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Rapid Communication

Cutaneous T-Cell Lymphoma Cells Employ a Restricted Range of T-Cell Antigen Receptor Variable Region Genes

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Monoclonal antibodies that recognize the proteins specified by two families of T-cell antigen receptor variable region genes were assessed as clonal markers in cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome). About 3% to 5% of tumors were expected to react with each antibody. However, 10 of 16 cases studied reacted with MAbs specific for the Vβ8 gene family. All the positive cases were examples of the plaque or tumor stages of CTCL. None of the cases of eczematoid or premycotic CTCL showed uniform reactivity with these antibodies. Unexpected use of one particular V gene family raises the possibility that CTCL derives from a distinct subpopulation of antigen- or virus-selected T cells. (Am J Pathol 1990, 136:17–21)

Cutaneous T-cell lymphoma is a distinctive clinical and pathologic entity in which the malignant T cells invariably invade the skin.[1] In its early stages it can be difficult to distinguish between CTCL and benign cutaneous inflammatory conditions because specific histologic features of malignancy may be difficult to demonstrate. Because of the difficulty in diagnosis there is a major dispute over the prevalence of CTCL. Some authorities consider it to be more common than Hodgkin's disease.[1]

The T-cell antigen receptor (TCAR) on most peripheral blood T cells is composed of two proteins called alpha and beta chains that are homologous to immunoglobulin molecules.[2] Both chains have variable, joining, and constant regions that are coded for by separate gene segments. During T-cell development these genes are rearranged to form the functional genes for each protein. The beta chain is formed from a relatively small pool of germ line variable region segments. Only about 20 families of V genes have been identified and most of these contain only a few distinct members.[3,4] Families of closely related V genes have been given numbers and their protein products can be identified by MAbs.[5,6] Such MAbs have been shown to identify clonal T-cell proliferation because all members of a particular clone must express the same V gene.[7] MAbs specific for the Vβ5 and 8 gene families were used to screen a series of skin biopsies from patients with CTCL.

Materials and Methods

All 16 cases of histologically diagnosed CTCL in the files of the Yorkshire Regional Cancer Organisation with stored frozen biopsy material were studied.

The MAbs specific for human Vβ5 and Vβ8 have been described.[8,9] The MX11 MAb used in this study is thought to be specific for products of the Vβ8 region for the following reasons. It was raised against the T-cell leukemia line Jurkat and reacts with 3% to 6% of normal peripheral blood T cells. A MAb with exactly these properties has been shown to recognize Vβ8.[5] In addition, a series of MX11-positive clones were raised and analysed and all were shown to use the Vβ8 gene segments.[11] MX11 is identical in specificity to the anti Vβ8 MAb MX6.[12] Extensive unpublished studies in our laboratory show that there is no overlap between Vβ5-positive and MX11-positive T cells. They were used in conjunction with a conventional immunoperoxidase technique. An anti-CD3 MAb was used as a positive control to identify all T cells and the primary antibody was omitted as the negative control.

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Table 1. *CTCL Cases Studied*

Case	Age/sex	Histologic pattern	Vbeta gene utilisation
1	?/M	Plaque	8
2	77/M	Tumor	8
3	74/M	Eczematoid	Mixed
4	64/M	Plaque	8 and 5
5	73/M	Plaque	8
6	85/M	Tumor	8
7	?/M	Eczematoid	Mixed
8	64/M	Plaque	8
9	69/M	Plaque	8
10	60/F	Tumor	8
11	61/M	Eczematoid	Mixed
12	41/M	Plaque	8
13	56/F	Eczematoid	Mixed
14	56/M	Eczematoid	Mixed
15	35/F	Tumor	8
16	?/F	Eczematoid	Mixed

Of the 16 cases studied 10 reacted with the monoclonal MX11. This implies that the tumor cells expressed a T-cell antigen receptor beta chain that was partly coded for by members of the Vbeta 8 gene family. This pattern of reactivity was confined to cases that showed plaque or tumor phase morphology. The pattern of reactivity in eczematoid CTCL was similar to that seen in reactive lymphoid proliferations.

