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Factor-XIIIa-expressing dermal dendrocytes in Kaposi's sarcoma

A comparison between classical and immunosuppression-associated types

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Summary. The histogenetic origin of Kaposi's sarcoma is a matter of controversy, with recent reports claiming it to derive from the factor-XIIIa-positive dermal dendrocyte rather than endothelial cells. We investigated the potential role of factor-XIIIa-positive dermal dendrocytes in the genesis of both classical (endemic) and immunosuppression-associated Kaposi's sarcoma. Thirteen cases of classical and 16 cases of immunosuppression (mostly AIDS)-associated Kaposi's sarcoma were immunostained with antibodies to factor XIIIa and to the blood-group antigen H, recognizing endothelial cells. Factor-XIIIa-positive cells were consistently antigen-H-negative and represented only a small percentage (usually less than 10%) of the proliferative cells. Their relative density tended to be decreased in immunosuppression-associated Kaposi's sarcoma when compared with that of the classical form. These results do not support the view that dermal dendrocytes may be the cells of origin of Kaposi's sarcoma; conversely, their decreased density in cases of immunosuppression-associated Kaposi's sarcoma could be related to immunosuppression and may account for more rapid tumour growth.

Key words: Kaposi's sarcoma – AIDS – Factor XIIIa – Dermal dendrocyte – Immunosuppression

Introduction

Kaposi's sarcoma (KS) is a proliferative disorder first described by M. Kaposi in 1872 under the term "multiple idiopathic haemorrhagic sarcoma".This so-called classical or endemic type of the disease (CKS) occurs predominantly in elderly men of Mediterranean descent; it manifests with red-bluish or brown plaques and nodules of the skin, located mostly on the lower extremities, and runs a rather indolent, chronic course. Over the last decade, two other forms of KS have been recognized that develop as a result of prolonged immunosuppression: immunosuppression-associated-KS (IAKS). These are KS associated with AIDS (AIDS-KS) (Krown 1988) and KS developing in organ (mostly renal) graft recipients (OG-KS) (Mauduit et al. 1987). Compared with CKS, IAKS occurs predominantly in young adults, manifests clinically with reddish patches and plaques with a more extensive distribution over the body and is characterized by rapid progression. Histologically, both CKS and IAKS comprise a proliferation of more or less well-differentiated vessels lined by endothelial cells, a proliferation of spindle cells, an inflammatory infiltrate of variable density and extravasation of erythrocytes.

Although the clinicopathological appearance of KS is characteristic and seldom causes diagnostic problems, its precise cell of origin has been a matter of controversy and is still not recognized unequivocally. A considerable amount of evidence, originating mostly from immunohistochemical investigations, support the endothelial (blood-vessel or lymphatic) origin of the proliferating cell in KS. More recently, it has been suggested that factor-XIIIa-expressing dermal dendrocytes (DD) could be the cell of origin of the spindle-cell population which characterizes KS (Nickoloff and Griffiths 1989a, b). DD are recently described bone-marrow-derived dendritic cells of the dermis found mainly in an angiocentric pattern within the reticular dermis (Headington 1986). They may be recognized immunohistochemically by antibodies directed against factor XIIIa, the enzymatic subunit of the coagulation factor that catalyzes the formation of cross-linking peptide bonds between glutamine and lysine residues of fibrin. DD also express a variety of immune-associated surface antigens shared with other members of the mononuclear-phagocyte system (Cerio et al. 1989); this observation, along with the fact that DD are endowed with phagocytic properties (Headington 1986), has lent support to the consideration of DD as members of the group of dendritic, antigen-presenting cells. We understook a comparative study of the distribution of factor-XIIIa-positive DD in CKS and

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IAKS in order to investigate the potential role of these cells in the histogenesis of KS.

