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Immunohistological diagnosis of "plasmacytoid T cell lymphoma" in paraffin wax sections

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Abstract

An immunohistological study of paraffin wax embedded tissue from three cases of plasmacytoid monocyte neoplasms, using a panel of antibodies which react with fixation resistant leucocyte markers, is reported. This neoplasm was found to have a distinctive antigenic profile, being negative for CD3 and elastase, but positive for CD43 and CD68. This immunological phenotype, coupled with its characteristic morphological features, should facilitate the recognition of this rare neoplasm in routinely processed tissue. Furthermore, the term "plasmacytoid monocyte sarcoma" is proposed to designate it because it is inappropriate to refer to it as a lymphoma. As all cases have been associated with a myeloproliferative disorder (usually an acute or chronic myeloid leukaemia), these tumours probably represent the accumulation in lymphoid tissue of neoplastic cells which have differentiated along the plasmacytoid monocyte pathway.

In 1958 Lennert and Remmele described unusual "nests of lymphoblasts" in reactive lymph nodes.[1] Based on their localisation in the T dependent areas of the lymph nodes,[2] expression of some T cell associated antigens,[3-8] and their well developed concentric rough endoplasmic reticulum,[9] these cells were initially referred to as "T-associated plasma cells" and then subsequently as "plasmacytoid T cells".[3] More recent immunophenotyping studies have shown, however, a profile of antigen expression by these cells which favours a monocyte origin,[6 7 10 11] and the term "plasmacytoid monocyte" is now increasingly used.[12 13] Additional evidence in favour of their cellular origin comes from the fact that each of the five published cases of "plasmacytoid T cell lymphoma" has been associated with a myeloproliferative disorder,[3 5 6 13 14] suggesting that the neoplastic cells which infiltrate the lymphoid tissue in these patients derive from the same bone marrow clone as the myeloid cells seen in the peripheral blood.

It would be interesting to collect further examples of this rare neoplasm as they might throw further light on the nature and physiological function of the plasmacytoid monocyte. Many of the antigenic markers which can be used to confirm the diagnosis of this neoplasm, however, are only detectable in fresh or frozen material.[3-12] In this paper we report an immunohistological study of three cases of "plasmacytoid T cell lymphoma" using a panel of antibodies which react with paraffin wax embedded tissues, and show that, even with the limited range of markers detectable in this type of material, this neoplasm has a distinctive antigenic profile which should facilitate the diagnosis of new cases in the future.

Methods

Paraffin wax embedded lymph node tissue from three cases of previously diagnosed "plasmacytoid T cell lymphoma" associated with myeloproliferative disorders formed the basis of this study (table 1). Two of the cases (cases 2 and 3) have been previously published.[6 14] Case 1 is an unpublished case which was diagnosed on the basis of the characteristic histological features of a biopsied enlarged lymph node, associated with an atypical myeloproliferative disorder.

The tissue samples used for this study had been fixed routinely in formalin or B5 fixative, and then processed and embedded in paraffin wax by conventional methods. Serial 5 μm sections were stained by the APAAP immunoalkaline phosphatase technique, using the antibodies summarised in table 2, as previously described.[15]

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Table 1 Cases of "plasmacytoid monocyte" lymphoma investigated in this study

Case No	*Age/Sex*	*Clinical history*	*Reference*
1	6 F	Presented at 3 years with atypical myeloproliferative disorder; responded to thioguanine and steroids; now well but has fluctuating lymphadenopathy, massive hepatosplenomegaly, and vasculitic rash	Unpublished
2	74 M	Lymphadenopathy, high white cell count $58{\cdot}3 \times 10^9$/l (60% neutrophils, 25% monocytes); acute myelomonocytic leukaemia diagnosed; died after six months	Beiske *et al*[6 10]
3	58 F	Twelve year history of stable idiopathic myelofibrosis, then developed lymphadenopathy (biopsy), hepatosplenomegaly, and CML-like blood picture (Ph'chromosome negative); died 28 months later	Koo *et al*[14]

