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Discordant Expression of Antigens Between Intraepidermal and Intradermal T Cells in Mycosis Fungoides

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Using immunohistochemical methods, the authors studied the expression of pan-T- and majority-T-cell antigens (CD5, CD2, CD3, TCR-β, CD7) and T-cell subset antigens (CD4, CD8) in cutaneous T cells in mycosis fungoides (MF) (177 biopsies from 124 patients) and a variety of inflammatory lesions (45 biopsies from 45 patients). The authors detected the absence of pan-T- or majority-T-cell antigens, or of both T-cell subset antigens, from T cells in the epidermis but not the dermis in 15 MF biopsies (8%) from 11 MF patients (9%), but in none of the inflammatory skin lesions. The opposite picture, characterized by lack of antigen expression by the dermal T cells only, was not seen in any of the MF or inflammatory lesions. The absence of antigen expression by epidermal but not dermal T cells, which the authors have termed antigen discordance, was most prevalent for CD5, CD7, and TCR-β, each being discordant in 6% to 7% of MF cases or patients tested. Among the MF biopsies showing antigen discordance, 14 of 15 biopsies (93%) from 10 of 11 patients (91%) were discordant for two or more antigens. Antigen discordance was not an artifact of treatment, because none of the patients showing discordance was receiving treatment at the time of their initial discordant biopsy. The discordance was the only immunophenotypic abnormality detected in 8 of 15 (53%) of the discordant MF biopsies. Thus, this antigen discordance was an important diagnostic feature that allowed the immunophenotypic distinction of MF from a variety of inflammatory skin lesions. (Am J Pathol 1990, 137:1447-1451)

Mycosis fungoides (MF) is a cutaneous T-cell lymphoma composed of cells that usually express a mature peripheral T-helper cell ($CD4^+$) immunophenotype.[1,2] Early skin lesions of MF are often difficult to diagnose because their clinical and histologic appearance can resemble that of some benign inflammatory dermatoses. Attempts at using immunophenotyping to separate MF from these inflammatory skin lesions can be disappointing because many lymphocytes in the inflammatory lesions share the mature T helper phenotype with MF cells.[1-4]

In many cases of MF, the neoplastic cells show the absence or deficiency of one or more of the pan-T-cell antigens (such as CD3, CD5, or CD2 found on all mature non-neoplastic T cells) or majority-T-cell antigens (such as CD7, Leu-8 or TCR-β found on the majority of mature non-neoplastic T cells), or of both T-cell subset (CD4, CD8) antigens.[1-4] Absence of pan-T-cell antigen, TCR-β, or both subset antigens provides strong support for a diagnosis of MF because such an abnormality is virtually never seen in inflammatory skin lesions.[3,4] Deficiency of the majority-T-cell antigen Leu-8 is nonspecific. Deficiency of CD7 provides some support for a diagnosis of MF in that such deficiency is more common in MF (60% to 70% of cases) than in inflammatory dermatoses (10% to 20% of cases).[2] In most cases of MF reported to show antigen deficiency, the deficiency is evident in the majority of the T cells throughout the skin lesion. We now report a study of 177 MF skin biopsies from 124 patients in which 15 biopsies from 11 patients exhibited absence of pan-T- or majority-T-cell antigens, or of both T-cell subset antigens, in the intraepidermal (IED) but not the intradermal (ID) T cells. In contrast, such antigenic discordance between

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the T cells in epidermis *versus* dermis was not seen in 45 biopsies of inflammatory skin lesions.

Materials and Methods

One hundred seventy-seven skin biopsies of MF (124 patients) and 45 biopsies of benign lymphocytic inflammatory skin lesions (45 patients) were examined. All lesions were diagnosed using standard clinical and histologic criteria.[2] The MF cases showed a dermal infiltrate with epidermotropism and either well-developed Pautrier's microabscesses or smaller focal intraepidermal collections of atypical mononuclear cells in the absence of spongiosis. One hundred seventy-three MF skin biopsies were classified as patch/plaque and four as tumor, based on clinicopathologic correlation.[5] The inflammatory lesions, selected to include only those in which the lymphocytic infiltrate involved both dermis and epidermis, were as follows: allergic contact dermatitis, 12 biopsies; erythrodermic psoriasis, nine; spongiotic dermatitis, seven; lichen planus, six; drug eruption, four; atopic dermatitis, three; nummular dermatitis, two; lichenoid keratosis, one; and pityriasis lichenoides chronica, one.