The hematoxylin and eoisin stained slides were examined and classified into eczematoid, plaque, or tumor stages as described.[13]

Results

The details of the 16 cases studied are shown in Table 1. There are two striking features: 10 cases react with the anti-Vβ8 MAb and these are all the cases with plaque or tumor histology (Figure 1). In addition, case 3 showed a population of cells reacting with the VB5 MAb as well. All the cases categorized as eczematoid stage show scattered cells that react with Vβ5 or Vβ8 reagents (Figure 2). These cases resemble the nonspecific chronic inflammation cases studied as controls.

Discussion

Previous studies using these MAbs in lymph-node-based T-cell lymphomas have implied that Vβ gene use is random. Of 58 tumors studied, six have reacted with one or the other of the two MAbs.[7,14] This accords well with studies of normal peripheral blood T cells in which approximately 10% of cells stained are reactive with this pair of reagents.

The results obtained in this study led to two conclusions. Most of the tumor cells in all of the cases with plaque or tumor-stage histology react with the same anti-Vβ MAb indicating that they all use a member of the Vβ8 gene family. In contrast, the staining in cases defined histologically as eczematoid stage show the pattern of staining found in nonspecific cutaneous inflammation. Cells that stain with either MAb are found scattered singly in no apparent geographical relationship to each other. This suggests that this stage is clearly distinct from the others and may represent a precursor of CTCL. Perhaps the eczematoid stage of CTCL is an inflammatory process from which one subpopulation is selected by oncogenic transformation. Alternatively, the malignant cells may be heavily diluted by reactive normal T lymphocytes and so cannot be recognized histologically.

The human Vβ8 gene family has been analyzed in detail.[17] There are 5 Vβ8 genes, 2 pseudo genes, and 3 functional segments. The functional genes are Vβ8.1, 8.2 and 8.3. The Vβ8.1 and 8.2 segments are about 3 kb apart while the Vβ8.3 is at least 23 kb distant from them. Restriction sites for the commonly used restriction enzymes EcoRI and BamHI occur between all 3 Vβ8 genes so that each segment has a unique restriction fragment. Each Vβ8 segment can recombine with either of the constant region gene segments with additional diversity generated by the use of joining (J) and diversity (D) gene segments. Thus many different restriction fragment sizes can be generated by members of the Vβ8 family. This probably accounts for the lack of a single β-chain gene fragment size common to different CTCL cases even though they use the same Vβ family. Diverse β-chain gene restriction maps can also be generated from the Vβ5 family,[10, 18] confirming that multiple patterns occur even though only one V gene family is expressed.

Unexpected exclusive use of Vβ8 in CTCL raises the question as to what mechanism underlies this phenomenon. In B cells the immunoglobulin variable region gene that lies nearest to the constant region gene segment on the chromosome is rearranged first.[15] More distal regions are used at later times during ontogeny. However, the Vβ8 family is not the V gene nearest to the β chain constant region so that simple physical factors seem to be an unlikely explanation.[16]

Restricted use of Vβ genes has been described for the T-cell response to a number of protein and autoantigens in mice.[19-21] Recent studies have shown that some staphylococcal enterotoxins can act as superantigens and activate all the cells bearing a particular Vβ gene family product.[22] Many cases of CTCL have been associated with previous exposure to a variety of environmental agents.[1] Perhaps these act as antigens and select particular T cells for malignant transformation through a coexistent carcinogenic effect.

A third possibility is that Vβ8 acts as a receptor for a virus with oncogenic potential. Such virus receptor interaction could either involve recognition of the antigen-combining site on Vβ8 or some other sequence conserved in this family. If the former were the case it might be expected that the virus protein involved should resemble an MHC molecule because the antigen-combining site on

