Expression of T-Cell Receptor Antigens in Mycosis Fungoides and Inflammatory Skin Lesions

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Using immunohistologic methods, we studied the expression of the T-cell receptor (TCR)-associated antigens CD3, TCR-β, and TCR-δ by cutaneous T cells in mycosis fungoides (MF) (36 patients) and a variety of inflammatory diseases (16 patients). Most T cells in the inflammatory diseases and patch/plaque mycosis fungoides expressed the immunophenotype characteristic of the vast majority of mature peripheral T cells: CD3+ TCR-β+ TCR-δ-. In contrast, abnormal CD3+/TCR-β- antigen expression was seen in 3 of 6 cases (50%) of tumor stage mycosis fungoides. Furthermore, we were able to document its evolution from the normal pattern present in earlier patch/plaque lesions of the two cases in which serial biopsies were available for study. Divergence of epidermal versus dermal CD3/TCR-β antigen expression was seen in 2 of 34 (6%) of biopsies of patch/plaque mycosis fungoides but not in inflammatory controls. The TCR-δ+ cells were generally rare regardless of diagnosis. We conclude that inflammatory skin diseases and most patch/plaque mycosis fungoides are typically composed of T lymphocytes that resemble mature peripheral T cells in regard to their expression of TCR-associated antigens. In contrast, aberrant patterns of TCR-associated antigen expression can be seen in tumor stage MF, and, more rarely in patch/plaque MF.

The T-cell antigen receptor (TCR), expressed on the surface of the vast majority of peripheral T cells, is a heterodimer composed of α and β subunits. This αβ dimer is noncovalently associated with the CD3 (T3, Leu4) antigen complex [1-3, reviewed in 4]. A small number of CD3+ peripheral blood T cells lack this αβ dimer [2]. Instead, CD3 is associated with a functionally competent γδ heterodimer TCR [5-9].

Several monoclonal antibodies (MAb) are available that react with various subunits of the TCR. Tissue section immunohistology with MAb β-F1 and anti-TCRδ1, specific for framework determinants on the TCR-β and TCR-δ chains respectively, has shown almost all lymphocytes in the T zones of peripheral lymphoid organs and inflammatory sites to express the TCR-β chain; a very small number of cells in these areas express the TCR-δ chain [2,3,10,11]. In contrast, almost one-third of peripheral T cell lymphomas in a large series lacked expression of either TCR-β or δ chain. The remaining two-thirds of the lymphomas expressed TCR-β but not δ chain [11].

Mycosis fungoides (MF) is a lymphoma composed of T cells that usually express a mature peripheral helper T cell immunophenotype [12,13]. This mature Thelper phenotype is generally shared by the majority of lymphocytes in a wide variety of inflammatory skin lesions [12,13]. In the current study, skin biopsies of MF and several different benign lymphocytic inflammatory lesions were examined immunohistochemically for TCR-associated antigens including CD3, TCR-β, and TCR-δ chains. The vast majority of T cells in inflammatory skin lesions and MF expressed the phenotype found on most peripheral T cells: CD3+ TCR-β+ TCR-δ-. An exception to this phenotype was seen in two types of lesions: 1) in 3 of 6 cases of tumor stage MF, the lymphoma cells lacked expression of TCR-β (2 cases) or CD3 (1 case) in addition to TCR-δ and 2) in 2 of 34 cases of patch/plaque MF, intraepidermal T cells lacked TCR-β (1 case) or CD3 and TCR β (1 case). These antigens, however, were expressed by the intradermal T cells.

MATERIALS AND METHODS

Forty skin biopsies of MF (36 patients) and 16 biopsies of benign lymphocytic inflammatory skin lesions (16 patients) were examined. Three sequential biopsies (two patch/plaque and one tumor) were obtained from each of two MF patients. The number of biopsies in each diagnostic category were as follows: patch/plaque MF (34), tumor MF (6), psoriasis (3), cutaneous lymphoid hyperplasia (3), allergic contact dermatitis (2), drug eruption (2), and one each of lichen planus, spongiotic dermatitis, lichenoid keratosis, pityriasis rubra pilaris, pityriasis lichenoides chronic, and pityriasis lichenoides et varioliformis acuta (PLEVA). All lesions were diagnosed using standard clinical and histologic criteria [13]. Histologic features of patch/plaque MF consisted of a lichenoid infiltrate of atypical mononuclear cells with epidermotropism. The MF tumors contained a deep dermal infiltrate as well. All MF cases contained either well-developed Pautrier's microabscesses or smaller focal intraepidermal collections of atypical mononuclear cells in the absence of spongiosis. All cases of MF were subclassified as patch/plaque or tumor based on clinicopathologic correlation [14].

