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Case report

Histiocytosis X arising in Hodgkin's disease: immunophenotypic characterization with a panel of monoclonal antibodies

Antonella Coli [1], Giulio Bigotti [1], and Soldano Ferrone [2]

[1] Department of Pathology, Catholic University of Sacred Heart, Largo F. Vito 1, I-00168 Rome, Italy
[2] Department of Microbiology and Immunology, New York Medical College, Valhalla, New York, USA

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Summary. This report describes the antigenic profile of the proliferating cells of pulmonary histiocytosis X (HX) in a patient treated with chemotherapy for Hodgkin's lymphoma; the association of pulmonary HX and Hodgkin's disease has rarely been described in the literature. The histopathological diagnosis of HX was confirmed with the aid of monoclonal antibodies (mAbs) to CD4, CD1a, and polyclonal serum anti S-100 protein. The phenotype of HX cells has been analysed using a panel of mAbs against HLA class I A, B, C monomorphic determinants, locus A and B, β2-microglobulin, HLA class II distinct monomorphic determinants, DP, DQ, DR, intercellular adhesion molecule-1 (ICAM-1) and vitronectin receptors. Our results indicate that HX cells express HLA class I and II, including locus A, locus B and DP, DQ, DR, like their normal counterpart (represented by Langerhans cells) and detectable levels of ICAM-1 but not vitronectin receptors. We would like to stress the possibility of the association of HX and Hodgkin's lymphoma extending the immunophenotypic profile of HX cells.

Key words: Histiocytosis X – Hodgkin's lymphoma – Immunohistochemistry – Intercellular adhesion molecule 1– Major histocompatibility complex

Introduction

Histiocytosis X (HX) is an uncommon disorder, presumably originating from the Langerhans cell (LC) (Beckstead et al. 1984), a type of cell usually residing in the epidermis (Murphy et al. 1986). The disease generally occurs in the young.

The histopathological diagnosis can offer a diagnostic challenge, since HX has to be distinguished from other histiocytic proliferations. It is usually confirmed by demonstration of markers such as CD1a and S-100 (Soler et al. 1985; Webber et al. 1985). Furthermore, the proliferating cells of HX have been shown to express HLA class I A, B, C common monomorphic determinants and HLA class II-DR, in a fashion analogous to the LC.

ICAM-1, a heavily glycosilated protein normally expressed in post-activated germinal centre cells and histiocytes but not in normal resting B and T lymphocytes, has not until now been investigated in the proliferating cells of HX, although recent evidence suggests that normal LC lacks CD11a/CD18 (LFA-1), the natural ligand of intercellular adhesion molecule-1 (ICAM-1) (De Panfilis et al. 1989).

Vitronectin receptors, one of the members of the supergene family of integrins (Hynes 1987), important in regulating cellular adhesion to extracellular matrix and ubiquitiously localized to loose connective tissue (Reilly and Nash 1988), have not been evaluated in this disorder.

We present here the association of Hodgkin's disease (HD) with pulmonary HX, analysing in detail the immunophenotype of HX proliferating cells.

Case report

This 42-year-old male smoker presented with right chest discomfort and increasing shortness of breath. Seven years previously he had developed symptomatic HD (mixed cellularity type) involving the left supraclavicular, right hilar and mediastinal lymph nodes. Treatment with cyclophosphamide, vincristine, procarbazine and prednisone had resulted in complete remission 1 year later.

Three years prior to admission he developed left axillary adenopathy for which he received local radiation therapy followed by chemotherapy, resulting in complete remission. During this time the patient noted progressive though intermittent dyspnoea on exertion. At the current admission the patient complained of recurrent chest pain and dyspnoea. Chest radiographs showed diffuse reticulonodular opacities.

After transbronchial biopsies had shown only minimal nonspecific inflammation, an open lung biopsy was performed.

Offprint requests to: A. Coli

Materials and methods

The lung biopsy specimens were examined using standard sections stained with haematoxylin and eosin; furthermore, paraffin sections were stained for acid fast bacilli and using a Gomori methenamine silver technique.