Table 2 Antibodies used for paraffin wax section immunophenotyping

Antibody	*Type*	*Specificity*	*Source/reference*
Anti-CD3	Polyclonal	CD3	Mason *et al*, 1989[16]
NP57	Monoclonal	Neutrophil elastase	Pulford *et al*, 1988[17]
KP1	Monoclonal	CD68	Pulford *et al*, 1989[18]
DF-T1	Monoclonal	CD43	Stross *et al*, 1989[19]
MAC387	Monoclonal	L1 antigen	Flavell *et al*, 1987[20]
Anti-lysozyme	Polyclonal	Lysozyme	Dako a/s

Results

Histological examination of the three specimens showed the previously described characteristic histological features (fig 1). The paracortical area was invaded by diffuse aggregates of uniform, pale staining, medium sized cells, rather larger than small lymphocytes. Residual reactive lymphoid follicles were present in each of the cases. In case 3 rare megakaryocytes could be seen within the tumour infiltrate in the lymph node, consistent with extramedullary haematopoiesis.

The immunological phenotypes of the neoplastic cells are summarised in table 3. In each case these cells were strongly positive for CD43 (antibody DF-T1) and for CD68 (antibody KP1) (figs 2 and 3). Both antibodies stained large aggregates of tumour cells as well as scattered plasmacytoid monocytes. Neither antibody was selective for the neoplastic cells, because CD43 could also be detected on normal T cells (fig 2) and CD68 on normal macrophages (fig 3). The tumour cells were negative for CD3 antigen (fig 2), and in this single case, tested for the myeloid marker elastase (fig 4). In this case scattered neutrophils were positive for the elastase antigen, but rare strongly elastase positive mononuclear cells—that is, myeloid precursors—were also present in the tumour (fig 4). Scattered megakaryocytes were also shown in this case (fig 4) by staining for platelet glycoprotein IIIa (antibody Y2/51).

Discussion

The cytology of plasmacytoid monocytes, as well as their immunological phenotype on frozen sections and cell suspension, has now been well documented,[1-14] allowing them to be distinguished from small germinal centres, from monocytoid B cells,[8 21] from aggregates of macrophages and from true plasma cells. Several studies have shown that they express CD4, CD36 (thrombospondin receptor), CD45 (leucocyte common antigen), the monocyte/macrophage marker CD68 and HLA Class II antigen, and show variable positivity for CD10 (CALLA).[2 4 7 11 22] They lack pan-T cell markers, the reactivity for CD4 being consistent with the expression of this molecule on monocytes/macrophages.[23] The same marker profile has been reported on neoplastic plasmacytoid monocytes, although variable expression of CD5 and CD2 has also been noted.[3 5 6 13 14]

Plasmacytoid monocytes are difficult to identify in routinely stained sections, being seen best in high quality Giemsa stained sections, and immunocytochemical labelling is of great value in their detection. In paraffin wax sections they have been reported by Facchetti *et al*[24 25] to express CD43, CD68, and CD74 (antibody LN2) and to lack CDw75 (antibody LN1) and the antigen detected by antibody MB2. A similar profile was found by the same laboratory in a single case of a plasmacytoid monocytic neoplasm.[13] In our report we have extended this work by using a panel of paraffin wax reactive antibodies to study three neoplasms arising from these cells. Neoplastic plasmacytoid monocytes in each of the three cases studied reacted strongly for CD68, a cytoplasmic antigen detectable in cells of myeloid/macrophage origin in paraffin wax sections with antibody KP1.[18] The neoplastic plasmacytoid monocytes in our cases also stained strongly for CD43 (DF-T1). This is in keeping with their proposed myelomonocytic origin, as CD43 is known from flow cytometric studies to be present on peripheral blood granulocytes and monocytes,[19] and antibody MT1, the anti-CD43 antibody most widely used by pathologists, has been reported from several laboratories as labelling myeloid cells in paraffin wax embedded tissue.[19 26] The expression of CD43 and CD68 agrees with the reports of Facchetti *et al*.[11 13]

