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The Expression of the Hodgkin's Disease-associated Antigen Ki-1 in Cutaneous Infiltrates

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Reactivity for Ki-1 antibody was studied in 145 patients with a large variety of cutaneous disorders. The antigen was consistently expressed and by a high proportion of tumour cells in infiltrates in which atypical cells revealed a 'histiocytic' appearance, i.e. lymphomatoid papulosis (LP), T-immunoblastic lymphoma with the characteristic of true histiocytic lymphoma, Hodgkin's disease, T-blast cell proliferation with giant multivesicular bodies, concurrent LP and mycosis fungoides (MF), and two cases of MF. Ki-1$^+$ cells with the usual morphology of atypical T-cells formed a major component in 2 other cases of MF, and a minor component in 7 other cases of MF. A possible non-neoplastic counterpart was found in small to medium-sized Ki-1$^+$ cells, including blast cells, which occurred occasionally in the T-cell infiltrates of eczema, actinic reticuloid, lichen planus and pityriasis lichenoides. Small Ki-1$^+$ cells which were observed in the reactive B/T cell component of lymphocytoma cutis but also in similar components occurring occasionally in non-epidermotropic cutaneous T-cell lymphoma, and malignant B-cell lymphomas, might be analogous to the Ki-1$^+$ cells in normal lymphoid tissue. *Key words: Skin; Ki-1; Malignant lymphoma; Mycosis fungoides; Inflammatory infiltrates.* (Received August 13, 1987.)

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Ki-1 (CD30) is a monoclonal antibody that reacts with an antigen which shows a unique pattern of expression on reactive and neoplastic lymphoid cells. The antibody was initially raised against Reed-Sternberg cells in Hodgkin's disease (HD) (1), and was subsequently also detected on tumour cells of equally uncertain lineage in disorders designated as true histiocytic lymphoma, angioimmunoblastic lymphadenopathy, regressing atypical histiocytosis and lymphomatoid papulosis (2–14). The expression of the Ki-1 antigen by cells of other than lymphocytic derivation has not been reported yet. Ki-1$^+$ cells appear to be usually in an activated and proliferative state (4, 15). The antigen was also found on perifollicular cells in normal lymphoid tissue and on normal peripheral blood lymphocytes especially if stimulated in vitro by antigen, mitogen, and some T- or B-cell lymphotropic viruses (4, 16, 17).

In the skin Ki-1 reactivity was consistently found on lymphoid cells resembling histiocytes in lymphomatoid papulosis (3–8, 14), on some large cell malignant lymphomas formerly designated as 'regressing atypical histiocytosis' (10, 12) and a spontaneously regressing T-blast cell proliferation with giant multivesicular bodies (18). It was detected in only a few cases of mycosis fungoides (MF), Sézary's syndrome (SS) and non-epidermotropic T-cell lymphomas (5, 12, 19, 20).

A normal Ki-1$^+$ cellular counterpart like the perifollicular cells in lymphoid tissue has not been found in the skin yet (5, 8, 11, 12). Knowledge about the normal and abnormal Ki-1$^+$ cell component may be quite valuable for differential diagnostic purposes, for the

analysis of aetiological factors and the identification of a possible precursor cell of the atypical lymphoid cells. We have therefore included the Ki-1 antibody in our panel of antibodies used for routine infiltrate analysis of skin biopsies in order to test it on a large number of specimens from miscellaneous disorders.

MATERIAL AND METHODS

Ki-1 reactivity of cells was tested in 145 cases of 156 consecutive punch biopsies from a wide spectrum of cutaneous disorders which were submitted to our laboratory for infiltrate analysis (Table I). Added were 19 specimens of MF and LP which had been stored for up to 7 years in −90°C.

Eight micrometer cryostat sections were stained with monoclonal antibodies using a three stage immunoperoxidase or alkaline phosphatase-anti-alkaline phosphatase (APAAP) technics (21). The monoclonal antibodies used in this study were directed against the Ki-1 antigen (4) and against antigens of T-cell subsets (Leu-1, -2a, -3a, -4; Becton Dickinson, Mountain View, CA; T11; CLB, Amsterdam, The Netherlands); of B-cells (Leu-14; Becton Dickinson); of monocytes/macrophages (M718; CLB, Amsterdam); of Langerhans' cells (OKT6; Ortho Diagnostic Systems, Inc., Raritan, NY) against a cell proliferation associated antigen (Ki-67; Dakopatts, Denmark) and against HLA-DR (Becton Dickinson).