A portion of each biopsy was frozen, cut, and immunostained using a three-stage avidin-biotin-horseradish peroxidase system as...
Table I. Immunophenotype of T Cells in Mycosis Fungoides and Benign Dermatoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
<th>CD3</th>
<th>TCR-β</th>
<th>TCR-δ</th>
<th>Location*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-patch/plaque</td>
<td>30</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF-patch/plaque</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
<td>10-20%+</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF-tumor</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF-tumor</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF-tumor</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF-patch/plaque</td>
<td>1</td>
<td>+</td>
<td></td>
<td></td>
<td>Epidermis</td>
</tr>
<tr>
<td>MF-patch/plaque</td>
<td>1</td>
<td>-</td>
<td></td>
<td></td>
<td>Dermis</td>
</tr>
</tbody>
</table>

* Location of T cells only in cases showing discordant phenotype of epidermal versus dermal T cells.

Each patient had two previous patch/plaque MF lesions (included in row 1 of this table) in which the T cells were CD3+TCR-β+TCR-δ-.

ND: not determined.

previously described [15]. A large panel of MAb directed against T-lineage differentiation antigens as well as other hematolymphoid antigens was applied to each biopsy [3,15]. These antibodies included anti-Leu-4, anti-Leu-3a, and anti-Leu-2a (anti-CD3, CD4, and CD8, respectively; Becton-Dickinson, Mountain View, CA), BF-1 (anti-TCR-β chain framework determinant; courtesy of Michael Brenner), and TCR-δ (anti-TCR-δ chain framework determinant; courtesy of M. Brenner). Primary antibodies were replaced with species and isotype matched irrelevant MAb as negative controls. Sections of reactive tonsils were used as positive controls [3,11]. Positive staining was interpreted to indicate cell surface or cytoplasmic expression, or both.

Sections were examined by light microscopy and the number and location of T cells, as defined by T cell markers including CD2 and CD5, was determined. Sequential sections were then evaluated for expression of CD3, TCR-β, and TCR-δ by the T cells. The CD3 and TCR-β expression was graded as positive (the vast majority of T cells expressing the antigen) or negative (<5% of T cells expressing the antigen). The TCR-δ expression was graded as negative as described above or, in the rare cases showing more than 5% TCR-δ+ T cells, as the estimated percent (rounded to the nearest 10%) of TCR-δ+ cells. In a few cases of MF, the phenotype of the T cells in the epidermis differed from that of the T cells in the dermis. In these cases, expression of each antigen was evaluated and reported separately for the intraepidermal versus the intradermal T cells.

RESULTS

The results are summarized in Table I. In almost every biopsy the majority of the lymphoid cells were T cells, except for the three biopsies of cutaneous lymphoid hyperplasia that contained intrader-
mal B cell follicles surrounded by large zones of T cells [16]. Thirty-five biopsies of MF had a T-helper (CD4+) phenotype, 4 MF lesions (from 2 patients) had a T-cytotoxic/suppressor (CD8+) phenotype, and 1 MF lesion failed to express either T-subset marker. Variable proportions of CD4+ and CD8+ T cells were seen in the inflammatory lesions [12,13,16].

In all inflammatory lesions and the majority of MF lesions, the T cells co-expressed CD3 and TCR-β (Fig 1). Cells expressing TCR-δ were extremely rare, accounting for less than 1–2% of the T lymphocytes in most biopsies (Fig 2). In 2 cases of MF, 1 case of PLEVA and 1 case of lichen planus, approximately 10–20% of the T cells expressed TCR-δ (Fig 2). Most CD3+ cells in these 4 cases were CD4+CD8-TCR-β+. There was no expression of TCR-δ by dendritic cells in the epidermis in any case, suggesting that the Thy-1+ TCR-δ+ dendritic cells found in mouse epidermis do not have a counterpart in human skin [17].

Six cases of tumor stage MF were studied. The T cells in biopsies of tumor lesions from 2 patients were CD3+TCR-β-TCR-δ- (Fig 3). Two previous patch/plaque MF biopsies were studied from each of these 2 patients. In both cases, most of the T cells in the patch/plaque lesions had the CD3+TCR-β+TCR-δ- phenotype (Fig 1). In the third case of tumor MF, the T cells had a markedly abnormal phenotype with loss of most pan T cell markers including CD3 but not TCR-β. The cells also failed to express TCR-δ chain antigen. The T cells in the final 3 cases of tumor MF were CD3+TCR-β+TCR-δ-.

In 2 cases of patch/plaque MF, the CD3/TCR phenotype of the T cells in the epidermis differed from that of the T cells in the dermis. In one of these cases, the dermal T cells were CD3+TCR-β+TCR-δ-. The epidermis and dermo-epidermal junction contained numerous T cells, as scattered single cells and small clusters of 2–3 cells, which showed loss of the TCR-β antigen as well as pan T cell antigens CD2 and CD5. In the other case, T cells in the dermis were CD3+TCR-β+TCR-δ-, whereas T cells in an overlying Pautrier's microabscess were CD3+TCR-β+. As the microabscess was present on only a few sections and single cell exocytosis was rare, the complete phenotype of the epidermal T cells could not be determined.