The pan-T cell marker CD3, demonstrated with a recently developed polyclonal antibody which detects this antigen in paraffin wax sections,[16] was absent, in keeping with the other evidence against their T cell origin. The lack of reactivity for elastase (antibody NP57) also agrees with previous reports that reactive plasmacytoid monocytes lack this granulocyte associated antigen.[11 14] It was interesting, however, that in case 3 rare elastase positive mononuclear cells were present (fig 4). These presumably represent immature myeloid

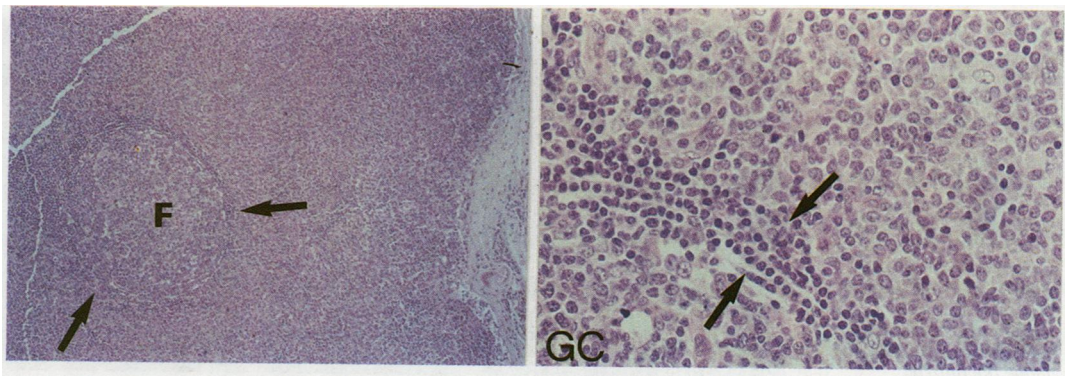

Figure 1 Typical histological appearance of neoplastic "plasmacytoid monocytes" (case 3). Low power view (left) shows diffuse replacement of much of the lymph node by neoplastic cells, and also a residual lymphoid follicle (arrowed) with a thinned marginal zone. Higher power view (right) shows the germinal centre (GC) and surrounding mantle zone (between arrows), and the adjacent neoplastic cells (haematoxylin and eosin).

Table 3 Immunoreactivity of "plasmacytoid monocyte" lymphoma studied in paraffin wax sections

Cases	Mac387	CD68	CD43	Elastase	CD3	Lysozyme
1	–	++	++	NT	–	NT
2	–	++	++	NT	–	–
3	–	++	++	–	–	–

NT = Not tested (due to limited number of slides available)

elements proliferating in the lymph node and resemble myeloid precursors that can be detected with this antibody in paraffin wax embedded tissues from cases of chronic myeloid leukaemia (unpublished observations). Myeloproliferative disorders are known to involve differentiation of the neoplastic cell along more than one bone marrow cell line—that is the granulocytic, megakaryocytic, and erythroid lineages. If plasmacytoid monocytes are related to the monocyte/macrophage cell lineage it is tempting to suggest that a myeloproliferative disorder could occasionally manifest itself (at least in tissue biopsy specimens) as a proliferation of this cell lineage, accompanied by less prominent proliferation of myeloid and megakaryocytic cells. This view is supported by the presence, in case 3, not only of elastase positive myeloid precursors, but also of megakaryocytes.

A final point concerns the appropriate nomenclature for this neoplasm. The term "plasmacytoid T cell lymphoma" is now well established from its widespread use[3 5 6] and this designation is understandable, given that these tumours (and the cells from which they arise) were first identified by histopathologists in the context of lymphoproliferative disorders. The cells are now accepted, however, as being of myeloid rather than T lymphocytic origin, so that not only would the word "monocyte" be an appropriate substitute for "T cell" in the term "plasmacytoid T cell lymphoma", but also their categorisation as a form of lymphoma is inappropriate. The tumour has parallels with chloromas and granulocytic sarcomas, in that it represents a myeloproliferative disorder manifesting itself as a lymph node based tumour. Hence "plasmacytoid monocyte sarcoma" seems the most appropriate term to use, and it is to be hoped that it will come to replace the traditional designation.