For immunohistochemistry tumour tissue samples were snap-frozen in liquid nitrogen and stored at −80° C until sectioning.

Sections of 4–6 μm thickness were cut, air-dried, fixed in acetone for 10 min at room temperature, then treated with chloroform to block the endogenous peroxidase activity and finally processed for the immunoperoxidase reaction using the avidin-biotin complex method as previously described (Hsu et al. 1981).

Polyclonal antiserum anti-S-100 protein (1:300) was employed as well as the following mAbs: anti-LCA (prediluted), OKT4 anti-CD4 (1:100), OKT6 anti-CD1a (1:20), OKM1 (1:2), CL 203.4 anti-ICAM-1 (1:10), TP 36.1 anti-vitronectin receptors (1:10), VF19-LL67 anti-locus A (1:20), Q6/64 anti-locus B (1:20), TP25.99 anti-HLA class I A, B, C monomorphic determinants (1:30), B7/21 anti-DP (1:10), SPV-L3 and anti-DQ (1:5), CL413 anti-DR (1:10), NAMB-1 anti-β2-microglobulin (1:30), Q5/6 (1:20) and Q5/13 (1:20) to distinct monomorphic determinants of HLA class II antigens.

OKT4 and OKT6 were obtained from the American Tissue Type Culture Collection; anti-LCA and anti-S-100 were purchased from Dako (Santa Barbara, Calif., USA). The remaining mAbs were supplied by one of us (S.F.) and previously described (Maio et al. 1989).

Normal horse serum was used as a control for the reaction instead of primary antibody.

Results

Microscopic examination of biopsies of the right upper and middle lobes shows consolidation of the lung parenchyma by numerous intra-alveolar histiocytes in a pattern resembling desquamative interstitial pneumonitis. Focal stellate areas of alveolar septal thickening are found, associated with interstitial and intra-alveolar collections of cells having the typical cytological features of LC (Fig. 1a). These are intermingled with mononuclear cells, neutrophils and small number of eosinophils. Two small, non-necrotizing granulomas are found in the upper lobe specimen, but no acid-fast or fungal organisms are seen on Ziehl-Neelsen or methenamine silver stains.

Immunoperoxidase-processed sections show positive staining of the proliferating cells with anti-CD4 and CD1a mAbs; the reactivity is mostly membranous. OKM1 stains only scattered, isolated cells without clustering. Anti-LCA does not stain any of the proliferating cells. Anti-S-100 protein stains the neoplastic cells strongly at the cytoplasmic level (Fig. 2). Antibodies against HLA class I and II distinct monomorphic determinants, anti-locus A (Fig. 3a) and B (Fig. 3b), anti-DP (Fig. 1c), DQ (Fig. 1d), DR (Fig. 1b) and anti-β2-microglobulin stain the proliferating cells with a membrane

