

Expression of the CD1a molecule in B- and T-lymphoproliferative skin conditions

BARBARA PIGOZZI, MATTEO BORDIGNON, ANNA BELLONI FORTINA,
GIORGIO MICHELOTTO and MAURO ALAIBAC

Unit of Dermatology, University of Padua, Via C. Battisti 206, I-35128 Padova, Italy

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Abstract. The skin immune system is characterized by the presence of two types of CD1a expressing cells: Langerhans cells and dermal dendritic cells, which are professional antigen processing and presenting cells. It is well established that several dermatoses are associated with T-cell mediated immune responses. In these pathological skin conditions, T-cells are activated by professional antigen presenting cells and dendritic cells are the most potent antigen presenting cells for both T-helper cells and T-cytotoxic cells. Therefore, it is plausible that dendritic cells are crucially involved in the pathogenesis of lymphoproliferative skin conditions characterized by the presence of a T-cell infiltrate. In this study, we examined the frequency and distribution of CD1a expressing cells and CD3⁺ cells in both the dermal and epidermal compartment in a wide range of lymphoproliferative dermatoses with a T-lymphoid infiltrate. In the skin conditions investigated, the CD1a molecule was highly expressed in mycosis fungoides, T-cutaneous lymphoid hyperplasia, lymphomatoid papulosis and parapsoriasis, whereas few CD1a-positive cells were observed in cutaneous B-cell lymphomas. Our study demonstrates a distinct pattern of CD1a staining in the infiltrates of cutaneous lymphoproliferative disorders which may reflect different immunoregulatory events involving T-lymphocytes and CD1a-positive dermal and epidermal dendritic cells.

Introduction

The CD1 family comprises small to moderate sized molecules of β_2 -microglobulin-associated, transmembrane glycoproteins. They are structurally related to the MHC-class I molecules but their genes are unlinked to the MHC locus and they do not have the extensive polymorphism of classical MHC encoded antigen presenting molecules (1,2). In humans, the CD1 family has five members: CD1a, CD1b, CD1c, CD1d

and CD1e proteins. Among these, it has been proposed that two or perhaps three distinct groups of CD1 proteins can be discriminated on the basis of sequence homologies, sites of expression and function. Thus, CD1a, CD1b and CD1c are typically classified as group 1 CD1 proteins, while the more divergent CD1d protein comprises group 2 (3). The CD1e protein is considered intermediate in terms of its homology to group 1 and group 2 CD1 proteins and its expression is confined exclusively to intracellular compartments of myeloid lineage cells. Thus, the CD1e protein is currently classified as a group 3 CD1 (4).

In contrast to MHC molecules, which present antigenic peptides for recognition by specific T cells, it has been demonstrated that CD1-presented antigens are predominantly or exclusively lipid in nature (5). In accordance with this capacity, the CD1 proteins are expressed primarily on antigen presenting cells (6). The skin immune system is characterized by the presence of two types of CD1 expressing cells: Langerhans cells and dermal dendritic cells, which are professional antigen processing and presenting cells; these cells are known to play pivotal roles during the induction phase of adaptive immune responses and were shown to express high levels of CD1a (7,8).

It is well established that several dermatoses are associated with T-cell mediated immune responses (9-12). In these pathological skin conditions, T-cells are activated by professional antigen presenting cells and dendritic cells are the most potent antigen presenting cells for both T-helper cells and T-cytotoxic cells (13). Therefore, it is plausible that dendritic cells are crucially involved in the pathogenesis of lymphoproliferative skin conditions with a T-cell infiltrate. In this study, we examined the frequency and distribution of CD1a expressing cells and CD3⁺ cells in both the dermal and epidermal compartment in a wide range of B- and T-lymphoproliferative dermatoses characterized by the presence of a T-lymphoid infiltrate.

Materials and methods

The study included tissue samples of 31 cases of lymphoproliferative skin conditions (Table I). All tissue samples had been obtained for diagnostic purposes. Our immunohistological experiments included sections of both normal human skin from 5 different subjects and 15 cases of inflammatory skin conditions as control. Tissues were snap-frozen in liquid nitrogen and stored at -80°C. Detection of CD1a was per-

Correspondence to: Professor Mauro Alaibac, Unit of Dermatology, University of Padua, Via C. Battisti 206, I-35128 Padova, Italy
E-mail: mauro.alaibac@unipd.it

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Table I. Lymphoproliferative dermatoses included in our study: a negative immunohistochemically analysis of the antigens CD1 and CD3 is shown as -; positivity is scored as + (low), ++ (moderate) and +++ (strong).