A portion of each biopsy was frozen, cut, and immunostained using a large panel of monoclonal antibodies (MAb) directed against B-cell, T-cell, and monocyte/macrophage antigens as previously described. Monoclonal antibodies against T-cell differentiation antigens included Leu-1 (CD5), Leu-2 (CD8), Leu-3 (CD4), Leu-4 (CD3), Leu-5 (CD2), Leu-8 (anti-lymphocyte homing receptor), Leu-9 (CD7) (Becton-Dickinson, Mountain View, CA), and βF-1 [anti-T-cell receptor beta chain (TCR-β)] (courtesy of Dr. Michael Brenner).[3] The number of MF biopsies (cases) stained for each antigen were as follows: 177 (124) were stained for CD5, CD8, CD4, CD3, CD2; 155 (118), CD7; 123 (95), Leu-8 antigen; and 48 (43), TCR-β. All 45 biopsies of inflammatory lesions were stained for each antigen except TCR-β, where 12 cases were stained.

Sections were examined by light microscopy and the number and location of T cells determined. These T cells were defined by expression of pan-T-cell antigens and included both malignant MF cells and benign lymphocytes. Sequential sections were then evaluated as previously described for estimated expression (rounded to the nearest 10%) of each of the above-listed T-cell differentiation antigens by T cells in these microenvironmental locations: 1) all T cells in the lesion, 2) ID T cells only, and 3) IED T cells only (including cells at the dermal–epidermal junction and within hair follicles).[3] A biopsy was labeled as discordant if 1) at least one pan-T- or majority-T-cell antigen differed in its expression between IED and ID T cells by at least 30%, or 2) the sum of the subset markers (CD4 + CD8) differed by at least 30% for IED *versus* ID T cells. Thirty percent was chosen as the cut-off value for discordance based on previous work in our laboratory examining accuracy and reproducibility of estimating lymphocyte subsets on tissue section immunohistochemistry slides.[6] A biopsy was considered to be antigen deficient if: 1) at least one pan-T- or majority-T-cell antigen was expressed by less than 50% of the T cells in the lesion, or 2) the sum of the subset markers on the T cells in the lesion was less than 50%.

Results

The 45 biopsies of inflammatory skin lesions were examined closely for discordance in expression of pan-T- and majority-T-cell markers CD5, CD2, CD3, TCR-β, Leu-8 antigen, and CD7, and the sum of the subset markers CD4 and CD8, between T cells in the epidermis *versus* those in the dermis. There was no discordance or overall deficiency in T-cell expression of CD5, CD2, CD3, TCR-β, or CD4 + CD8 in any of these inflammatory lesions. None were discordant for CD7, although five (11%) of the lesions were CD7 deficient (Figure 1). Discordant expression of the Leu-8 antigen was found in 18 (40%) of the lesions, half of which also showed an overall deficiency of Leu-8. All 18 biopsies were characterized by loss of Leu-8 antigen by IED T cells with respect to the ID T cells. Six (13%) lesions were Leu-8 deficient but not discordant, whereas 21 (47%) lesions were neither deficient nor discordant.

Because discordance for CD5, CD2, CD3, TCR-β, CD7, or the sum of CD4 and CD8 was not seen in any

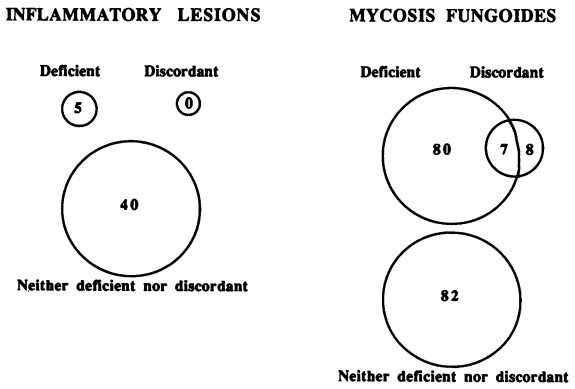

Figure 1. *Venn diagram of discordance and deficiency for pan-T and majority-T-cell antigens (CD5, CD2, CD3, TCR-β, CD7) and T-cell subset antigens (CD4 and CD8) in cutaneous T cells.* **Left:** *There was no evidence of discordant antigen expression between epidermal versus dermal T cells in 45 biopsies of inflammatory lesions. Five biopsies showed an overall deficiency of CD7.* **Right:** *Discordance for one or more antigens was seen in 15 of 177 biopsies of MF. Seven of these fifteen biopsies also showed an overall deficiency for at least one antigen.*