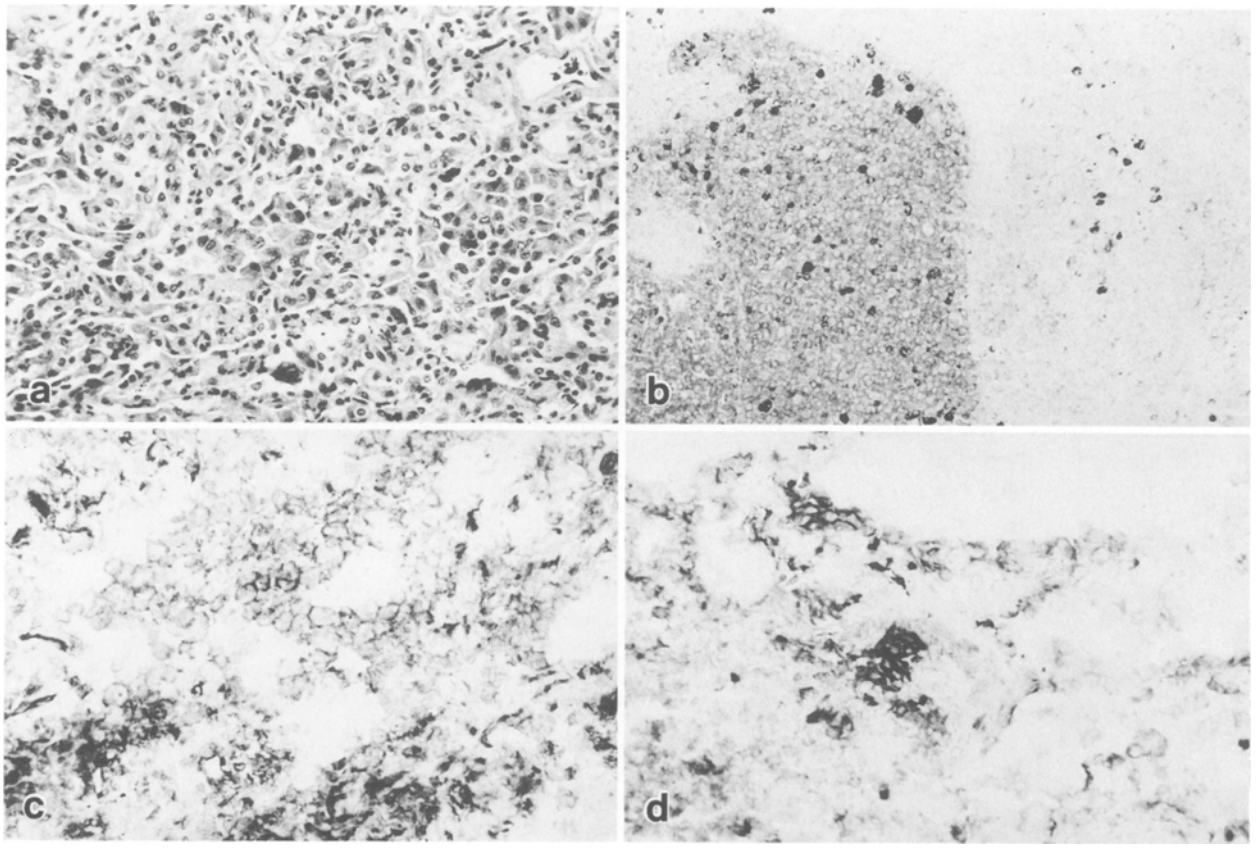

Fig. 1. **a** Histological appearance of histiocytosis X (HX): proliferation of cells with typically folded and grooved nuclei. Distribution of HLA class II DR (**b**), DP (**c**) and DQ (**d**) on cryostat sections of pulmonary HX detected by the IP-ABC method. Staining with anti-DP, DQ, DR is localized in the membrane of proliferating cells. **a, c, d** ×250; **b** ×100