Materials and methods

Thirteen biopsy or excision specimens of CKS and 16 biopsies of IAKS of the skin were obtained from 15 immunocompromised patients (13 AIDS-KS and 2 OG-KS). These specimens had been collected over the last 6 years in our dermatopathology laboratory, fixed in formalin and paraffin embedded. The diagnosis had been made on sections stained with haematoxylin and eosin (H & E) and Perl's stain for iron, when necessary.

On examination of H & E-stained sections, each case was classified as belonging to the nodular or the patch/plaque type. Nodular KS was defined by rather well-circumscribed dermal nodules consisting of aggregations of spindle-shaped cels, surrounding vascular slits lined by endothelial cells. The patch/plaque type of KS was characterized by the presence of an ill-defined proliferation of anastomosing, thin-walled vascular spaces with jagged outlines, surrounded by fibroblast-like cells diffusely infiltrating the dermis, but lacking compact spindle-cell aggregations. The density of the inflammatory mononuclear round-cell infiltrate (lymphocytes, plasma cells) was scored in each case as 1 (sparse), 2 (moderate) or 3 (dense).

Immunohistochemical staining was performed by applying a labelled streptavidin-biotin-peroxidase technique (LSAB kit Dako, Copenhagen, Denmark). Briefly, 3-μm-thick sections were deparaffinized and rehydrated; endogenous peroxidase was blocked by 0.3% hydrogen peroxide. Thereafter the sections were incubated with: (a) normal goat (blocking) serum, (b) primary antibody (see below), (c) secondary antibody (a mixture of biotinylated antibodies to rabbit and mouse immunoglobulins), (d) peroxidase-conjugated streptavidin, (e) chromogen substrate (aminoethylcarbazole), (f) Mayer's haematoxylin as counterstain. Primary antibodies used were: (a) a rabbit polyclonal antibody to factor-XIIIa (Behringwerke, Marburg, FRG), diluted 1:300, and (b) a mouse monoclonal antibody to blood-group antigens H and Y, recognizing the membrane of blood endothelial cells (clone BNH9; Immunotech, Marseille, France), diluted 1:10. The relative density of factor-XIIIa-positive cells within the KS lesions was scored as 1 (< 1%), 2 (1–5%), 3 (5–10%) or 4 (over 10%).

The mean scores of factor-XIIIa-positive cells and the density of the inflammatory infiltrate were compared by the Student's *t*-test. The frequency of nodular and patch/plaque types in CKS versus IAKS was compared using Fisher's χ^2 test.

Results

The results of our study are shown in Table 1.

The great majority of CKS lesions belonged to the nodular type (12:13, 92.3%), whereas lesions of IAKS were predominantly (11/16, 68.75%) of the patch/plaque type ($P<0.01$ by the χ^2 test). Both types of KS contained a mononuclear-cell inflammatory infiltrate, comprising mainly lymphocytes and, to a lesser degree, plasma cells; its density was higher in CKS (mean score 2.38 ± 0.65) than in IAKS (mean score 1.69 ± 0.70) ($P<0.01$ by the *t*-test).

The antibody to factor XIIIa decorated dendritic cells located around blood capillaries of the papillary dermis and around the secretory tubules of eccrine sweat glands of the deep dermis. In some cases, spindle-shaped cells with a wavy appearance observed within dermal nerves (most likely endoneural fibroblasts) were also stained. These normal cellular components served as internal positive controls.

When the KS lesions were examined, it was seen that factor-XIIIa-positive cells were found mostly at the periphery, and more rarely within the area comprising the vascular proliferation. They had a morphology similar to that of normal DD (elongated, fusiform cells with a cytoplasm more scanty than that of spindle-shaped cells of KS), and were consistently antigen-H-negative, as seen on serial sections (Figs. 1, 2). Their relative density, when compared with the density of the remaining proliferating dermal cells (excluding the mononuclear inflammatory infiltrate) was low, varying in most cases between 1% and 5%, with only one case of CKS comprising over 10% (15%) of factor-XIIIa-positive cells. It thus seemed that factor-XIIIa-positive cells represented DD either entrapped by or simply surrounding

