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γδ T cells as potential contributors to the progression of parapsoriasis to mycosis fungoides

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Abstract. It has recently been demonstrated that γδ T lymphocytes play a role in innate immunity to the neoplastic process; however, the significance of this subset of T lymphocytes in the pathophysiology of the most common form of cutaneous T cell lymphomas, mycosis fungoides, has yet to be investigated. In order to identify whether γδ T lymphocytes play a role in the progression of a pre-lymphomatous stage (parapsoriasis) to frank lymphoma (mycosis fungoides), we evaluated their presence in the skin biopsies of patients affected by mycosis fungoides and parapsoriasis. The skin biopsies of ten patients with mycosis fungoides and nine patients with parapsoriasis were analyzed using immunohistochemistry with a panel of different antibodies for T cell-associated markers (CD3, CD4, CD5, CD8 and γδ TCR). The percentage of T cells expressing γδ T cell receptors (TCR) was similar in the biopsies from both groups of skin disorders, ranging from 1 to 5% of the total T cell population. We observed a constant presence of T cells bearing γδ TCR in parapsoriasis and mycosis fungoides that did not differ between the two conditions. This presence is consistent with the role played by this subgroup of T cells of the innate immunity system in the development and maintenance of the two skin disorders. However, the contribution of γδ T cells to the progression of parapsoriasis to mycosis fungoides remains to be elucidated.

Introduction

γδ T cells account for 2-5% of $CD3^+$ peripheral T cells, and are important contributors to innate immunity against cancer (1,2). The importance of γδ T cells is demonstrated by their two main functions: reactivity to tumor cells and regulatory interaction with αß T cells (1). In particular, it seems that innate immunity through γδ T cells could lead to the establishment of acquired immunity by the selection of proper antigens and their best elimination. γδ T cell receptors (TCR) recognize novel ligands that are not seen by αß T cells, providing an important additional pathway of local immunosurveillance with direct relevance for tumor defence (3,4). It has been demonstrated that the response of αß T cells towards the T helper 1 or 2 phenotype is seemingly guided in its definition by γδ T cells, indicating that the close relationship between the two types of T cells is bidirectional (5). Most γδ T cells display a CD4 and CD8 double negative phenotype [in accordance with their lack of major histocompatibility complex (MHC) restriction] and express surface NKG2D, a molecule found on two other major subsets of cells with cytotoxic potential: $CD8^+$ αß T cells and NK cells. The engagement in humans of NKG2D by one of its various recognized ligands, including MHC class I chain-related A and B, provides a co-stimulatory function and targets cellular destruction (6,7). This cytotoxic activity and the production of high levels of cytokines, such as interferon-γ and tumor necrosis factor α, has been demonstrated in various types of tumors. This establishing that, among innate effector lymphocytes, γδ T cells represent a unique subset of unconventional lymphocytes due to their shared features with both conventional αß T and NK cells (8).

Several lines of evidence indicate that the immune system plays a major role in the surveillance of lymphoid malignancies (9,10). Experimental mouse models in particular indicate that MHC-unrestricted innate effector lymphocytes, such as NK and γδ T cells, play a critical role in the immunosurveillance of lymphoproliferative disorders, especially when MHC class I expression is reduced or missing (11).

Recruitment of tumor infiltrating lymphocytes (TILs) at the tumoral site is an essential tool for the development of a valid anti-neoplastic lymphocyte response (12). In order to investigate the role of γδ T cells in the innate immunity response to lymphoproliferative disorders, it may be relevant to determine their distribution at the tumoral site. Several studies have analyzed the presence of γδ TILs in relation to infiltrating neoplastic cells, with conflicting results (8,13,14). To the best of our knowledge, no studies concerning the presence of γδ TILs in cutaneous lymphoproliferative disorders have been conducted.