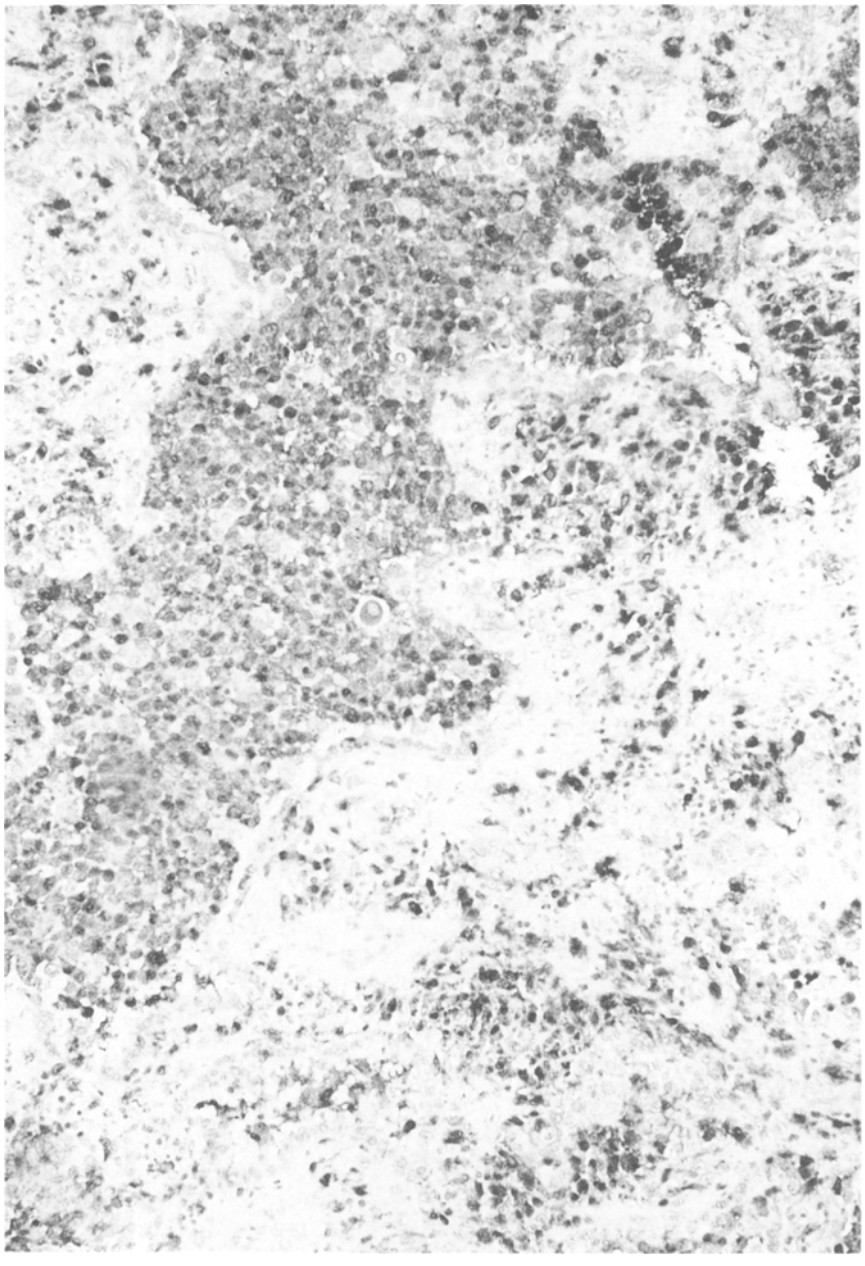

Fig. 2. Section of pulmonary HX treated with anti S-100 protein antiserum. Immunoreactivity is detected in the proliferating cells at the cytoplasmic level. ×100

distribution. Anti-HLA class I and II also stain the hyperplastic alveolar epithelium (type II pneumocytes) entrapped among the proliferating cells; the endothelium of the vessels but not the wall, is ubiquitiously stained by both the latter mAbs; the alveoli, unaffected by the process and localized in the thin rim of the surrounding lung parenchyma, are stained only by anti-HLA class I, with both locus A and B being represented.

mAb CL203.4 anti-ICAM-1 stains most of HX cells with membrane and partially cytoplasmic pattern (Fig. 3d); focally residual hyperplastic alveolar epithelium (type II pneumocytes) is also stained (Fig. 3c).

Furthermore, the examination of sequential sections stained with anti-HLA class I and II mAbs as well as with anti-ICAM-1 mAb reveals that the same clusters of cells positive for S-100 protein and CD1a are staining.

No staining is detected by TP 36.1 anti-vitronectin receptors either in the proliferating cells or in the collagen surrounding them; the only positive staining observed with this latter mAb is localized to the subendothelial layers and around smooth muscle cells of the vessels as well as perivascularly and in the sub-basement membrane region of both normal and hyperplastic alveolar epithelium. The stronger staining is observed in the latter.