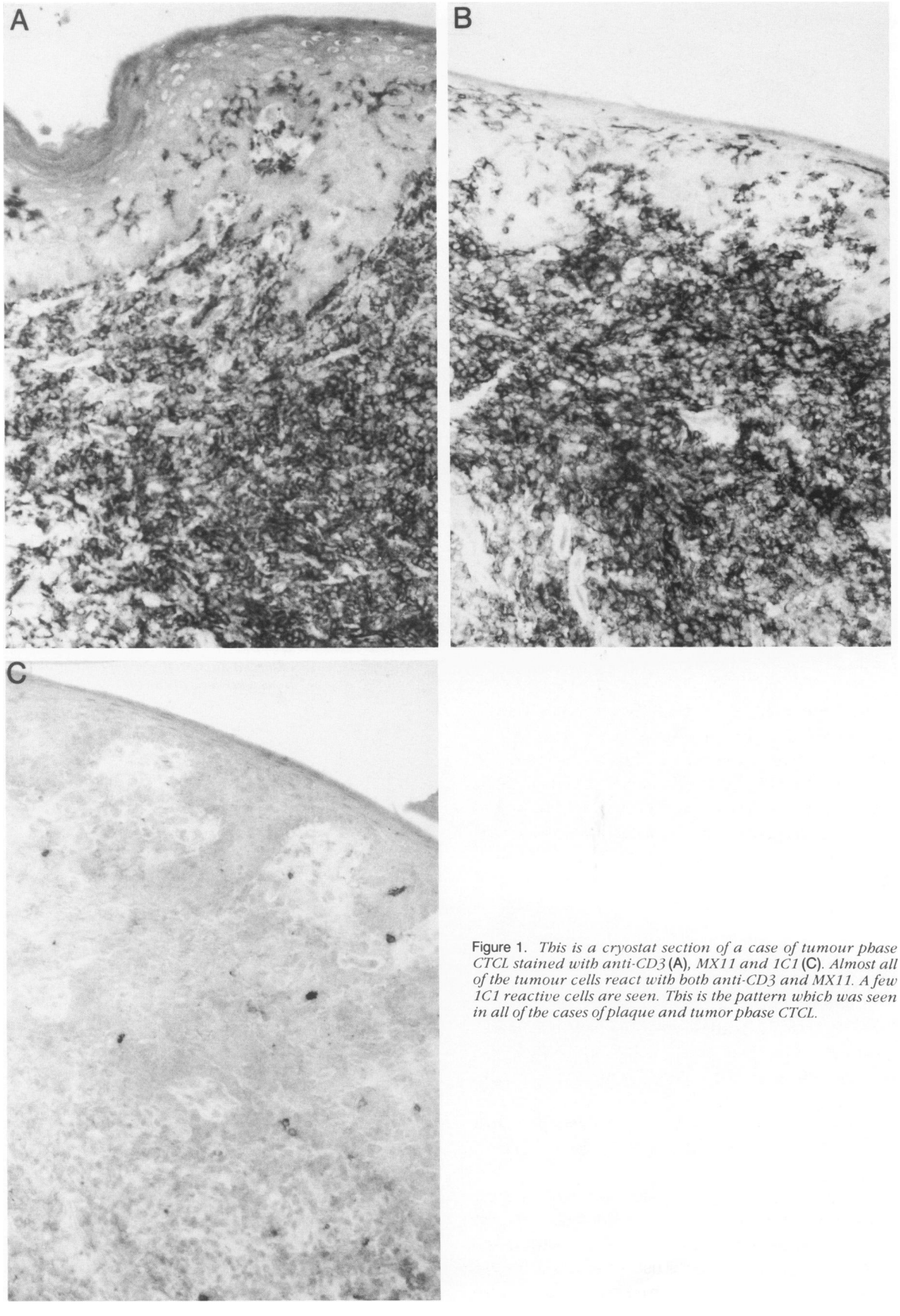

Figure 1. *This is a cryostat section of a case of tumour phase CTCL stained with anti-CD3* (A), *MX11 and 1C1* (C). *Almost all of the tumour cells react with both anti-CD3 and MX11. A few 1C1 reactive cells are seen. This is the pattern which was seen in all of the cases of plaque and tumor phase CTCL.*