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Mycosis fungoides is the most common T cell cutaneous lymphoma. It generally follows a slow progressive clinical course comprised of three different clinical stages: the patch, the plaque and the tumor stage. Histologically, mycosis fungoides is characterized by a dermal infiltrate of $CD3^+$/$CD4^+$/$TCR\alpha\beta^+$ T cells associated with a variable degree of epidermotropism of neoplastic cells (15). It has also been demonstrated that mycosis fungoides, particularly in its early stages, is immunologically and phenotypically very close to contact dermatitis; often it is difficult to distinguish between these two conditions. There is therefore clinical and experimental evidence to correlate the development of mycosis fungoides with chronic inflammation caused by a reaction to continual antigen stimulation (16). We previously demonstrated that, in these situations, $CD1a^+$ dendritic cells are equally distributed (17).

Parapsoriasis is a group of disorders characterized by the presence of persistent and chronic scaling inflammatory patches and plaques of unknown aetiology. Patients with parapsoriasis en plaque, mainly of the large type, can develop overt mycosis fungoides. Consequently, there are authors in favor of including parapsoriasis within the disease spectrum of mycosis fungoides (18). In contrast, other authors consider parapsoriasis to be a pre-mycosis fungoides stage with histologic, immunophenotypic and genotypic features indistinguishable from those observed in inflammatory dermatoses (19).

In this report, we investigated the presence of γδ T cells in the reactive T lymphoid infiltrate of skin biopsies from patients affected by mycosis fungoides and parapsoriasis. Our aim was to evaluate a possible role of this subset of T cells in the progression of a pre-lymphomatous stage (parapsoriasis) to frank lymphoma (mycosis fungoides).

Materials and methods

The study included tissue samples of parapsoriasis, mycosis fungoides and inflammatory dermatoses (eczema, psoriasis and lichenoid dermatoses). All diagnoses were made on the basis of clinical, histological, immunophenotypical and, in some cases, molecular data. Immunohistological analysis was performed on sections from ten cases of mycosis fungoides and nine cases of parapsoriasis, with twenty cases of inflammatory skin conditions as a control. Tissues were snap-frozen in liquid nitrogen and stored at -80˚C. The following monoclonal antibodies (mAbs) were also used: CD3 (clone UCHT1), CD4 (clone MT310), CD5 (clone DK23), CD8 (clone DK25) and CD19 (clone HD37) (all from Dako, Glostrup, Denmark) and γδ TCR (clone B1.1). γδ TCR mAb can be utilised on frozen sections only; therefore, air-dried acetone-fixed frozen sections were incubated overnight with the mAbs and, after washing, processed using a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Rabbit anti-mouse Ig (Dako) was applied for 30 min. After washing, the sections were incubated with APAAP-complex (Dako) for 30 min. Naphtol-AS-MX phosphate, along with Fast Red TR salt, was used to develop alkaline phosphatase. Endogenous alkaline phosphatase was blocked by adding levamisole to the substrate. Sections were counterstained for 5 min with Mayer's hematoxylin. Negative controls were performed by omitting the primary mAb on samples or by replacing the primary antibody with another irrelevant mAb of identical isotype. The presence of γδ TCR was categorized as follows: -, absence of T cells bearing γδ TCR; +, T cells bearing γδ TCR in 1-5% of $CD3^+$ cells; ++, T cells bearing γδ TCR in 5-10% of $CD3^+$ cells; +++, T cells bearing γδ TCR in >10% of $CD3^+$ cells.

Results and Discussion

In this study, we analyzed the presence of $CD3^+$ cells bearing γδ TCR in nine cases of parapsoriasis and ten cases of mycosis fungoides (Figs. 1 and 2). The control group comprised twenty cases of inflammatory dermatoses, notably eczema, psoriasis and lichenoid dermatoses. The cases of mycosis fungoides comprised different stages of the neoplasia: cases 1, 2, 3, 4, 5, 6 and 8, the patch stage; case 7, the tumor stage; cases 9 and 10, the erythrodermic stage. The results of the analysis regarding the presence of $CD3^+$ cells bearing γδ TCR in mycosis fungoides and parapsoriasis are summarized in Tables I and II.

