Editorial review

Immunodiagnosis in cutaneous T cell lymphoma: how does gene expression of the variable region of the T cell receptor fit into the diagnostic and pathophysiological picture of T cell neoplasia

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Mycosis fungoides (MF) is a cutaneous T cell lymphoma (CTCL) which can present as localized indolent patches and plaques that clinically resemble inflammatory dermatoses, such as psoriasis, or as rapidly progressive tumors with infiltration of lymph nodes and viscera (1). The immunology of CTCL has involved multifaceted studies of T cell regulation. In this editorial review, we focus on the latest application of new technology designed to exploit the scientific advances related to the ontogeny of T cell clonal expansion in human disease processes.

In advanced MF cases, survival has generally been poor (2, 3). However, some reports have suggested a curative potential (defined as being disease-free eight years after treatment is discontinued) in early (Stage 1) MF with topical nitrogen mustard and/or total skin electron-beam therapy (2–8). Others have reported combined modality approaches with encouraging results in the early stages of MF, but no significant improvements in overall survival (5–11). Despite the failure of these large randomized trials to cure the majority of patients, the potential for cure in some patients with early MF provides great impetus for establishing this diagnosis as early in its development as possible. However, a patient considered to have MF clinically may require many years and numerous biopsies before the diagnostic histology is recognized at the light microscopic level (12). The principle obstacle is the light microscopic assessment of early CTCL in the differentiation by the pathologist of the lymphoproliferative T cells of MF from benign, reactive T cells associated with inflammatory skin disease. In an effort to overcome these limitations in recognizing early MF, several different techniques, including T cell receptor (TCR) gene rearrangement studies and immunophenotyping of T cell markers, have been proposed as useful tools available to help the diagnosis of MF (13, 14). Molecular-based techniques have attempted to discern a clonal population of T cells that was not apparent on review of routine hematoxylin/eosin-stained sections.

In addition to diagnostic problems related to CTCL, the etiology of CTCL has remained largely unknown. One theory involves chronic environmental antigenic stimulation (15). Recent reports of increased numbers of antigen-presenting cells in MF would tend to support the notion that chronic antigenic stimulation is a factor in the development of MF (16). A case-controlled study, however, showed no associated environmental factors in 100 patients (17). Only those cases of CTCL associated with the HTLV-1 retrovirus have been conclusively related to a specific, transmissible, exogenous agent (18).

What is known about MF/CTCL is that it is a clonal proliferation of T cells which are usually CD3+, CD4+ (helper), CD29+, CD45RO+, CD45RA memory T cells (12–14). Most cases of

advanced disease have a clonal gene rearrangement in the TCR as detected by Southern blot analysis (12, 19). Early lesions, however, are usually observed to contain the TCR in its germline configuration, suggesting that there may be too few monoclonal neoplastic cells for detection by conventional Southern blot analysis (13, 20). Even more established plaque stage MF lesions may have TCR germline configurations (21).

The human TCR receptor is a heterodimer of alpha and beta chains which are non-covalently associated with the CD3 molecule on the T cell surface (22). The alpha and beta chains are structurally similar to immunoglobulins (23, 24). The DNA sequences and genes which encode these chains are a complex cluster of variable (V), constant (C), diversity (D), and joining (J) segments, all separated by non-transcribed exons. During the normal process of gene rearrangement after antigenic exposure, the beta chain has each segment adjoined by the action of the recombinase enzyme system so that a unique, functional V-D-J-C gene is assembled (22–24). A similar process occurs for the alpha chain except no D segments are involved (22). The process of rearrangement is thought to occur in the thymus. The V region of the beta chain (Vβ) represents a cluster of some 60 functional genes which have been grouped into 20 families based on $>$ 75% sequence homology (24). This limitation of diversity is compensated for by varying combinations of J and D genes along with the random insertion of non-germline nucleotides (25).

This limited number of Vβ gene families has allowed development of family specific MoAbs. These antibodies are an exciting advancement in immunopathology as they provide a way to dissect the biologic nature of T cell infiltrates. These MoAbs have the potential to recognize monoclonal vs oligoclonal vs polyclonal dermatoses and assist in correlations between etiology and morphology (26, 27).

