Immunohistochemical staining for CD45R isoforms in paraffin sections to diagnose mycosis fungoides–type cutaneous T-cell lymphoma

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The definitive diagnosis of mycosis fungoides (MF)–type cutaneous T-cell lymphoma (CTCL) is difficult because a cumulative set of information is typically required: clinical features, histopathology, and special diagnostic tests (typically immunophenotyping and T-cell receptor gamma [TCRγ] gene rearrangement). Fresh tissue is not always available for the special tests. We report a simple and readily available procedure evaluating the staining pattern on formalin-fixed, paraffin-embedded skin that can help with the diagnosis of patch/plaque stage MF. We reviewed 92 cases of MF or probable MF that had clinical information, immunophenotyping and TCRγ gene rearrangement studies and that had been evaluated in our multidisciplinary lymphoma conference. We used antibodies to the isoforms of CD45, CD45RO for mature T cells and CD45RB for subsets of T cells. When atypical CD45RB-positive/CD45RO-negative cells were seen in nonspongiotic epidermis, the individuals had a high cumulative clinical and histologic score for MF. In contrast, 15 cases of known contact dermatitis showed a reactive pattern of both CD45RB- and CD45RO-positive cells in spongiotic epidermis. We compared the epidermal CD45RB-positive/CD45RO-negative staining pattern with CD7 deficiency by immunophenotyping and TCRγ gene rearrangement, two commonly used methods in the diagnosis of MF. The epidermal CD45RB-positive/CD45RO-negative staining pattern is comparable and may be better in equivocal cases of possible MF. Therefore immunostaining for CD45RB and CD45RO on paraffin sections is a simple, reliable, and convenient modality in the diagnosis of MF. (J Am Acad Dermatol 2007;56:635-42.)

Mycosis fungoides (MF)–type cutaneous T cell lymphoma (CTCL) is typically an indolent primary cutaneous lymphoma in which a malignant clone of CD4-positive ($CD4^+$) T helper cells infiltrates the skin and epidermis. The mechanism by which the malignant T cells home to epidermis is unknown. The diagnosis of MF-type CTCL has traditionally been made by correlating clinical criteria with routine histopathology. A definitive pathologic diagnosis depends on identification of clusters of small cells with wrinkled hyperchromatic nuclei (Pautrier microabscesses) in the epidermis. Other histologic features of MF include haloed lymphocytes, exocytosis of lymphocytes, disproportionate epidermotropism, epidermal lymphocytes larger than dermal lymphocytes, hyperconvoluted intraepidermal lymphocytes, and lymphocytes aligned within the basal layer.[1,2] Molecular studies are increasingly sophisticated and helpful in the diagnosis of MF-type CTCL and are available in many major centers. Southern blot analysis of T-cell rearrangement (TCR) β or γ chain genes and

Abbreviations used:

CTCL:	cutaneous T-cell lymphoma
MF:	mycosis fungoides
PCR-DGGE:	polymerase chain reaction denaturing gradient gel electrophoresis
TCR:	T-cell receptor

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denaturing gradient gel electrophoresis of polymerase chain reaction products of γ chains (PCR-DGGE) are now a part of routine evaluation for CTCL.[3] PCR-DGGE can identify a malignant clone with high sensitivity (as few as one malignant cell/100 total T cells).[4] Immunophenotyping studies on frozen or paraffin sections of skin that demonstrate a loss of mature T-cell markers such as CD3 and CD7 are also used for diagnosis of suspected CTCL.[5] Flow cytometry is increasingly used now when fresh tissue is available.

The major problem with the diagnosis of early MF-type CTCL is that patients with minimal clinical disease (a few patches of atrophy or erythema in bathing trunk areas) often have subdiagnostic disease as well. The lack of consensus for standardized diagnostic criteria for the early stage of MF produces variability among pathologists' diagnosis of MF.[6] The disease may progress by slow evolution over a long period before a definitive diagnosis can be made, often after a series of biopsies. In addition, many pathology laboratories do not have the molecular and immunophenotyping studies readily available. Although CD3, CD4, CD7, and CD8 antibody immunostaining methods on paraffin sections are also now available, in our hands, immunophenotyping with these antibodies is not as helpful as the method we report herein.