It was followed by haematoxylin nuclear counterstaining which was performed after postfixation of the sections in a mixture of absolute ethanol (85%), acetic acid (5%) and formalin (10%) to improve

Table I. *K-1 reactivity in neoplastic and non-neoplastic cutaneous diseases*

Lymphomatous diagnosis	Number of positive cases: Present study	Number of positive cases: Literature[a]	Inflammatory diagnosis	Number of positive cases: Present study	Number of positive cases: Literature[a]
Hodgkin's disease	1 (1)[b]	1 (1)	Non-epidermotropic pseudo T-cell lymphoma	0 (3)	
'True histiocytic' lymphoma	1 (1)		Lymphocytoma cutis (lymphadenosis benigna cutis)	3 (6)	
T cell proliferation with giant multivesicular bodies	1 (1)		Pityriasis lichenoides	2 (7)	0 (12)
Regressing atypical histiocytosis		8 (8)	Actinic reticuloid	3 (9)	
Lymphomatoid papulosis	5 (5)	46 (52)	Lichen planus	1 (2)	0 (2)
Lymphomatoid papulosis and concurrent mycosis fungoides	1 (1)	3 (3)	Diverse types of chron. dermatitis (eczema)	3 (23)	0 (1)
Mycosis fungoides	14[c] (48)	9 (31)	Insect bite reaction	0 (1)	
Non-epidermotropic T-cell lymphoma	1[d] (2)	6 (9)	Small plaque parapsoriasis	0 (1)	
Adult T-cell leukemia	0 (1)	0 (4)	Jessner's lymphocytic infiltration of the skin	0 (3)	
Sézary syndrome	0 (2)	3 (7)	Molluscum contagiosum	0 (1)	
Malignant B-cell lymphoma	4[c] (17)		Graft-versus-host disease	0 (3)	
			Scleroderma	0 (2)	
			Psoriasis	0 (3)	0 (3)
			Xanthogranuloma	0 (2)	
			Leucocytoclastic vasculitis	– –	0 (1)

[a] Ralfkiaer et al. (1985) have reported negative results in patch test, eczema, psoriasis, lichen planus, lymphocytoma cutis, pityriasis lichenoides acuta, and large plaque parapsoriasis without giving the numbers of cases tested.
[b] Total number of cases tested within parentheses.
[c] Ki-1 positivity probably in the non-neoplastic component in two cases.
[d] Ki-1 positivity probably in the non-neoplastic component.

nuclear detail. Sections from the same specimens without nuclear counterstaining revealed the same reaction pattern. Immunohistochemical findings were further correlated to cytological features by studying either HE sections of paraffin embedded, routinely processed specimens or trichrome stained semithin section of Epon 812 embedded material from the same patients. With respect to MF, clinical and histopathological data were compared between cases with and without Ki-1 reactivity.

RESULTS

The most marked expression of the Ki-1 antigen was found in a group of disorders in which the abnormal cells often revealed a more or less 'histiocytic' appearance (Table I). Their nuclei often tended to bi- and multinucleation as was most markedly shown in Reed-Sternberg cells (Fig. 1). This category comprised a spontaneously regressing T-blast cell proliferation with giant multivesicular bodies, a T-immunoblastic lymphoma with the characteristics of 'true histiocytic lymphoma', a primary manifestation of nodular sclerosing Hodgkin's disease in the skin, five cases of LP, a case with concurrent LP and MF, and two cases of clinically characteristic MF. These two MF cases differed from all others in that their tumour cells tended to exhibit a Reed-Sternberg-like cell morphology (Figs. 2 and 3). In the cases of this category a majority of the abnormal cells was Ki-1 positive although in some cases of LP a considerable number of abnormal cells, mostly small lymphocytic variants, was unreactive. In contrast, in both the MF cases and in the patient with concurrent LP and MF a predominance of such lymphocytic variants were Ki-1$^+$.