In this issue, Gilks et al. (28) report that eight of eight patients with CTCL lacked any restricted (i.e. clonal) Vβ gene usage. This contradicts the report by Jack et al where 10/16 patients showed a predominance of $V\beta_8$ expressing T cells (29). The restricted Vβ gene usage amongst different cases of CTCL is of potential etiopathological as well as diagnostic significance, because it may indicate that some common antigen is involved in these T cell neoplasias (29). Recognition of infiltrates which predominately express a single Vβ gene product allows recognition *in situ* of clonal proliferations. This technique could also eliminate the costly routine use of Southern analysis of DNA in search for clonality in T cell neoplasia. $V\beta_5$ was expressed by 99% of circulating cells in one patient with Sezary syndrome and also by the majority of cutaneous infiltrating T cells in this patient (30). Nine of 10 MF patients showed no predominance of Vβ gene product expression, or even aggregations of positive cells that might suggest proliferation of clone(s) locally using a panel of seven Vβ MoAbs (30). Clark et al reported similar low rates of Vβ expression in a 1986 report where 2/24 T cell lymphomas were $V\beta_8+$ and 1/24 was $V\beta_5+$ (31). Charley et al and O'Grady et al screened a total of 38 T cell neoplasms and found 6/38 with restricted Vβ expression (26, 27) Ralfkier et al (32) studied 45 CTCL patients and found $V\beta_8$ in 2/45, $V\beta_5$ in 1/45, $V\beta_6$ in 1/45, and $V\beta_{12}$ in 1/45 patients, but no predominant Vβ family expression in the other 40 patients.

To summarize these reports, a total of 142 CTCL patients were screened with from 2–7 Vβ specific MoAbs and 25/142 (18%) cases showed restricted Vβ expression. Of these, 18 were $V\beta_8$, 4-$V\beta_5$, 2-$V\beta_6$, and 1-$V\beta_{12}$. Thus, it currently appears that considerable variability in Vβ expression exists in CTCL, but that a sub-population of CTCL cases can be associated with $V\beta_8$ expression. These findings in CTCL are somewhat similar to a report of non-Hodgkin's T cell lymphoma in which in 29% of TCR positive lymphomas, the neoplastic cells demonstrated a restricted Vβ usage using a panel of 7 different reagents (33). It should be noted that just identifying a cell population with restricted Vβ gene expression does not necessarily prove either a monoclonal or malignant process. Certain antigens (i.e. staphylococcal superantigens (34) may provoke an oligoclonal response, and increased populations of Vβ 8+ cells have already been observed in sarcoidosis and Crohn's disease (35, 36).

There is also considerable previous work in CTCL using the presence or absence of various cell surface antigens (e.g. Leu-8 and Leu-9) for diagnostic purposes. Leu-8 (the peripheral lymph node homing receptor) and Leu-9 (CD-7) are normally expressed by the majority of resting T cells, but are down-regulated during activation along with HLA-DR up regulation (37). Reports suggesting absence of these markers as sufficient criteria for the diagnosis of CTCL have been found in only a minority of cases (38). Moreover, the recognition that Leu 8 may be lost during local T cell activation in the skin has suggested the need to re-examine the diagnostic sensitivity of these markers in distinguishing benign from malignant T cell infiltrates (37). This discrepancy has been partially resolved through the use of stricter criteria to define what is meant by deficiency. Picker et al report that only CTCL will be recognized if $>$ 50% loss of CD7/Leu-9 or Leu-8 is used as a cutoff (14). Similar stringent criteria need to be developed before Vβ antigen expression can be routinely incorporated into the analysis of potential CTCL specimens. We

hope that a wide variety of both acute and chronic T cell reactive cutaneous eruptions such as allergic contact dermatitis, psoriasis, atopic dermatitis, and pityriasis lichenoides cases will be fully explored for Vβ usage to complement the publication of CTCL cases, so as to avoid some of the immunogical pitfalls in distinguishing CTCL from benign processes.

Why should there be so much variability amongst these different reports on the expression of Vβ gene products in CTCL? Several possible explanations must be considered in attempting to answer this question. The first is purely technical and it may well be that different laboratories use different techniques and different MoAbs, as discussed by Gilks et al in this issue (28). Also, the potential unmasking of certain $V\beta_8$ epitopes during fixation procedures may make the MX11 antibody more reactive with lymphocytes in tissue section (29). Tissue cross reactivity and restriction of epitopes may also be different between the various $V\beta_8$ clones used in these studies. As many as five $V\beta_8$ sub-families have been described (25), thus it is quite conceivable that different MoAbs may well recognize differing epitopes between these sub-families. Another recent study by Clark and Boylston, however, demonstrated that MX11 MoAb was randomly expressed in reactive lymph node in a cell surface/pattern (39). Thus, it is unlikely that the 10/16 MX11+ MF cases reported by Jack et al (29) were solely the results of nonspecific staining.