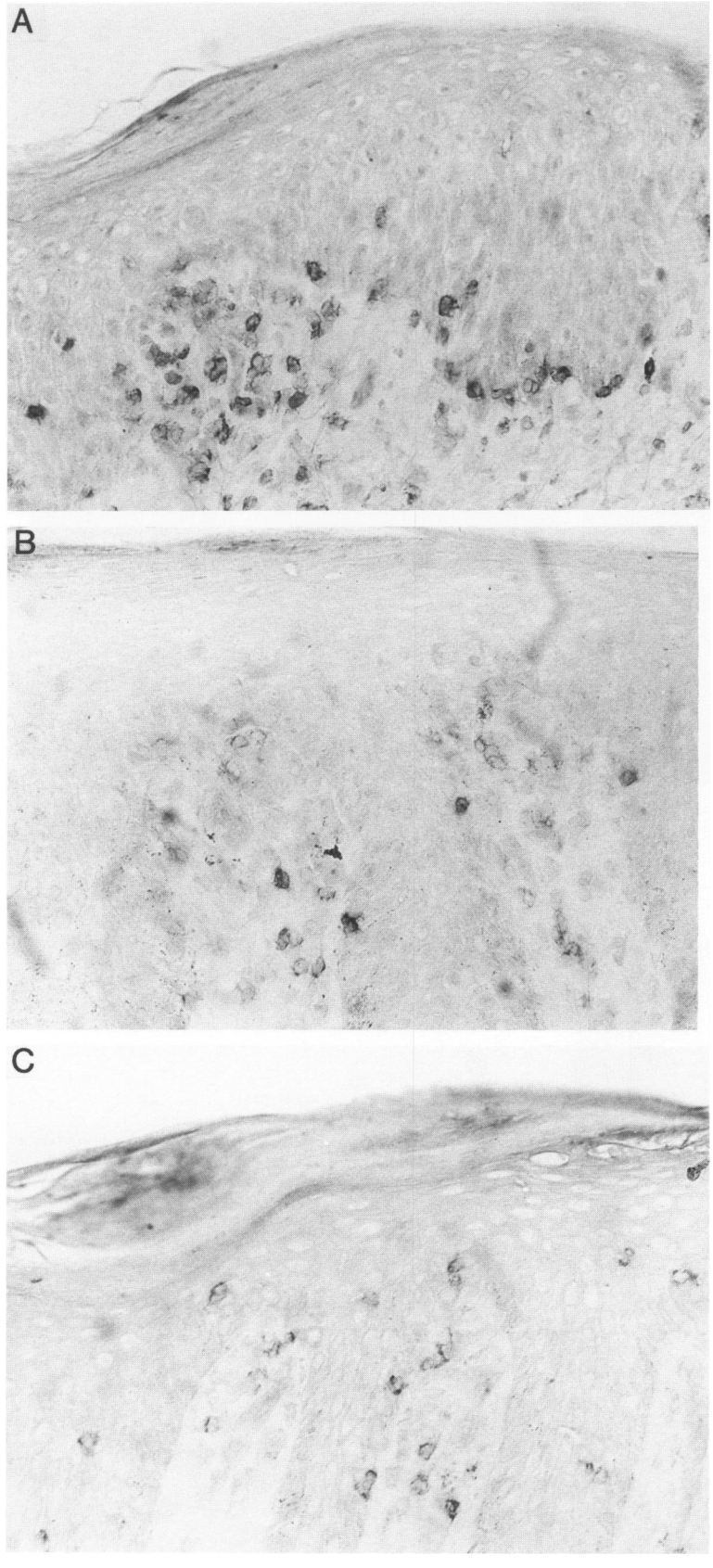

Figure 2. *This is a case of eczematoid CTCL stained with anti-CD3* **(A)**, *MX11* **(B)** *and 1C1* **(C)**. *Most of the lymphoid cells react with anti-Cd3 and a minority with either MX11 or 1C1. This is the pattern which is observed in reactive lymphoid proliferations with these antibodies.*

TCAR interacts with MHC complexed to a small antigenic peptide.[23] Cytomegalovirus has an MHC-like protein that combines with $\beta 2$ microglobulin on its target cell.[24] Alternatively, the oncogenic virus might infect antigen-presenting cells in the skin.[25] Specific recognition of infected cells by Vβ8-positive T cells might be the mechanism that transfers the virus to one T-cell family. A similar mechanism has been proposed for the transfer of HIV1 from monocytes to T cells in Acquired Immunodeficiency Syndrome.[26]

Regardless of the underlying mechanism, the frequent use of Vβ8 makes it possible to investigate the biology of CTCL using MAbs as tumor-specific markers. In addition it provides a useful tool for identifying malignant cells during staging. Whether it has any role in the differential diagnosis of cutaneous lymphoid infiltrates is unclear.

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