This immunostaining method for paraffin sections allows discrimination of early patch/plaque stage MF-type CTCL using markers for CD45 isoforms, CD45RO, and CD45RB, which are in use in most surgical pathology laboratories. Both antigens are high molecular weight glycoproteins produced by alternative RNA splicing of three exons (A, B, and C) of a single gene on human chromosome 1.[7,8] CD45 is expressed on all hematopoietic cells except red blood cells and platelets. CD45RO (UCHL1) recognizes distinct antigens on activated and mature memory T cells. CD45RB (common leukocyte antigen) is expressed in high amounts on peripheral B cells, cytotoxic and suppressor T cells, a subset of T helper cells, and most thymocytes.[9-13] CD45RB expression decreases as T cells progress from naïve to more mature memory cells, suggesting that it is expressed predominantly on immature T cells.[14,15]

We studied the staining pattern in biopsy specimens from 92 patients with known or suspected MF and in biopsy specimens from 15 known patients with contact dermatitis. We show herein that an increased number of atypical $CD45RB^+$, CD45RO-negative ($CD45RO^-$) cells in nonspongiotic epidermis correlated with the clinical and the routine histologic diagnosis of MF, and with special diagnostic tests. In contrast, biopsy specimens of known contact dermatitis showed a nonspecific staining pattern, in which both $CD45RB^+$ and $CD45RO^+$ cells were seen in spongiotic epidermis. We compared this epidermal $CD45RB+/CD45RO^-$ staining pattern with TCRγ gene rearrangement and CD7 deficiency by immunophenotyping on frozen sections of affected skin, two well-established and commonly used methods, and found that this $CD45RB^+/CD45RO^-$ staining pattern on paraffin sections has similar sensitivity and specificity. This unique staining pattern that presumably reflects loss of mature T-cell markers is helpful in the diagnosis of MF in biopsy specimens in which very subtle epidermotropism is present. It is well accepted that MF cells are $CD45RO^+$ in frozen sections and by flow cytometry. The absence of CD45RO staining of epidermotropic T cells in paraffin sections of MF may be due to the different method (paraffin embedding vs frozen tissue), but is nevertheless useful, as we demonstrate in this study. Because fresh tissue is not always available for immunophenotyping studies on frozen sections and flow cytometry, the CD45RB/CD45RO staining pattern can be performed on the same tissue block used for routine histology. This method also allows retrospective studies on archived material with routinely available antibodies on paraffin sections of skin.

PATIENTS AND METHODS

Patients

All patients in this study (N = 92) were enrolled in the Cutaneous Lymphoma program at University Hospitals, Case, Cleveland, Ohio and were drawn from a referral area that covers most of northeastern Ohio. Many are referred by clinicians in the community for diagnosis and therapy. We selected the patients who had known classic $CD4^+$ patch- or plaque-stage MF or suspected MF, had phenotyping studies on frozen skin, and TCRγ gene rearrangement by PCR—DGGE on frozen skin. We limited the MF group to only those individuals who had all 3 types of analysis performed in the same manner (histology read by A. G.; immunophenotyping on frozen sections performed in the laboratory of G. W. and read by G. W. and S. S., and gene rearrangement by PCR-DGGE performed in the laboratory of and interpreted by G. W.). The individuals in this unique data set are a small percentage of the total numbers of patients who have been studied by using the CD45RO/D45RB staining method. These patients were all evaluated at least once in a multidisciplinary lymphoma conference in which all clinical, pathologic, and laboratory studies were reviewed.[16] We compared immunostaining results with antibodies to CD45RB and CD45RO on paraffin-embedded biopsy

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