DISCUSSION

Early lesions of MF are often extremely difficult to diagnose because their clinical and histologic appearance can resemble that of some benign dermatoses. Immunoperoxidase studies using certain MAb can sometimes be helpful in making this diagnostic distinction [13]. The current data, however, suggest that such studies using antibodies against CD3 or TCR would be of limited usefulness in distinguishing patch/plaque MF from reactive lymphocytic infiltrates in that the majority of T cells in each of these two lesions have the phenotype characteristic of most peripheral T-cells (CD3+TCR-β+TCR-δ+) [2,3,11]. These anti-CD3/TCR antibodies would have been useful in making a diagnosis of MF in only 2 of 34 biopsies of patch/plaque MF in this study. In these two lesions, T cells within the epidermis (but not within the dermis) had an abnormal T cell phenotype suggestive of malignancy (CD3+TCR-β-TCR-δ-).

Individual components of the TCR-CD3 complex may be present in the cytoplasm of maturing thymocytes without other components being present [18–20]; however, T cells with aberrant (discordant) CD3/TCR phenotypes were not seen in Pautrier's microabscesses. As the microabscess was present on only a few sections and single cell exocytosis was rare, the complete phenotype of the epidermal T cells could not be determined.

In contrast to patch/plaque MF, discordant TCR phenotypes were present in 3 of 6 MF tumors. Furthermore, 2 cases of tumor
MF expressing an abnormal TCR phenotype (CD3+TCR-β-TCR-δ-) had prior patch/plaque lesions expressing the typical mature TCR phenotype (CD3+TCR-β+TCR-δ-). This demonstrates that loss of TCR antigens in MF can occur with progression of disease, as has been previously shown for antigens such as CD5, CD3, and CD11 [12].

The current TCR data, in conjunction with previously published immunophenotypic data, suggest that the neoplastic cells of patch/plaque MF tend to resemble mature peripheral helper T lymphocytes while the neoplastic cells of tumor MF often resemble those of peripheral T cell lymphomas [2,3,11-13,21,22]. The CD3-TCR discordance has been reported in as much as one third of the latter lesions [3,23]. This latter resemblance is also reflected in the cutaneous nodule formation, lack of epidermotropism, and propensity for extracutaneous involvement shared by patients with tumor MF and those with non-MF peripheral T cell lymphomas [11-14,21,22]. These findings suggest important differences between patch/plaque MF and tumor MF that cross clinical, histopathologic, and immunophenotypic boundaries; they may help to explain the more aggressive clinical behavior of tumor stage MF in terms of a biologic similarity to non-MF peripheral T cell lymphomas.

TCR-δ+ T cells, most of which lack the CD4 and CD8 subset markers, are generally rare in the peripheral blood T cell population [1,6,8,9]. However, there is wide individual variation (1-18%) in the number of peripheral blood lymphocytes that react with TCR-δ1 [9]. Because recent studies suggest this antibody is a pan-TCR-δ marker [9], TCR-δ1+ cells should accurately reflect the proportion of T cells expressing the TCR-γδ heterodimer. The TCR-δ+ cells were extremely scarce in most of the biopsies examined in the current study, suggesting that generally there is no selective recruitment of these cells to skin lesions. Additional studies are warranted, however, because two biopsies of patch/plaque MF and the solitary biopsies of lichen planus and pityriasis lichenoides et varioformis acuta that were studied did contain 10-20% TCR-δ+ cells.

The TCR-δ+ cells may play a role in immune surveillance against tumors by means of cytotoxic activity [7,8,24]; however, the rarity of TCR-δ+ cells in most MF lesions suggests they are not typically a major host response factor in MF. An exception may be in the 2 cases of MF in this study in which 10-20% of the T cells expressed TCR-δ chain. In these 2 cases, there may be selective recruitment of TCR-δ-bearing T cells into the MF lesions. Alternatively, these 2 patients may have high circulating levels of TCR-δ+ T cells [9]. If these cells are in fact important in tumor-related immune responses, these 2 patients might be expected to pursue a favorable clinical course.

Interestingly, one of these patients (T1N0M0B0 at diagnosis) is alive with limited patch/plaque disease at 10 years after treatment with electron beam radiation and topical nitrogen mustard; the other (T1N0M0B0 at diagnosis) is alive without disease at 6 years after treatment with topical nitrogen mustard. However, studies comparing the numbers of TCR-δ+ T cells in MF lesions with that in each patients’ peripheral blood, as well as long-term follow-up of additional patients, will be needed to clarify this issue.

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