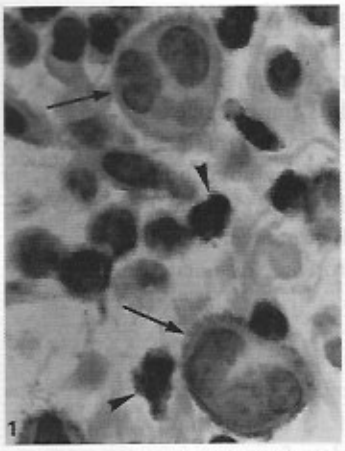
Of ten other cases of MF, Ki-1$^+$ cells formed a large majority of the tumorous infiltrate in two cases, were few in number but regularly found in three, and only occasionally detected in the specimens from five patients. Most Ki-1$^+$ cells were medium-sized to large T-immunoblasts. In five cases occasional Ki-1$^+$ cells were small and mainly situated in B-cell dominated areas with the formation of lymph follicles in one biopsy; and it seemed likely that they were part of the reactive cell component. A similar situation was found in a patient with a non-epidermotropic T-cell lymphoma, and in two cases of malignant B-cell lymphoma. The other cases of malignant lymphoma did not reveal Ki-1$^+$ cells.

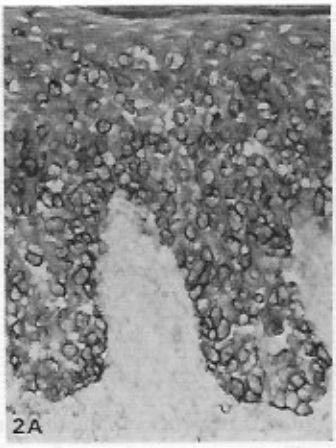
The remaining specimens containing Ki-1$^-$ cells were from non-lymphomatous cutaneous disease (Table I). The highest frequency was observed in specimens from lymphadenosis benigna cutis which showed in three of six patients small Ki-1$^+$ cells often situated just outside the germinal centers (Fig. 4). Occasional Ki-1$^+$ cells were also observed in cases of pityriasis lichenoides, lichen planus and few cases of chronic dermatitis, i.c. contact dermatitis. These were often larger and had a blast cell appearance (Fig. 5). In the remaining 44 specimens with inflammatory infiltrates no Ki-1 reactivity was detected.

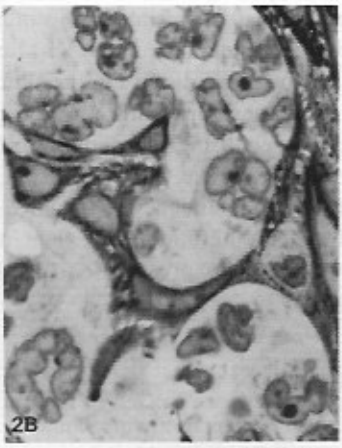
Ki-1$^+$ cells were confined to the dermal infiltrates, with the exception of one MF case in which Ki-1$^+$ tumour cells with a RS-like appearance were almost exclusively present in the epidermis (Fig. 2), another MF case with few positive cells in hair follicle epithelium, and a case of pityriasis lichenoides.

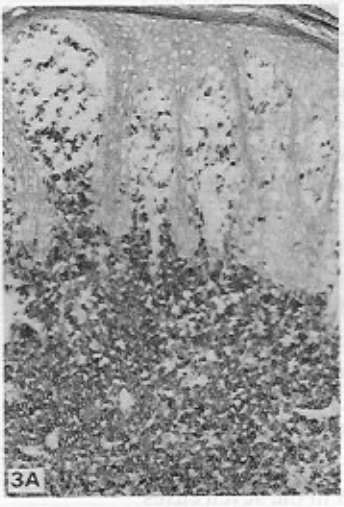
The reaction product was on the cellular membrane in all cases (Fig. 1). In the case of the spontaneously regressing T-blast cell proliferation with giant multivesicular bodies and in one of the MF cases with RS-like cells, there was also intracytoplasmic staining related to extensive intracytoplasmic multivesicular systems.

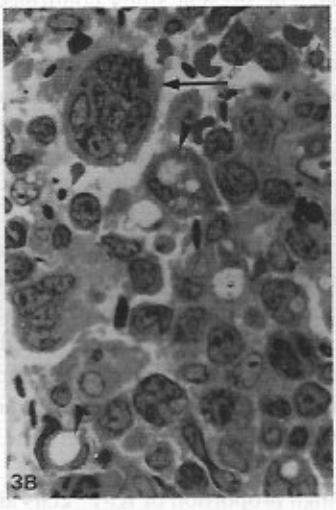
A high proportion of the Ki-1$^+$ cells showed expression of HLA-DR. Correlation of the reactivity of Ki-1 and the proliferation marker Ki-67 in ten specimens which were available for Ki-67 testing and contained sufficiently large numbers of Ki-1$^+$ cells gave widely diverging results. Good correlation was found in only two cases and no correlation at all in four. In the remaining four specimens some areas in the Ki-1$^+$ infiltrate revealed good correspondence with Ki-67, whereas other parts showed hardly any Ki-67 reactivity.