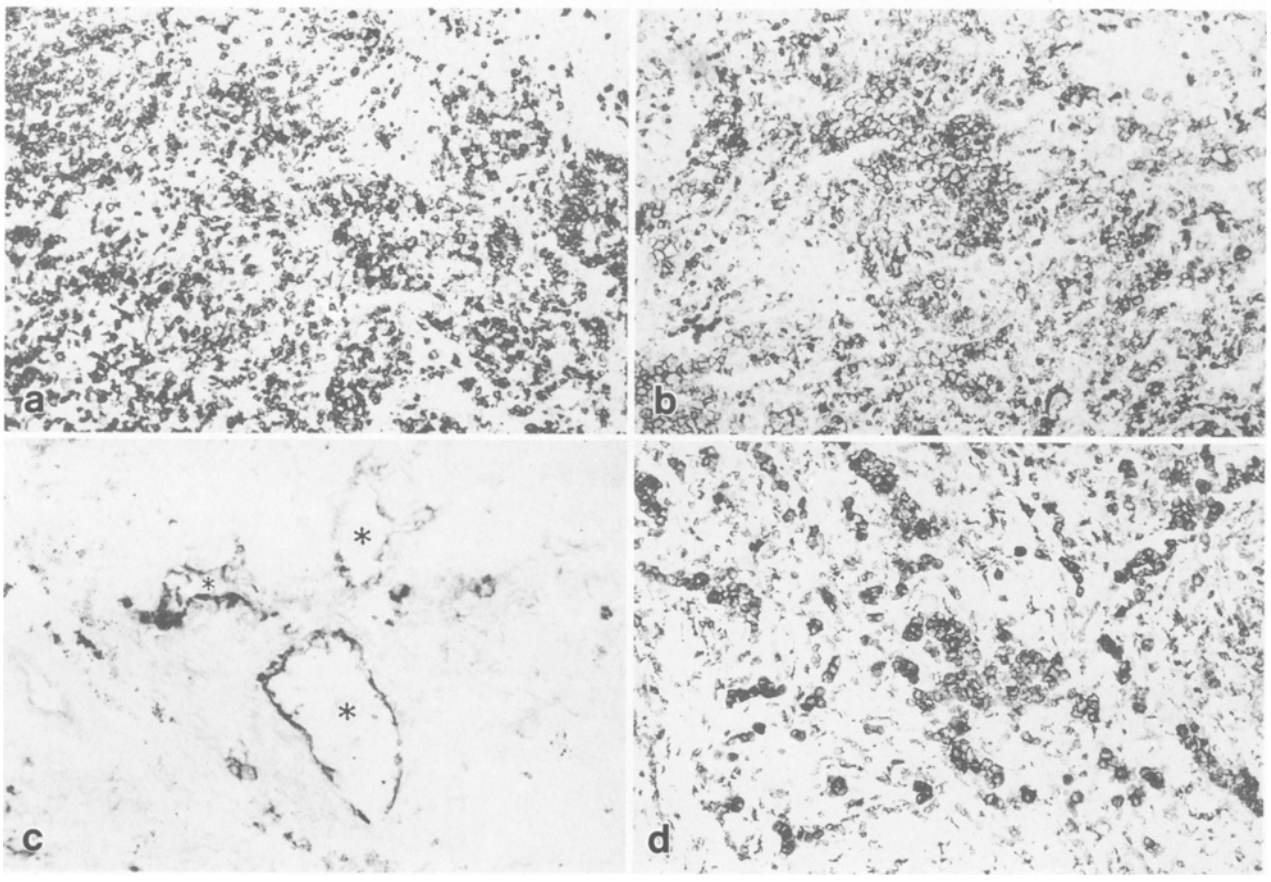

Fig. 3. Cryostat sections of pulmonary HX treated with anti-locus A of HLA class I (**a**), anti-locus B (**b**) and anti-ICAM-1 (**c, d**). Staining with anti-locus A and anti-locus B is confined to the membrane of HX cells. Anti-ICAM-1 stains focal residual hyperplastic alveolar epithelium surrounding alveolar lumina (*asterisks*) (**c**) and the membrane of HX cells (**d**). ×250