Diagnosis	Antigen	Epidermis	Derma
Cutaneous B-cell lymphoma			
Case 1	CD1a	+++	+
	CD3	-	+++
Case 2	CD1a	+	+++
	CD3	-	+++
Case 3	CD1a	++	-
	CD3	-	++
Case 4	CD1a	++	+
	CD3	-	+
Case 5	CD1a	++	++
	CD3	-	+++
Cutaneous CD30⁺ anaplastic lymphoma			
Case 1	CD1a	+	+
	CD3	-	+++
Case 2	CD1a	++	+++
	CD3	+	+++
Case 3	CD1a	+++	++
	CD3	+	+++
Mycosis fungoides (patch/plaque stage)			
Case 1	CD1a	++	++
	CD3	+	+++
Case 2	CD1a	+++	+++
	CD3	+++	+++
Case 3	CD1a	+++	++
	CD3	+++	++
Case 4	CD1a	+++	+++
	CD3	-	+++
Case 5	CD1a	++	++
	CD3	++	+++
Case 6	CD1a	++	++
	CD3	+	+++
Case 7	CD1a	++	++
	CD3	++	+++
Case 8	CD1a	+++	++
	CD3	+	+++
Case 9	CD1a	+++	+++
	CD3	++	+++
Case 10	CD1a	++	++
	CD3	+	++
Case 11	CD1a	++	++
	CD3	++	+++
Case 12	CD1a	+	+
	CD3	+	+++

Table I. Continued.

Diagnosis	Antigen	Epidermis	Derma
Lymphomatoid papulosis			
Case 1	CD1a	++	+++
	CD3	-	+++
Case 2	CD1a	+++	+++
	CD3	-	+++
Parapsoriasis			
Case 1	CD1a	+	++
	CD3	-	++
Case 2	CD1a	+++	+++
	CD3	+++	+++
Case 3	CD1a	++	++
	CD3	-	++
Case 4	CD1a	+++	+++
	CD3	+	+++
T-cutaneous lymphoid hyperplasia			
Case 1	CD1a	+++	++
	CD3	-	++
Case 2	CD1a	+++	+++
	CD3	-	+++
Case 3	CD1a	++	++
	CD3	-	++
Sezary syndrome			
Case 1	CD1a	+	+
	CD3	++	++
Case 2	CD1a	+	+
	CD3	-	+++

formed on frozen sections from reactive and neoplastic skin conditions using an anti-CD1a monoclonal antibody (mAb, clone NA1/34; Dako). The following mAbs were also used in the study: CD3 (clone UCHT1; Dako, Glostrup, Denmark), CD4 (clone MT310; Dako), CD5 (clone DK23; Dako), CD7 (clone DK24; Dako), CD8 (clone DK25; Dako) and CD19 (clone HD37; Dako). Air-dried, acetone-fixed frozen sections were incubated overnight with the mAbs and, after washing, processed with a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Rabbit anti-mouse Ig (Dako) was applied for 30 min and, after washing, the sections were incubated with APAAP-complex (Dako) for 30 min. Naphtol-AS-MX phosphate along with Fast Red TR salt were used for the development of alkaline phosphatase. The 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 consisted of replacement of the primary antibody with another irrelevant mAb of identical isotype. We evaluated

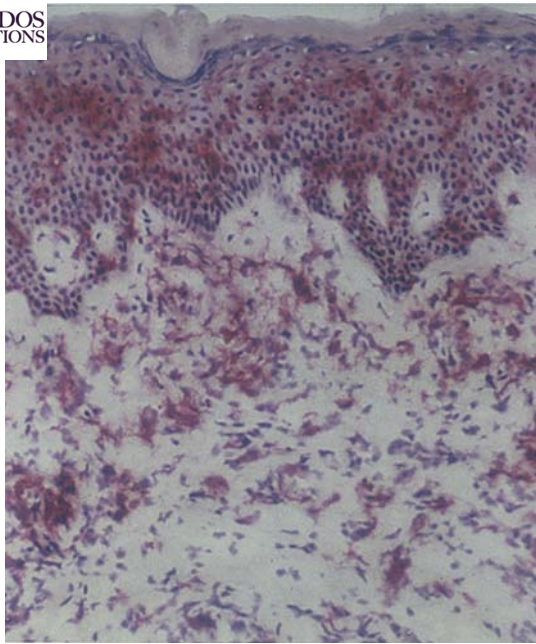


Figure 1. Representative immunostaining of a case of mycosis fungoides showing numerous CD1a⁺ cells in both the epidermal and dermal compartment (APAAP, x40).