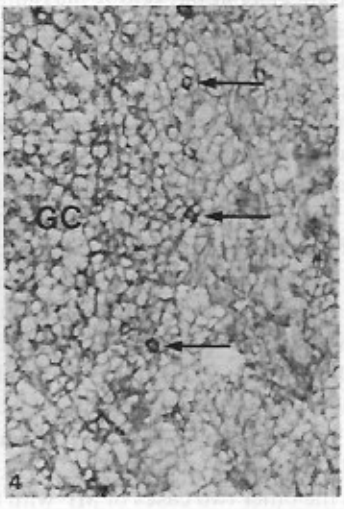












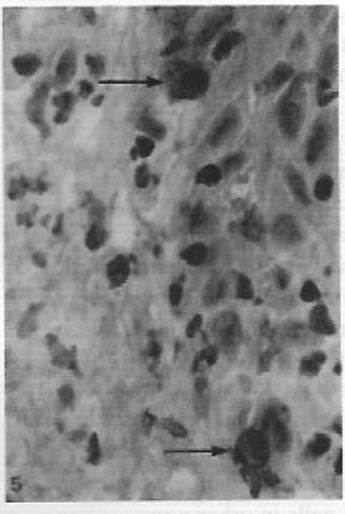


Fig. 1. Ki-1 reactivity expressed on the cell membranes of Reed-Sternberg-like cells (*arrows*) and small atypical lymphoid cells (*arrow heads*) in lymphomatoid papulosis. Glycolmethacrylate embedding after block incubation with the Ki-1 antibody (×675).

Fig. 2. Case of mycosis fungoides revealing dense infiltration of the epidermis by Ki-1$^+$ lymphoid cells (A) (×105). These cells exhibit nuclei with an intermediate morphology between Reed-Sternberg-like cells and Sézary cells (B) (×750).

Fig. 3. Case of mycosis fungoides showing a diffuse dermal infiltrate of Ki-1$^+$ cells (A) (×43). The tumour cells population comprised Reed-Sternberg-like cells (*arrow*) and cells with giant multivesicular bodies (*arrow heads*) resulting in a signet ring cell appearance in some cells (B) (×450).

Fig. 4. Lymphocytoma cutis. Small Ki-1$^+$ cells (*arrows*) are situated in the mantle of lymphocytes surrounding a large germinal center (*GC*) (×135).

Fig. 5. Pityriasis lichenoides. Some large Ki-1$^+$ lymphoid blast cells (*arrows*) are situated near the dermo-epidermal junction of a rete ridge (×335).

Immunohistochemical testing of B- or T-lymphocyte associated antigens showed absence of such markers on the Ki-1$^+$ cell population in the patient with a primary manifestation of Hodgkin's disease in the skin, and in two patients with LP. There were aberrant T-cell immunoprofiles with a variety of deficiencies in the patient with T-immunoblastic lymphoma with the morphology of 'true histiocytic lymphoma', in three other patients with LP, the patient with concurrent LP and MF, and four patients with MF including both patients with a tendency to form RS-like cells. In the other patients with MF, and in the T blast cell proliferation with giant multivesicular bodies a 'usual' helper/inducer T cell phenotype was expressed. Ki-1$^+$ cells did not express monocyte/macrophage-associated antigens.

DISCUSSION

Our observations in a wide range of cutaneous disorders indicate that Ki-1 expression by infiltrate cells in the skin is uncommon. The present study confirmed data from previous investigations in the skin about the consistent Ki-1 reactivity on atypical cells in lymphomatoid papulosis, some large cell lymphomas and Hodgkin's disease (2–14). These disorders, although clinically different, share some immunological and cytological features. Immunohistochemically, most reveal T-cell-associated antigens on the atypical cells although with aberrant phenotypes as compared with normal T-lymphocytes. Cytologically, the atypical cells show a histiocytic appearance and a tendency to bi- and multisegmentation of the nuclei forming Reed-Sternberg (-like) cells.