The data reveal no difference between the presence of $CD3^+$ cells bearing γδ TCR in mycosis fungoides and in parapsoriasis. In most of the cases, γδ T cells were present in 1-5% of the CD3 and $CD5^+$ T cells. These findings are consistent with the number of γδ T cells found in peripheral blood, as well as with the percentages found in our control group of inflammatory dermatoses.

We additionally observed that, in both parapsoriasis and mycosis fungoides, T cells bearing γδ TCR were essentially located in the dermal compartment in association with the $CD4^+$/$CD3^+$ T cell lymphoid infiltrate characteristic of these cutaneous lymphomas. However, in some cases an evident epidermotropism of γδ T cells was observed. In three cases of parapsoriasis and one case of mycosis fungoides, T cells bearing γδ TCR were entirely absent (Tables I and II).

Several studies on murine epidermis (20,21,22) have suggested that γδ T cells play a role in the immunosurveillance of cutaneous neoplasia. In particular, it has been demonstrated that γδ TCR knock-out mice have an increased risk of developing cutaneous neoplasia. In the present study, we observed an almost constant presence of γδ T cells in both mycosis fungoides and parapsoriasis. This is consistent with the role of this subset of T cells in the immunopathogenesis of these conditions (23).

Though γδ T cells were observed in similar percentages in both mycosis fungoides and parapsoriasis, a role for them in the neoplastic progression of the pre-lymphomatous stage (parapsoriasis) to frank lymphoma (mycosis fungoides) cannot be excluded. The role of innate immunity in the immunopathogenesis of mycosis fungoides is currently under investigation. It was recently observed that keratinocytes involved in mycosis fungoides present a higher expression of toll-like receptors (TLRs) 2, 4 and 9, whereas in parapsoriasis these receptors are only weakly expressed (24). TLRs are type I transmembrane proteins involved in innate immunity. They are characterized by their activation of cellular phagocytosis, cytokine production and the expression of MHC and co-stimulatory molecules (25,26). Recent studies on the murine system indicate that certain subsets of γδ T cells

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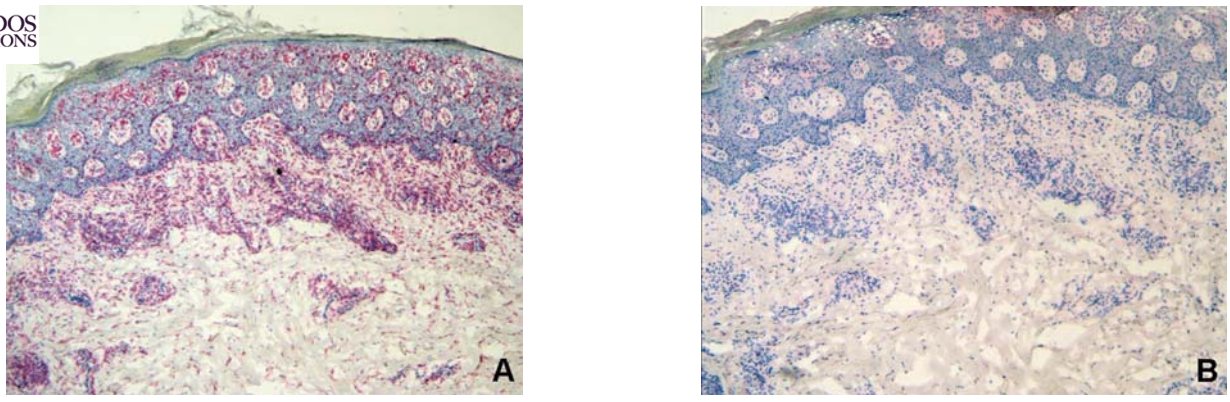

Figure 1. Case of mycosis fungoides. (A) CD4+ T cells; (B) T cells bearing γδ TCR (A and B, magnification x4). Limited T cells bearing γδ TCR are detectable in the dermal compartment.

