL cell surface continues to express the altered antigen. Second, the antigen could indeed cease to be expressed during different phases of disease. Against this possibility is the observation that in patient 1, Table 3, the T-cell phenotype of both peripheral blood and skin-infiltrating cells was similar. Third, the fact that otherwise homogeneous CTCL epidermal T-cell infiltrates in patients 3, 6, 9, and 11 (Table 3) were to varying degrees 3A1\(^+\) suggests that the microenvironment may in some way be promoting intracutaneous T-cell phenotypic changes. The notion that CTCL malignant cells arise exclusively from 3A1\(^+\), OKT4\(^-\) cells at a site of normal intrathymic differentiation seems unlikely, since in many patients the majority of dermal and epidermal-infiltrating CTCL cells were 3A1\(^+\), OKT4\(^-\). Finally, a possibility that cannot as yet be excluded is that in skin there is a large component of nonmalignant 3A1\(^-\) T cells intermixed in the dermis and epidermis along with 3A1\(^+\) CTCL cells. That this was the case in many infiltrates seems likely, although in other cases, nearly all lymphocytes in both the epidermis and dermis were 3A1\(^+\). Thus, a major limitation of our study is the inability to differentiate on a cell-to-cell basis between malignant CTCL cells and benign reactive inflammatory T cells.

It is important to note that HTA-1\(^+\) Langerhan’s cells made up a significant portion of the dermal and epidermal infiltrate in both benign and malignant cutaneous T-cell infiltrates, yet HTA-1\(^+\) Langerhan’s cells were absent in the cutaneous infiltrates of a patient with B-cell lymphoma. A similar observation has recently been made in a preliminary report by Chu et al.\(^{16}\)

It was of particular interest to study patients with lymphomatoid granulomatosis and normal subjects with classic cutaneous delayed hypersensitivity reactions as a comparison to the CTCL phenotype. Previous functional studies on OKT4\(^+\) and OKT8\(^-\) normal peripheral T-cell subsets have shown that OKT4\(^+\) cells produced lymphokines and are the inducer T-cell subset for both cytotoxic subsets have shown that OKT4\(^+\) cells produced lymphokines and are the inducer T-cell subset for both cytotoxic T-cell generation and for B-cell activation.\(^{14}\) In contrast, OKT8\(^-\) peripheral T cells function both as cytotoxic and suppressor T cells in vitro.\(^{14}\) Moreover, OKT4\(^+\) T cells predominate in normal thymus and lymph nodes, while OKT8\(^-\) cells predominate in normal human bone marrow.\(^{16}\) At this point one must emphasize that until functional studies are performed on the infiltrating T cells teased from skin tissue, only conjunctional statements regarding their function in vivo can be made. However, in light of these previous observations, it is not surprising that in classic delayed hypersensitivity reactions (Tables 4 and 5), the predominant infiltrating T cell was OKT4\(^+\), OKT8\(^-\), Ia\(^+\), the phenotype of the inducer.
T-cell subset that in peripheral blood is the major producer of various lymphokines. Similarly, one case of granuloma annulare, one case of contact dermatitis, and two cases of lymphomatoid granulomatosis each had predominantly (though not exclusively) OKT4+, OKT8− T-cell infiltrates.

It should be pointed out that while most of the CTCL skin T-cell infiltrates had a homogeneous staining pattern (i.e., all T cells in a particular area of the dermis or epidermis stained with one or another subset of T-cell reagent), frequently a complex pattern of T-cell subsets was seen. These patterns ranged from that seen in the skin in contact dermatitis with the infiltrate 80% OKT4+ T cells and 20% OKT8− T cells to the more complex patterns seen in the PPD skin test (Fig. 5, A–D; patient 1, Table 4). Thus, only long-term clinical studies coupled with serial phenotyping of cutaneous T-cell infiltrates will be able to answer the question of whether one phenotype versus another in CTCL has a better prognosis or clinical course, or whether a particular T-cell phenotype in those cutaneous T-cell diseases that have been reported to progress to T-cell malignancies is more or less associated with malignant transformation.17 It should be pointed out that all reagents used in our study define surface antigens of normal T cells. Hopefully, more selective anti-CTCL reagents will become available for diagnostic use.

Thus, our study shows the complex nature of T-cell antigen patterns in inflammatory and malignant skin infiltrations. Information obtained in this study should aid in the study of the relevant pathophysiologic events associated with malignant T-cell transformation and in the development of skin involvement in diseases in the spectrum of cutaneous T-cell lymphoma.

ACKNOWLEDGMENT

The authors wish to thank Drs. Gerald Lazarus, Wayne Rundles, Joseph Moore, and Robert Gilgor for the referral of patients; Drs. Ron Levy, Gideon Goldstein, Ellis Reinherz, and John Hansen for exchange of reagents; Dr. Richard Metzgar for performing terminal deoxynucleotidyl transferase determinations and peroxidase-anti-peroxidase labeling of cells; and Joyce Lowery for expert secretarial assistance.

REFERENCES


27. Trowbridge I, Omary B: Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. Proc Natl Acad Sci USA (in press)


Phenotypic characterization of skin-infiltrating T cells in cutaneous T-cell lymphoma: comparison with benign cutaneous T-cell infiltrates

BF Haynes, LL Hensley and BV Jegasothy