Table 1. Histological and immunohistochemical findings in classical (CKS) and immunosuppression-associated (IAKS) Kaposi's sarcoma

	CKS ($n=13$)	IAKS ($n=16$)
Nodular type	12	5
Patch/plaque type	1	11
Infiltrate density	2.38 ± 0.65	1.69 ± 0.70
Factor-XIIIa density	1.92 ± 1.12	1.81 ± 0.75

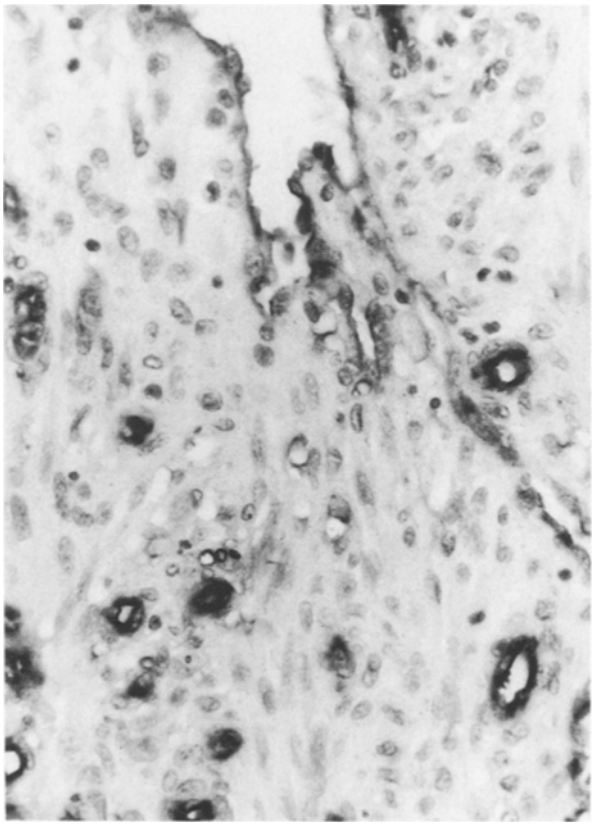

Fig. 1. Immunolabelling of a nodular lesion of classical Kaposi's sarcoma for blood-group antigen H: all cells lining vascular channels are labelled; however, no reactivity is seen on spindle-shaped cells. Immunoperoxidase, ×250

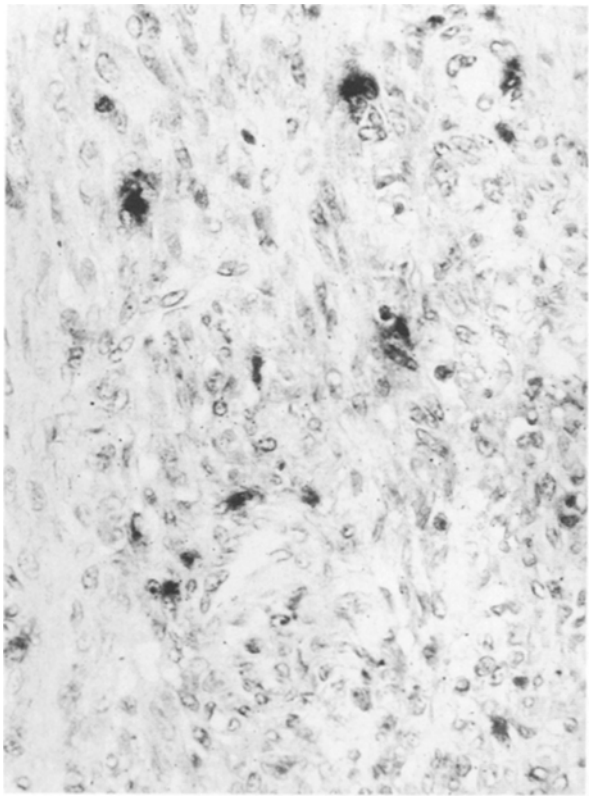

Fig. 2. Immunolabelling of a nodular lesion of classical Kaposi's sarcoma with anti-factor-XIIIa antibodies: scattered reactive cells, surrounding vascular channels, are seen within the tumour. Immunoperoxidase, ×250