Other possible explanations for the disparate expression of certain Vβ gene families in CTCL involves variations in tumor biology. It is possible that the different groups have studied patients from different environmental circumstances The patients of Jack et al were presumably predominantly Scottish, whereas Ralfkiaer et al studied mainly Danish patients, Gilks et al mainly Canadian patients, Clark et al primarily British and Hunt et al were primarily from Pittsburgh (26–30). If an exogenous factor(s) is/are involved in the pathogenesis of CTCL, different antigenic stimulants in these different regions could lead to different malignant clones expressing antigen-specific Vβ gene products. Thus, it is conceivable that the group of $V\beta_8$+ patients reports by Jack (all Scottish) along with the scattered other $V\beta_8$+ cases all represent a common antigenic stimulant and/or common ancestry. The underlying assumption in this regard is that during the evolution of CTCL, which begins as an inflammatory reactive (i.e. polyclonal) process, a subpopulation of T cells is selectively stimulated to proliferate (i.e. clonal expansion) via a transformation event mediated by an external agent.

Alternatively, these cases may represent random sampling of $V\beta_8$ from the 20 Vβ families. This would explain why other cases are $V\beta_5$+ and $V\beta_{12}$+. The Gilkes report and the Ralfkiaer report, however, have the vast majority of patients as Vβ− with their respective MoAb panels (28, 32). If antigenic stimulation is responsible for the majority of CTCL, it would seem that unique antigens are implicated in most of these cases. To support the hypothesis of chronic antigenic stimulation, all of these cases should have a predominance of some Vβ gene products expressed by the malignant T cells. The apparent lack of positive Vβ staining in these cases may be simply explained by incompleteness of the screening panel of Vβ gene family MoAbs. It is also quite possible that at least some of these T cell lymphomas have lost TCR expression similar to other antigenic loss characteristic of CTCL (40–42).

An equally valid interpretation of these various studies is that Vβ gene restriction is not present in MF/CTCL. This conclusion is supported by DNA studies that have inconsistently shown TCR gene rearrangements in lesional CTCL (12, 20, 21). If all cases of CTCL represent a clonal expansion of a unique, transformed T cell, then a rearranged TCR gene representing a single Vβ gene product should be recognized by Southern analysis, as well as tissue staining of frozen sections. The lower limit of sensitivity for Southern analysis is that the rearranged DNA must account for 1–5% of the total cellular DNA of the sample (12). Thus, some false negatives are expected. The lower limit of sensitivity with immunoperoxidase staining should also be 5%, as up to 5% of normal lymphocytes can express any given Vβ gene product (25, 26).

Variabilities of gene expression, antigenic stimulus, antibody specificities, patient demographics, and laboratory technique are all factors which appear to make the current use of Vβ gene products in the diagnosis of MF/CTCL suspect. These factors are also reasons to scrutinize what may be a high rate of "false negative" DNA Southern analyses in these patients. When a comprehensive panel recognizing all 20 Vβ families becomes available, then and only then will Vβ MoAbs become useful in searching for clonal populations in cutaneous infiltrates. Inexpensive, rapid analysis via immunoperoxidase or immunofluorescence of tissue sections could then be performed by applying a cocktail of 3–4 Vβ MoAbs to a series of sections (33). If prominent staining is seen with any one, then that would be further stained with each MoAb individually. This technique would allow rapid dissection of Vβ gene product expression in tissues and would also minimize the use of valuable tissue and expensive reagents. Of course, certain subsets of T cell lymphomas (particularly large cell types) may not express TCR despite CD3 expression, and thus in these varieties, this approach would not be helpful (33).

Future applications of Vβ gene family probes for clonality in cutaneous T cell-mediated disease include the use of Vβ family specific primers combined with polymerase chain reaction (PCR) technology to increase sensitivity and specificity. McCarthy et al recently reported the use of this technology on paraffin-embedded specimens from variety of T cell neoplasms and suggested sensitivity limits of 0.05% (42). Similar landmark molecular dissection of CTCL by Lessin et al was recently reported in which patient specific primers and probes were produced to enable very sensivitive analysis of early lesions, as well as retrospective evaluation of nondiagnostic specimens (43).

For the moment, the light microscopic recognition of early CTCL remains the "gold standard", but it is anticipated that, as molecular biological and immunological based probes are developed (potentially combined with PCR), the "midas touch" may be passed on from the anatomic pathologist to the molecular biologist and back to the immunodermatopathologist. Carefully controlled, large series of both benign, reactive, as well as malignant cutaneous lymphoproliferative lesions need to be evaluated before we can seriously consider abandoning our traditional hematoxylin-eosin stained slides.

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The work supported in part by NIH grants AR38957, AR40065, AR01823, and AR40488 awarded to Brian J. Nickoloff.

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