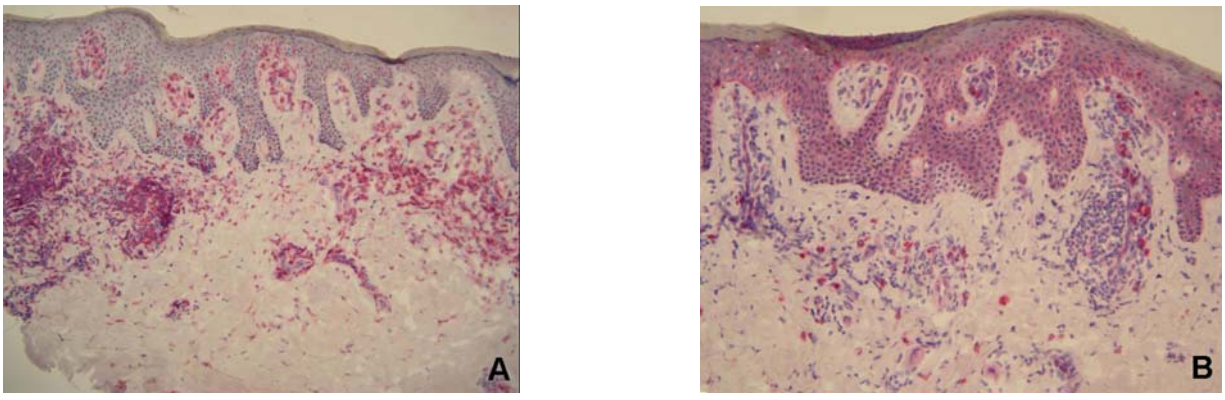

Figure 2. Case of parapsoriasis. (A) CD4+ T cells; (B) T cells bearing γδ TCR (A and B, magnification x4). Several γδ T cells are observable in this case.

Table I. Expression of γδ TCR in the ten cases of mycosis fungoides analyzed.

Mycosis fungoides	TCR γδ
Case 1	+
Case 2	++
Case 3	+
Case 4	+
Case 5	+
Case 6	+
Case 7	+
Case 8	+
Case 9	-
Case 10	+

-, absence of T cells bearing γδ TCR; +, 1-5% of T cells bearing γδ TCR; ++, 5-10% of T cells bearing γδ TCR; +++, >10% of T cells bearing γδ TCR.

Table II. Expression of γδ TCR in the nine cases of parapsoriasis analyzed.

Parapsoriasis	TCR γδ
Case 1	-
Case 2	++
Case 3	+
Case 4	+
Case 5	+
Case 6	+
Case 7	-
Case 8	+
Case 9	-

-, absence of T cells bearing γδ TCR; +, 1-5% of T cells bearing γδ TCR; ++, 5-10% of T cells bearing γδ TCR; +++, >10% of T cells bearing γδ TCR.

constitutively express specific TLRs which, after interacting with their ligands, determine their activation (27). This observation suggests that TLRs, which are overexpressed in mycosis fungoides, may be involved in the chronic activation of T cells in mycosis fungoides due to their interaction with γδ T cells. Several immunoregolatory circuits are consequently activated to potentiate and sustain the coordinated activation of the different populations of innate immunity, and to determine the cytokine milieu responsible for the proliferation and activation of neoplastic γδ T cells.

In conclusion, immunohistochemical analysis performed on the frozen sections of biopsies from patients with parapsoriasis and mycosis fungoides demonstrated the constant presence of T cells bearing γδ TCR in both conditions. This observation is consistent with the role played by this subgroup of T cells of the innate immunity system in the development and maintenance of the two skin disorders. However, the contribution of γδ T cells to the progression of parapsoriasis to mycosis fungoides remains to be established, as does the role of innate immunity in the immunosurveillance of cutaneous T cell lymphomas.

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