the vascular proliferation. When the relative density of factor-XIIIa-positive cells in the two groups of KS was compared, it was found that IAKS contained fewer such cells (mean score 1.81 ± 0.75) than CKS (mean score 1.92 ± 1.12), but this difference did not reach statistical significance ($P > 0.05$ by the t-test).

On the other hand, the antibody to H and Y antigen in all cases decorated normal endothelial cells of dermal capillaries and cells lining vascular lumina within the tumour, irrespective of their size or degree of differentiation. Neither spindle-shaped cells of KS nor factor-XIIIa-positive DD were labelled by this antibody in any of the specimens studied.

Discussion

KS is a vascular proliferative disorder with particular clinicopathological features that has raised considerable debate concerning its histogenesis. Indeed, while cells lining vascular channels within the tumour are regarded as endothelial cells, the nature of the spindle cells is less clear and their immunohistochemical phenotype controversial. For instance, the immunoreactivity of spindle cells for factor-VIII-related antigen, the most specific endothelial marker, has been reported to be frankly positive (Nadji et al. 1981; Modlin et al. 1983; Bendelac et al. 1985; Corbeil et al. 1991), negative (Burgdorf et al. 1981; Sehested and Hou-Jensen 1981; Miettinen et al. 1983; Beckstead et al. 1985; Mayer da Silva et al. 1987), or weak and focal, both in CKS (Akhtar et al. 1984; Flotte 1984; Guarda et al. 1981; Ordonez and Batsakis 1984; Leu and Odermatt 1985; Little et al. 1986; Russel-Jones et al. 1986; Suzuki et al. 1986; Hashimoto et al. 1987; Dictor and Anderson 1988; Scully et al. 1988; Sankey et al. 1990; Gray et al. 1991) and in AIDS-KS (Flotte et al. 1984; Russel-Jones et al. 1986; Schulze et al. 1987; Gray et al. 1991). Similarly, the reactivity of the spindle-cells for the *Ulex europaeus* agglutinin lectin-I (UEA-I), recognizing α-L-fucose residues present on the surface of endothelial cells, has been reported to be (weakly) positive by some (Ordonez and Batsakis 1984; Beckstead et al. 1985; Bendelac et al. 1985; Little et al. 1986; Russel-Jones et al. 1986; Hashimoto et al. 1987; Schulze et al. 1987; Dictor and Anderson 1988; Gray et al. 1991) and negative by others (Miettinen et al. 1983; Suzuki et al. 1986; Mayer da Silva et al. 1987; Sankey et al. 1990). Variable results have also been reported regarding OKM5 (CD36) reactivity, found to be strongly expressed by spindle cells of AIDS-KS (Rutgers et al. 1986) or negative in cases of CKS (Suzuki et al. 1986). These conflicting results may be due, at least in part, to the different material (frozen versus formalin-fixed) used in each of these studies. Other immunohistochemical markers that have been found expressed to a variable degree by KS cells include EN3-EN4 (Russel-Jones et al. 1986; Corbeil et al. 1991), BNA-120 (Hashimoto et al. 1987), E92 (Rutgers et al. 1986), HC1 (Rutgers et al. 1985; Scully et al. 1988), B271 and E431 (Scully et al. 1988), QBEnd 10 (Sankey et al. 1990) and the human progenitor-cell antigen CD34 (Nickoloff 1991). Following the characterization of the factor-XIIIa-positive DD, Nickoloff and Griffiths (1989a) reported that AIDS-KS comprised a significant proportion (up to 50%) of these cells, which they regarded as the possible cells of origin for the spindle-cell population of KS. Other authors, however, did not corroborate this finding. The present study was undertaken in an attempt to clarify the involvement of DD in the genesis of IAKS and CKS.