In conclusion, we describe an antigenic

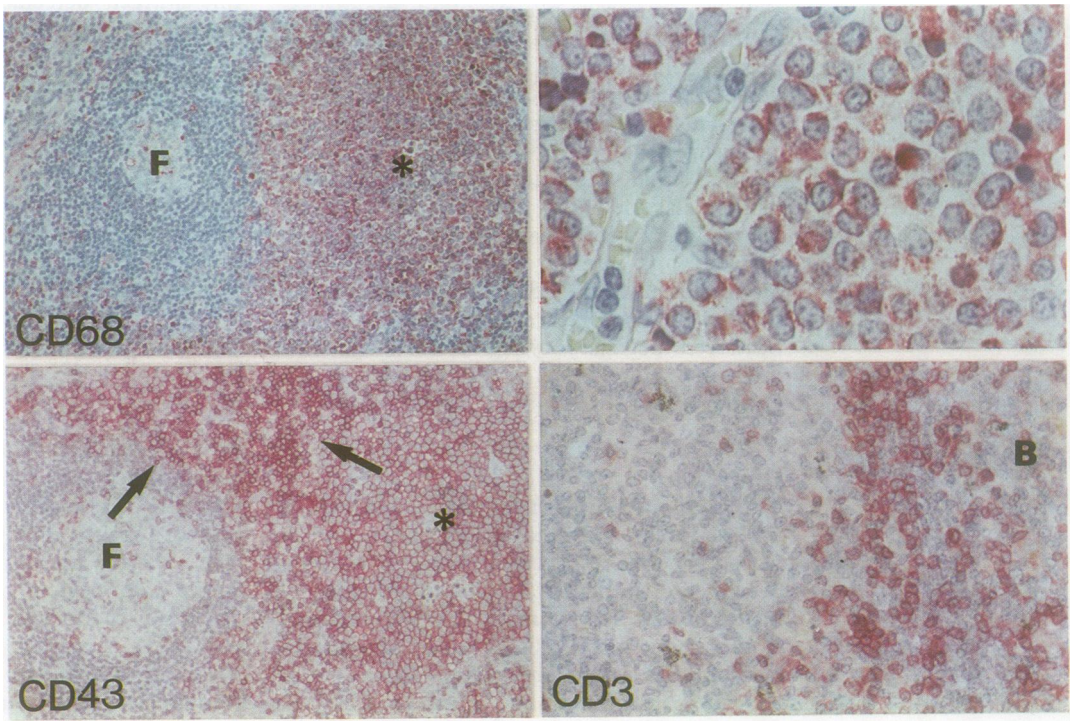

Figure 2 Case 1: CD68: *lower power illustration on the left shows a large area of neoplastic plasmacytoid monocytes (*) which express this monocytic/macrophage marker lying beside an unstained B cell follicle (F). The illustration on the right shows the neoplastic cells at higher power.* CD43: *asterisk marks an area of neoplastic plasmacytoid monocytes expressing this antigen. It is also found on extrafollicular T cells (arrowed), which could be distinguished from the neoplastic cells because of their smaller size. A B cell follicle (F) is unstained.* CD3: *reactive T cells are stained, but B cells (B) and neoplastic plasmacytoid monocytes (to the left of the illustration) are unstained.*