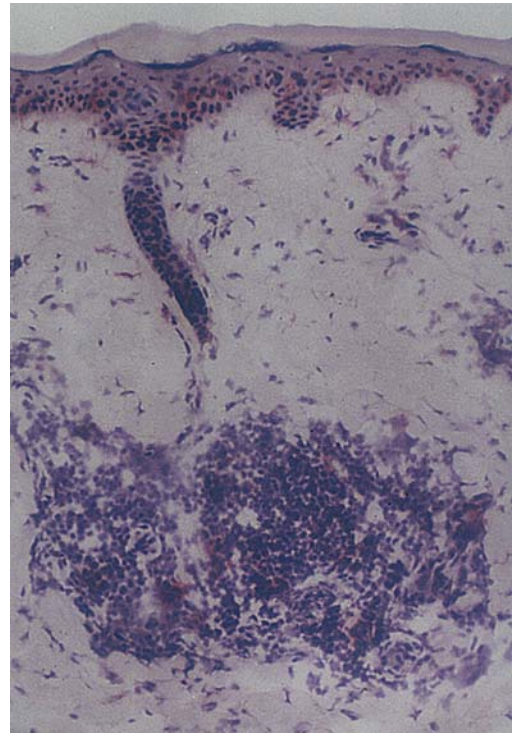


Figure 2. Representative immunostaining of a case of cutaneous B-cell lymphoma demonstrating only a few epidermal and dermal CD1a⁺ dendritic cells (APAAP, x40).

CD1a and CD3 reactivity according to the following grading system: -, absent; +, low; ++, moderate; +++, strong.

Results

On frozen sections of normal skin, CD1a-positive cells formed an evident reticule in epidermis and they were not generally noticed in the dermis. CD3-positive cells were present only in the perivascular dermal and the epidermal expression of the CD3 molecule was observed only in a few isolated lymphocytes.

In lymphoproliferative skin conditions, the CD1a molecule was highly expressed in the cases of patch/plaque-stage mycosis fungoides investigated in both the epidermal and dermal compartment (Fig. 1). In this condition, a dense CD3-positive cell dermal infiltrate was present and it was in some cases associated with epidermotropism of CD3-positive cells. A strong dermal and epidermal expression of the CD1a molecule was also detected in T-cutaneous lymphoid hyperplasia, lymphomatoid papulosis and parapsoriasis. In these conditions, an abundant dermal cellular CD3-positive infiltrate was present, whereas epidermotropism of CD3⁺ cells was generally modest or absent. In the 5 cases of cutaneous B-cell lymphoma, we generally observed a strong dermal infiltrate of T-lymphocytes, but few CD1a⁺ cells in both the dermal and epidermal compartment (Fig. 2). A similar situation was detected in one of the 3 cases of cutaneous CD30⁺ anaplastic large cell lymphoma included in the study, but not in the other 2 cases. A low expression of CD1a was also detected in the 2 cases of Sezary syndrome investigated. The results concerning the expression of the CD1a molecule in lymphoproliferative dermatoses are summarized in Table I.

We also investigated as control a small group of inflammatory skin conditions. In these cases, CD1a⁺ dermal dendritic cells were densely distributed within the lymphoid infiltrate in

the affected dermis of contact dermatitis, psoriasis and lichen planus. A strong expression of CD1a was also observed in the epidermal compartment, although epidermotropism of CD3⁺ cells was not generally observed.