Table 1. *Mycosis Fungoides: Antigen Discordance Between Intraepidermal and Intradermal Lymphocytes*

Antigen (antibody)	No. of patients discordant for antigen/No. of patients with any discordance stained for antigen
CD5 (Leu-1)	8/11 (73%)
CD7 (Leu-9)	7/10 (70%)
CD2 (Leu-5)	5/11 (45%)
TCR-β (βF-1)	3/7 (43%)*
CD3 (Leu-4)	1/11 (9%)
CD4 (Leu-3) plus CD8 (Leu-2)	3/11 (27%)
Two or more antigens	10/11 (91%)

* TCR-β/CD3 immunophenotype on two patients was previously reported.[4]

of the inflammatory lesions, these antigens were emphasized in the evaluation of the MF lesions. Discordance for at least one of the pan–T- or majority–T-cell antigens, or for the subset antigens, was found in 15 of the 177 biopsies of MF (8%) from 11 of the 124 patients (9%) ($P < 0.1$ for patients with MF *versus* inflammatory lesions; chi-square test with Yates correction) (Table 1, Figure 2). All 15 biopsies were of patch/plaque MF. Discordance for two or more antigens was seen in 14 of 15 biopsies (93%) from 10 of 11 patients (91%). The antigens most likely to show discordance were CD5, CD7, and TCR-β, each being discordant in 6% to 7% of the patients or biopsies examined. CD3 discordance was the least prevalent, being seen in less than 1% of patients and biopsies.

In each of the 15 MF biopsies showing discordance, the antigen loss was by IED T cells. The reverse picture, characterized by loss of antigen by ID T cells with preservation of antigen expression by IED T cells, was not seen in any of the biopsies. In all discordant MF cases, differences in expression of at least one antigen between IED and ID T cells exceeded the threshold of 30% needed to classify a case as discordant. For these cases, the mean percent difference (range) in ID *versus* IED T cells expressing the discordant antigen was as follows: CD5 = 75 (50 to 100); CD2 = 73 (50 to 90); CD3 = 100 (one case); TCR-β = 73 (50 to 90); CD7 = 52 (30 to 80); CD4 + CD8 = 92 (80 to 100). In 14 of 15 (93%) cases showing antigen discordance, the discordance was an important feature in lending immunologic support to a diagnosis of MF; in eight of these biopsies antigen discordance was the only immunophenotypic abnormality present, whereas six biopsies showed antigen discordance plus CD7 deficiency.[2] The remaining discordant biopsy showed deficiency for CD5 in addition to CD7 (Figure 1).

One biopsy was available for immunophenotyping in each of seven patients with discordant MF. None of these patients was receiving specific treatment at the time of biopsy. Five of these patients had not undergone specific treatment before biopsy. One patient had completed therapy with topical nitrogen mustard 1.5 years before biopsy, whereas another patient had completed intramuscular methotrexate therapy and photochemotherapy with 8-methoxypsoralen and long-wave ultraviolet A (PUVA) more than 2 years before biopsy.

Sequential biopsies were available for study in 4 of the 11 patients with discordant MF. Patient 1 had four biopsies over a 9-week period. The first biopsy was taken before any specific treatment; biopsies 2 through 4 were taken after initiation of PUVA therapy. There was discordance for CD5, CD7, and subset antigen expression in all four biopsies, and for CD2 expression in biopsies 3 and 4. CD7 was deficient in biopsies 1, 3, and 4. Patients 2, 3, and 4 had two biopsies each. The initial biopsies were taken before any specific treatment, whereas the second biopsies were taken after initiation of specific therapy, as described below. The initial biopsy from patient 2 was