The combination of Ki-1 reactivity and a Reed-Sternberg cell appearance was striking and has been stressed by previous investigators (4, 6, 8, 19, 20). In the present study, it was particularly well demonstrated by a case with concurrent LP and MF lesions and such a distinct presence of RS-like cells in skin lesions and in an involved lymph node, that it simulated HD (22), and by two other cases which were characteristic MF in clinical features and in the architecture of the infiltrates, but showed a tumour cell morphology that was intermediate between MF and LP. Moreover, in none of the Ki-1 negative cases, RS-like cells were detected.

In the other two cases of MF with a high proportion of Ki-1$^+$ cells and in the seven cases with small numbers of Ki-1$^+$ cells these cells showed the usual morphology of T-immunoblast cells. The low incidence of Ki-1 expression in MF corresponds well with earlier statements (4, 5, 8, 12, 19). Correlation of clinical and histological data from MF cases with and without Ki-1$^+$ cells did not reveal any difference.

Unlike previous studies (5, 8, 11, 12) our investigation did reveal Ki-1 reactivity in non-lymphomatous disorders, but the number of positive specimens and the number of Ki-1$^+$ cells in those specimens were very small. The presence of these occasional Ki-1$^+$ cells may be related to the minute Ki-1$^+$ lymphocytic population that has been identified in the normal peripheral blood (4). On the other hand does the relative frequency of Ki-1$^+$ cells in dermal infiltrates with a substantial B-cell component as shown in lymphocytoma cutis indicate that such Ki-1$^+$ cells might be homologue to the Ki-1$^+$ cells in normal lymphoid tissues (2, 15). As for the Ki-1$^+$ cells in infiltrates which lack a B-cell component it may be argued that they represent the normal counterparts of the atypical cells in Ki-1$^+$ T-cell disorders. The often blast cell appearance of these cells and the presence of some Ki-1$^+$ cells in pityriasis lichenoides, which has much in common with lymphomatoid papulosis, seem in agreement. The absence of Ki-1 reactivity in twelve cases of pityriasis lichenoides reported (8, 11, 12) and the rarity and inconsistent occurrence of Ki-1$^+$ cells in other non-neoplastic T cell proliferation, however, indicate that more study is needed.

The Ki-1 antigen was most consistently expressed by large blast cell variants of the

lymphoid cells which was in accordance with earlier observations (4–6, 8–12, 14, 18–20). Small lymphocytic variants within the same abnormal T-cell population, which could be identified by their incomplete immunoprofiles, were often Ki-1 negative. On the other hand Ki-1 reactivity did occur on such variants as was proven by immunoelectron microscopy for Sézary cells (6) and well-illustrated by two of our MF cases which showed a predominance of such Ki-1$^+$ lymphocytes.

The preferential expression of the Ki-1 antigen by blast cells seems in line with the frequent co-expression of the proliferation associated antigen Ki-67 as was also observed by others (6, 8, 11, 15). However, it appeared from the marked discongruity in many other cases, that Ki-1 is not exclusively expressed by cells linked to mitotic division. The co-expression of HLA-DR by the Ki-1$^+$ cells confirmed earlier observations which indicated that Ki-1 is expressed by activated cells (4–6, 8–12, 14, 18–20). But from the present study which showed a complete absence of Ki-1 reactivity in many infiltrates of highly activated lymphoid cells it seems that this association is more complex than may be concluded from the development of Ki-1 expression on a high proportion of normal blood lymphocytes after exposure to phytohaemagglutinin, lymphotropic viruses and *Staphylococcus aureus*.

Our observations indicate that special, still unknown, conditions are needed to make at least a proportion of the abnormal T-cells express Ki-1 reactivity. They do not give the answer yet to the crucial question if such Ki-1$^+$ cells are derived from special small subpopulations of lymphocytes which tend to express Ki-1 reactivity when properly stimulated, or originate from more common lymphocytes brought to Ki-1 expression by special influences, or if both mechanisms are operating. The answer may be of great importance for the understanding of the nature of diseases with a consistent Ki-1 reactivity.

The present study and data from previous investigations indicate that, at the moment, the application of the Ki-1 antibody for diagnostic purposes in cutaneous disorders is of help in the identification of LP, Hodgkin's disease, some 'histiocytic' T cell lymphomas and, to a lesser degree, MF. With respect to research in this field seems testing of the Ki-1 antibody important because it may help to discover cases which by a freak of nature offer those clues which lead to the elucidation of the function of the Ki-1 antigen, and from there to a better insight into the nature of the Ki-1$^+$ cell disorders which are on the whole poorly understood and display an often confusing biological behaviour.

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