Our results showed a remarkable difference in the histological appearance of CKS versus IAKS. Indeed, the former comprised almost exclusively (92.3%) nodular lesions, whereas IAKS was more frequently (68.75%) of the patch/plaque type. This finding, which is in keeping with previous reports (Gottlieb and Ackerman 1982), seems to correlate with the clinical aspect of KS that manifests with raised tumours in the classical form and with more flat patches or plaques in the immunosuppression-associated form. Another histological finding that differed significantly between the two forms is the intensity of the peritumour inflammatory reaction, which is less extensive in the IAKS as compared to the CKS group. This finding, which has already been noted for skin carcinomas developing in renal homograft recipients (Mullen et al. 1976), seems to be related to the immunosuppression and could contribute to the more rapid tumour progression.

When the DD population within the KS lesions was considered, it was found that these cells did not consti-

tute a significant percentage of the proliferating cells (even after exclusion of the mononuclear round-cell infiltrate) in either type of KS; indeed, factor-XIIIa-positive cells represented less than 10% of the total spindle-cell population in almost all lesions, with only one case (of CKS) having around 15% of these cells. Factor-XIIIa-positive cells had a morphological appearance reminiscent of normal DD more than of KS spindle cells and were consistently antigen-H-negative. They were thus clearly distinct from the endothelial population lining vascular channels. These results are in disagreement with those of Nickoloff and Griffiths (1989a) and do not support the view that factor-XIIIa-positive DD may be the parental cell of origin in KS, be it of the classical or the immunosuppression-associated type. Instead, we feel these cells could represent DD entrapped within the proliferative process. This is in agreement with the results of Cerio et al. (1990) and Gray et al. (1991), whose work appeared in the literature during preparation of the present manuscript.

DD are bone-marrow-derived dendritic cells expressing a panel of membrane antigens (such as HLA-DR/DQ, CD36/OKM5, CD14/LeuM3, LFA-1) involved in immune reactions; they also share other cytochemical characteristics with cells of the mononuclear-phagocyte system (expression of non-specific esterase and lysozyme) and furthermore display phagocytic properties (Headington 1986; Cerio et al. 1989); hence it seems likely that DD may be "part of an immunologically competent system indigenous to the dermis and either supplementary or complementary to immunologically functional cells of the epidermis" (Headington 1986). Noteworthy in this context is the fact that in our study we found the relative density of factor-XIIIa-positive DD to be reduced in IAKS as compared to CKS. Even though the difference failed to reach statistical significance, we believe that, like the intensity of the mononuclear inflammatory infiltrate, the decrease of DD in IAKS when compared with CKS may be related to the immunodeficiency. Indeed, it is conceivable that the iatrogenic and the HIV-induced immunosuppression lead to a reduction in the density of DD surrounding the tumour proliferation, with an ensuing decrease of the local immune surveillance mechanisms. This may account, at least in part, for the more rapid tumour progression of IAKS which contrasts with the usually chronic and indolent course of CKS. This hypothesis lends further support to the consideration of DD as immunocompetent cells involved in local immune defence mechanisms, and relates to the function of another cutaneous dendritic, antigen-presenting cell, the epidermal Langerhans cell. In some cutaneous malignancies of mesenchymal origin it has been shown that decreased numbers of Langerhans cells may correlate with a more rapid tumour growth and a shorter survival (Meissner et al. 1990). We are tempted to speculate that a similar correlation could exist with respect to the density of the peritumour factor-XIIIa-positive DD.

In conclusion, the results of our study do not support the contention that DD may be the cell of origin of KS; we feel that they rather represent part of the inflammatory peritumour infiltrate. Our finding of reduced numbers of DD in the group of KS associated with immunosuppression when compared with classical KS suggests that these cells could be effectively involved in local immune-defence mechanisms, the decrease of which may promote tumour progression.

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