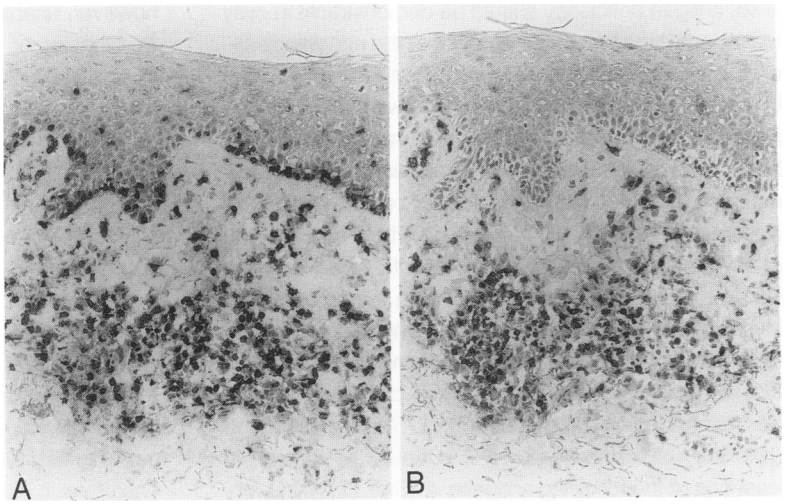

Figure 2. *Antigen discordance in MF.* **A:** *Skin biopsy demonstrating CD3⁺ T cells in both epidermis and dermis (40×).* **B:** *Serial section showing CD2 discordance. Intradermal T cells are CD2⁺, while intraepidermal cells are CD2⁻ (40×). Immunoperoxidase with methylene blue.*

discordant for CD5 and CD7, while the second biopsy, taken 3 years later during topical nitrogen mustard therapy, was deficient in CD7. The initial biopsy from patient 3 showed discordance for CD5, CD2, and CD7. The second biopsy, taken 6 months later, after the patient had completed electron beam radiation and begun topical nitrogen mustard therapy, showed discordance for CD2, discordance and deficiency for CD5, and deficiency for CD7. Patient 4 showed discordance for CD7 in the first biopsy, while a second biopsy, taken 1 year later during topical nitrogen mustard therapy, showed deficiency of CD7. CD5 and TCR-β, although discordant in the first biopsy, were expressed by the majority of the IED and ID T cells in the second biopsy.

Discussion

Immunophenotypic analysis is often used in an attempt to distinguish early lesions of MF from a variety of benign inflammatory skin lesions, which can have similar clinical and histologic findings. In many instances, however, immunophenotyping fails to separate MF from inflammatory lesions because most lymphocytes in both groups of lesions have a mature T-helper cell phenotype.[1-4]

Deficiency of T-cell antigens normally expressed by most non-neoplastic T cells can occur in MF. Unfortunately, this deficiency is frequently seen in skin biopsies of tumor MF, which is generally not a diagnostic problem, but appears to be less common in patch or plaque lesions.[1-4] This apparent loss of antigen expression with neoplasm progression may be due to mutation or changes in gene expression, or to emergence of a new neoplastic clone. Alternatively, the antigen deficiency might be present in MF cells in early lesions but is not easily detectable using standard tissue section immunophenotyping. In part, this may be due to tumor cells in the MF lesion being obscured by an infiltrate of non-neoplastic T cells. If this is so, antigen deficiency should be demonstrable in early MF lesions if the immunophenotype of the non-neoplastic cells is evaluated separately from that of the MF cells. Unfortunately, cytologic differences between MF cells and non-neoplastic lymphocytes are generally so slight that frozen-section immunoperoxidase staining does not allow definition of individual cells as neoplastic or non-neoplastic. Another approach to separate MF cells from reactive lymphocytes is by their microenvironmental location in the skin. As many of the lymphoid cells in the epidermis of MF lesions are cytologically abnormal and might represent MF cells, we chose to examine the immunophenotype of these cells to determine if they exhibited antigen loss that was not apparent in the underlying ID T cells. We found deficiency of expression by IED but not ID T cells of at least one of the following antigens in 15 of 177 skin biopsies of MF but not in any of 45 biopsies of inflammatory skin lesions: pan-T markers CD5, CD2, and CD3; the majority T-cell markers CD7 and TCR-β; and the sum of the subset markers CD4 and CD8. Furthermore, discordance for two or more antigens was present in 14 of 15 skin biopsies (93%). In over half of the 15 MF skin biopsies showing antigen discordance, no immunophenotypic abnormalities would have been noted if only the overall immunophenotype of the T cells in the skin lesion was determined.