Discussion

The association between HX and Hodgkin's lymphoma has already been reported in the past (Lyon et al. 1985), either as simultaneous involvement of the same lymph node (Burns et al. 1983; Kjeldsberg and Kim 1980) or arising in the lungs after chemotherapy for treatment of HD (L'Hoste et al. 1982; Sajjad and Luna 1982). Pulmonary HX occurring in HD seems to be quite rare, and it has been previously described in only 5 patients (Flint and Smid 1987; L'Hoste et al. 1982; Neumann and Frizzera 1986; Sajjad and Luna 1982). Furthermore, a study of 28,462 patients with HD revealed the occurrence of other types of second tumours, but HX has never been documented (Kaldor et al. 1987).

The association between HX and Hodgkin's lymphoma is not well understood, but the possibility that T-cell deficits, as observed in these patients (Cimino et al. 1988), might lead to an uncontrolled proliferation of LC has also to be considered as a possible contributing mechanism and as a link between the two disorders.

In our article we describe the occurrence of pulmonary HX subsequent to the chemotherapeutic treatment of HD, with the purpose of stressing the possibility of this association and of extending the immunophenotypic characterization of HX proliferating cells with the aid of a panel of different mAbs.

Our results show that proliferating cells of HX express HLA class I A, B, C monomorphic determinants, β2-microglobulin as well as locus A and B, although HLA class I A, B, C and DR have been already detected in the proliferating cells of HX (Azumi et al. 1988; Flint et al. 1986). This is the first time that locus A and B of HLA class I have been demonstrated separately in such cells. Furthermore, we have demonstrated strong staining of HX cells by anti-HLA class II and in particular anti-DP, DQ and DR.

While in the normal human tissue loci A, B and C are contemporaneously expressed on most of the nucleated cells, some solid tumours have been shown to exhibit selective loss of the antigenic product of one or the other locus (Natali et al. 1989); this has been true also for the HLA class II loci products (Natali et al. 1987). We have therefore checked the differential expression of the loci encoding for the antigenic products of HLA class I and II in the proliferating cells of HX; our results indicate that HX cells maintain a phenotype similar to LC with all the loci investigated present. This immunophenotypic similarity, even if it should be confirmed on a greater number of cases, lends support to

the opinions of some authors that the proliferating cells of HX represent activated LCs (Kawanami et al. 1981) or, as Burns et al. (1983) suggest, that HX may be a peculiar localized response to the lymphomas, perhaps resulting from the abnormalities of the immune system associated with this disorder.

Proliferating cells of HX have displayed staining with anti-ICAM-1. This is mostly of membrane type and much less evident in the cytoplasm; this finding could be related to the role of the adhesion molecules in guiding the migration and localization of these cells. Of note was the strong staining pattern of hyperplastic alveolar epithelium entrapped within the tumour infiltrate by anti-ICAM-1, contrasting with the non-reactivity of normal alveolar epithelium outside the tumour; this might represent a synthesis de novo, possibly under the stimulation of cytokines such as interleukins or gamma interferon (Dustin et al. 1986; Rothlein et al. 1988) or, alternatively ICAM-1 might be passively picked up by the alveolar epithelium.

In the present case, proliferating cells and the surrounding collagen were not stained by anti-vitronectin receptor, raising the possibility of loose adherence of such cells to extracellular matrix; however, the attachment of HX cells to extracellular matrix could be mediated by other adhesion molecules.

In conclusion, we feel that HX should always be considered when evaluating a histiocytic proliferation in patients with past or present Hodgkin's lymphoma in order to avoid a mistaken diagnosis of relapse and to permit appropriate therapy. The present contribution, extending the immunophenotypic profile of the proliferating cell in HX, is of interest especially in view of the incomplete current knowledge about the relationship between dendritic cells, HX and lymphomas.

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