Discussion

Mycosis fungoides is the most common T-cell cutaneous lymphoma and it generally shows a slow progressive clinical course. Histologically, mycosis fungoides is characterized by a dermal infiltrate of CD3-positive/CD4-positive cells associated with the epidermotropism of neoplastic cells (14). In our study, we demonstrate in patch/plaque-stage mycosis fungoides a strong correlation between the number of CD3-positive T-cells in the dermal compartment and the expression of the CD1a molecule in both the dermis and epidermis. This situation is in agreement with previous reports (15,16) and very similar to T-cell mediated inflammatory dermatoses such as contact dermatitis (17). Mycosis fungoides, particularly in its early stages, is immunologically and phenotypically very close to contact dermatitis and often these two situations are difficult to discriminate. There is, thus, clinical and experimental evidence that correlates the development of mycosis fungoides with a situation of chronic inflammation derived from a reaction to continual antigen stimulation. This analogy with contact dermatitis supports the hypothesis that this cutaneous lymphoma could take origin from a neoplastic transformation of a T-helper cell clone involved in a cutaneous immunological response (18). Our data about the density of dendritic cells expressing the CD1a molecule and T-cells are in agreement with this immunopathological model. An original finding has been the observation that the density of epidermal CD1a-

positive cells was not associated with the epidermotropism of CD3-positive cells, suggesting that epidermal Langerhans cells are equally operative in this situation, even if only a pre-dominant dermal involvement is present.

In the two cases of Sezary syndrome investigated in our study, we found a low number of CD1a-positive dendritic cells in both the dermal and epidermal compartment. Sezary syndrome is an aggressive variant of cutaneous T-cell lymphoma with a poor prognosis if compared with mycosis fungoides. Our findings probably reflect the different clinical behaviour of these lymphoproliferative skin conditions.

Expression of the CD1a molecule in parapsoriasis was similar to that observed in mycosis fungoides. This cutaneous disorder is characterized by clinical and histological features analogous to early mycosis fungoides and is considered by some authors to be a pre-mycosis stage (19,20). This similitude is probably indicative of a common immunopathological mechanism characterized also in this condition by chronic antigen stimulation, which determines T-cell proliferation with mycosis fungoides-like histological and immunophenotypical features. A similar situation concerning CD1a expression was observed in T-cutaneous lymphoid hyperplasia which has been proposed to be the benign end of a continuum of lymphoproliferative disorders with cutaneous T-cell lymphoma at its malignant extreme (21).

In cutaneous CD30⁺ anaplastic large cell lymphomas, *in situ* immunohistochemical analysis demonstrated, in 1 out of 3 cases, a low expression of the CD1a molecule in both the dermal and epidermal compartment and, however, the presence of a strong CD3⁺ infiltrate. This type of cutaneous lymphoma is characterized by clinical and histological differences from mycosis fungoides (22). The variable expression of the CD1a molecule in this cutaneous lymphoma suggests that, in this condition, an immunopathological mechanism different to that proposed for mycosis fungoides is involved. This hypothesis is consistent with the clinical course of cutaneous CD30⁺ anaplastic large cell lymphomas, characterized by spontaneous remissions and disease reactivation, whereas in mycosis fungoides the course is usually progressive (23,24). It has been shown that T-cells expressing the CD30 molecule have a cytokine profile characterized by a high production of IL-10. Particularly, a marked expression of this cytokine has been found in nodal anaplastic large cell lymphomas, characterized by the proliferation of CD30-positive cells (25). IL-10 is shown to inhibit the CD1a molecule (26,27) and it is possible that, in a reactivation phase of the disease, an increase in IL-10 secretion by CD-30⁺ neoplastic cells may have determined a low expression of CD1a in one of the 3 cases of cutaneous CD30⁺ anaplastic large cell lymphomas. However, numerous CD1a⁺ cells were observed in the lesions of the other two patients and also in lymphomatoid papulosis, which is an indolent form of cutaneous CD30-positive T-cell lymphoma. This suggests that the presence or absence of CD1a in these conditions may reflect a different stage of disease progression and/or biological behaviour.

In cutaneous B-cell lymphomas, the infiltrate frequently showed a moderate expression of CD1a which was generally confined to T-cell-rich areas as previously reported (28). However, the number of CD1a-positive cells only in one case correlated with the expression of CD3⁺ cells. These findings

suggest that the recruitment of CD1a cells in T-cell-rich areas is probably hampered by neoplastic B-cell proliferation. It is plausible that neoplastic B-lymphocytes produce cytokines inhibiting the recruitment of CD1a⁺ dendritic cells in the skin and, consequently, the local response by T-cell mediated anti-tumor activity.

In conclusion, we have investigated the expression of the CD1a molecule in a group of well-characterised cutaneous lymphoproliferative conditions. The relationship between expression of CD1a protein and the cytokinic milieu may help to clarify the immunopathological mechanisms which are operative in these disorders.

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