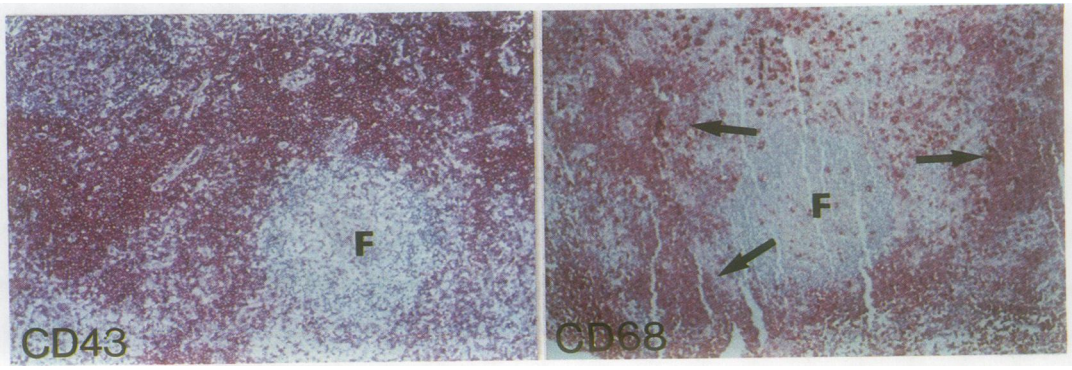

Figure 3 Case 2. CD43: *there is extensive staining of extrafollicular areas, representing both neoplastic plasmacytoid monocytes and T cells. A B cell follicle (F) is unstained.* CD68: *large clusters of neoplastic plasmacytoid monocytes (arrowed) express this monocyte/macrophage marker. A B cell follicle (F) is unstained. Scattered reactive macrophages are seen above the follicle.*

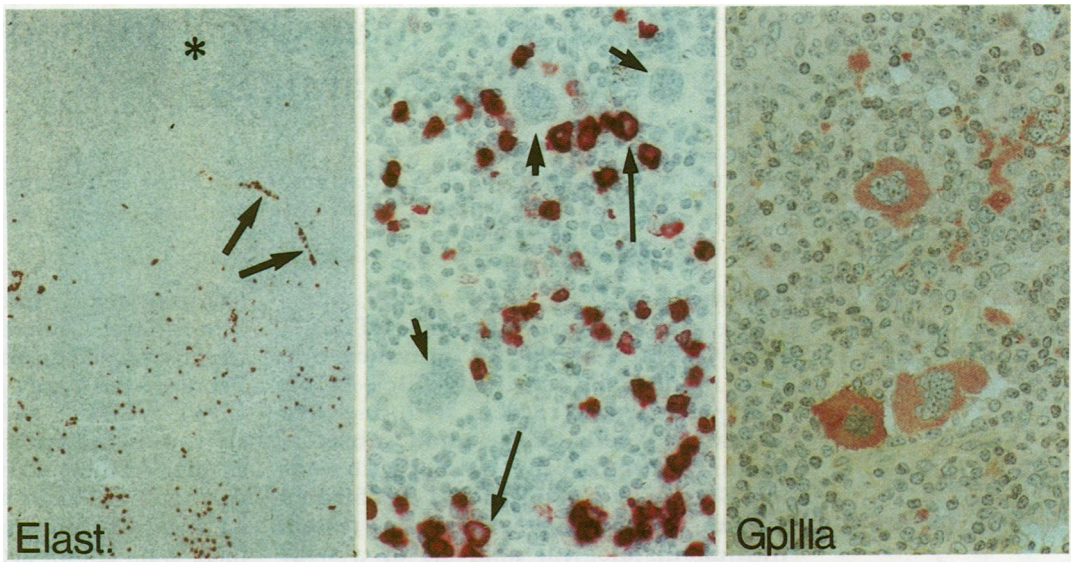

Figure 4 Case 3. Elastase: low power illustration on the left shows that the tumour and also a B cell follicle (*) are unstained, but scattered elastase positive cells are present in vessels (arrowed) and within the tumour. Higher power illustration (centre) shows that many of the elastase positive cells are mononuclear myeloid precursors (long arrows). Scattered megakaryocytes are seen (short arrows). GpIIIa: This platelet/megakaryocyte marker picks out scattered megakaryocytes lying within the tumour.

profile (CD43 and CD68 positive, CD3 and elastase negative) for neoplastic plasmacytoid monocytes in paraffin wax sections. This profile, coupled with characteristic architectural and morphological features, should facilitate the recognition by pathologists of this neoplasm in the future.

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