This immunophenotypic discordance was not an artifact of treatment, because none of the 11 patients whose biopsies showed discordance was receiving treatment at the time of their initial discordant biopsy. In fact, only two of these patients had ever received any prior therapy for their MF other than topical corticosteroids, and these patients had been off such therapy for more than 1.5 years before biopsy. Furthermore, among the four patients with serial biopsies, discordance was usually decreased or unchanged, rather than increased, in biopsies obtained during specific therapy with topical nitrogen mustard or PUVA.

In this series, the antigens most frequently lost by the IED T cells in MF were CD5, CD7, and TCR-β. This correlates with previous reports showing overall deficiency of these antigens in MF.[3] Although there are isolated reports of discordant reactivity of MAbs HML-1 (raised against human intraepithelial lymphocytes; reacting with an activation antigen) and Ki-67 (reacting with a proliferation antigen) with IED *versus* ID T cells in MF, the usefulness of these findings in the differential diagnosis of early MF from inflammatory skin lesions has not been defined.[7-10] There is one report suggesting possible antigen discordance for CD2, CD4, or CD5 in three cases of pityriasis lichenoides.[11] Unfortunately, this report contains no quantitative immunophenotypic data or clinical details regarding these cases, eg, whether they include lymphomatoid papulosis cases and whether there is any potential treatment effect on biopsy results. We were, however, unable to demonstrate antigen discordance in 45 biopsies of inflammatory skin lesions obtained from 45 patients, including a single case of pityriasis lichenoides chronica.

TCR-β, defined by MAb βF-1, is technically not a pan–T-cell antigen because a small number (usually less than 5%) of mature T cells instead express the gamma delta TCR.[12] In reality, however, TCR-β functions as a pan-T antigen in the differential diagnosis of peripheral T-cell lymphomas (including MF) from reactive T-cell proliferations in that loss of TCR-β antigen is strongly suggestive of a diagnosis of lymphoma.[3,4]

Antigenic discordance for Leu-8, characterized by loss of Leu-8 by IED T cells relative to ID T cells, was seen in multiple skin biopsies of inflammatory lesions and MF. Therefore, it was a diagnostically nonspecific feature of

cutaneous T-cell infiltrates. The Leu-8 antigen was recently shown to be the human homolog of the mouse MEL-14–defined lymphocyte 'homing receptor' which is involved in lymphocyte migration to lymph nodes.[13–15] Activation of lymphocytes by antigen or mitogen causes down-regulation of Leu-8 antigen expression, suggesting that the Leu-8–negative T cells seen in epidermis may be activated.[14,15] Alternately the Leu-8 antigen may be involved in some way in lymphocyte migration to dermis but not epidermis.

CD7 discordance was noted in 6% of our MF biopsies, but in none of 45 inflammatory lesions. One should be cautious in interpreting isolated loss of CD7 by epidermal T cells as a definitive sign of malignancy, however, as CD7, a majority T-cell marker, can be deficient among combined epidermal and dermal T cells in inflammatory skin lesions (11% of the cases in this study).[2,3]

Essentially all mature non-neoplastic T cells are $CD4^+CD8^-$ (T helper) or $CD4^-CD8^+$ (T cytotoxic).[16] Thus, the sum of $CD4^+$ and $CD8^+$ lymphocytes in an inflammatory lesion should be equal to the number of T cells. The same would be true in most cases of MF, as MF cells are generally $CD4^+$ $CD8^-$. In the rare cases of MF that fail to express either subset antigen, the sum of the $CD4^+$ and $CD8^+$ MF cells would be 0. As evaluation of IED *versus* ID T cells for discordance in either subset marker alone could show selective migration of T-cell subsets rather than antigen loss, we evaluated the sum of the $CD4^+$ + $CD8^+$ lymphocytes in IED *versus* ID T-cell populations. There was no discordance for expression of the sum of these subset antigens in the 45 inflammatory skin lesion biopsies and in most biopsies of MF in this series. In 3% of the MF lesions, however, IED T cells failed to express either of these markers, suggesting loss of subset antigen by the malignant cells in the epidermis but not the dermis.

In summary, deficiency of pan-T or majority-T antigens, or of both T-cell subset antigens, by T cells within the epidermis but not in the underlying dermis, is not uncommon in MF, being present in 9% of cases. Our data suggest that this discordant expression is an important diagnostic feature that may allow the immunophenotypic distinction of MF from a variety of inflammatory skin lesions.

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