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Cytokeratin-immunoreactive cells of human lymph nodes and spleen in normal and pathological conditions

An immunocytochemical study

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Summary. The occurrence and the distribution of cytokeratin (CK)-immunoreactive reticulum cells in a series of normal and pathological human lymph nodes and spleens are documented. The immunoreactive cells exhibit morphological and immunophenotypic features of so-called fibroblastic reticulum cells, with or without myoid differentiation. They invariably co-express vimentin and, to a lesser extent, desmin and muscle-specific actin isoforms. These CK-immunoreactive cells are apparently a normal subpopulation of reticulum cells, being detectable from the early stages of spleen and lymph node development. They are distributed mainly in the paracortical and medullary regions of the lymph nodes and at the periphery of the white pulp in the spleen. Their number and distribution are highly variable in different neoplastic and non-neoplastic pathological conditions but the changes are not disease specific. CK-immunoreactive reticulum cells are easily identifiable in both frozen and fixed lymphoid tissue and in cytological smears of fine-needle aspirates, provided that monoclonal antibodies whose spectrum of reactivity includes cytokeratins 8 and 18 are used. The awareness of the occurrence of CK-immunoreactive cells in normal lymphoid tissues is of particular relevance in the search for micrometastatic foci using anti-CK antibodies.

Key words: Cytokeratins – Reticulum cells – Lymph nodes – Spleen

Introduction

Cytokeratins (CKs) are the most reliable immunocytochemical epithelial markers detectable for diagnostic purposes. Alone, or in association with other intermediate filament (IF) proteins, they are invariably though not exclusively expressed by normal human epithelial cells and their tumours irrespective of the degree of differentiation (Gabbiani et al. 1981; Osborn and Weber 1983; Debus et al. 1984; Battifora 1987, 1988a, b). Therefore, in diagnostic histopathology, CK immunolocalisation is a useful adjunct in the diagnosis of poorly differentiated malignant tumours and in the identification of small metastatic foci, which can escape detection on routinely stained sections (Battifora 1988a, b).

Recently, however, Franke and Moll (1987) reported expression of CK polypeptides 8 and 18 in a subpopulation of extrafollicular reticulum cells of human lymph nodes, tonsils and spleen. Most of these cells were shown to co-express CKs and vimentin, while some of them were also immunoreactive for desmin and smooth muscle-specific alpha actin. Since this investigation was performed only on frozen tissue sections of a limited number of normal adult samples, we have evaluated the prevalence and the distribution of CK-immunoreactive cells in a large series of fetal and adult lymph nodes and spleens in normal and in different pathological conditions. These experiments have been done both on frozen and on fixed and embedded tissues, using 11 different commercially available monoclonal antibodies (mAbs) to CKs, in order to provide easily reproducible results.

We have immunostained adjacent sections for the localisation of CKs, vimentin, desmin and muscle-specific actin isoforms, to ascertain if the CK-immunoreactive cells also include the stromal cells with myoid features recently described in normal and pathological lymphoid organs (Pinkus et al. 1986; Toccanier-Pelte et al. 1987).

Materials and methods

This investigation was performed on 241 tissue samples (Table 1) including fetal, neonatal and adult lymph nodes and spleens with-

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Table 1. Tissues used in the current investigation

Tissue	No. of cases
Normal lymph nodes	
adult	85
neonatal and infantile	12
fetal	30
Normal spleens	
adult	15
neonatal and infantile	2
fetal	8
Pathological lymph nodes	
micrometastases-bearing	15
cat scratch disease	5
tuberculosis	4
florid sarcoidosis	3
toxoplasmosis	3
post-lymphangiographic lymphadenopathy	3
necrotising lymphadenitis	1
Castleman's disease	3
AIDS-related complex lymphadenopathy	5
Hodgkin's disease[a]	8
B-cell non-Hodgkin's malignant lymphoma[b]	19
T-cell non-Hodgkin's malignant lymphoma[c]	7
Pathological spleens	
congestive splenomegaly[d]	5
myeloid metaplasia	1
polycythaemia vera	1
non-Hodgkin's malignant lymphoma[e]	4
idiopathic thrombocytopenic purpura	2

[a] Mixed cellularity, 3 cases; nodular sclerosis, 4 cases; nodular lymphocytic predominance, 1 case
[b] Non-Burkitt's lymphoblastic, 1 case; chronic lymphocytic leukaemia, 3 cases; centrocytic, 2 cases; follicular centroblastic-centrocytic, 2 cases; lymphoplasmacytoid immunocytoma, 3 cases; centroblastic, 4 cases; immunoblastic, 4 cases
[c] T-zone, 2 cases; AILD type, 3 cases; pleomorphic medium and large cell type, 2 cases
[d] Four cases of liver cirrhosis (spleen weight 450–750 g) and 1 nodular regenerative hyperplasia of the liver (spleen weight 4950 g)
[e] Two centrocytic lymphomas; 2 centroblastic-centrocytic follicular lymphomas

out evidence of specific inflammatory changes or of neoplastia (normal tissues) and adult lymph nodes and spleens exhibiting different pathological changes. Papanicolaou-stained cytological specimens of 10 fine-needle aspirates of hyperplastic but otherwise normal lymph nodes were also decolorised and investigated immunocytochemically.

Fetal lymph nodes (from superficial and deep sites) and spleens were obtained following spontaneous or therapeutic abortion at different gestational ages (17-, 19-, 22-, 25-, and 32-week fetuses); three additional spleens of 11-, 12-, and 13-week fetuses were also investigated. Neonatal and infantile lymph nodes and spleens were collected at autopsy from a 1-month-old boy who died of pulmonary distress and a 1-year-old girl who died with the sudden infant death syndrome. Some of the lymph nodes of each case were embedded in OCT (Miles, Naperville, Ill.), snap-frozen in liquid nitrogen and stored at $-70°$ C until use for immunocytochemistry, while the remaining nodes were fixed either in 10% buffered formalin or in methacarn and embedded in paraffin. Different samples of all the spleens were frozen or fixed and embedded in a similar manner.

Adult lymph nodes ($n=85$) were obtained from 20 patients of both sexes and a broad range of ages (32–75 years) undergoing surgery for neoplastic or non-neoplastic diseases, exhibiting only reactive changes (non-specific lymphadenitis).

Thirty of the 85 lymph nodes were bisected and one half of each node was embedded in OCT and frozen, whereas the other was fixed in formalin (15 cases) or in methacarn (15 cases) and embedded in paraffin. Special attention was paid to flat embedding of the two moieties of each lymph node in such a manner that sections from the mirror cut surfaces could be obtained and the number of immunoreactive cells in corresponding fields of the two parallel samples could be compared. The remaining 55 lymph nodes were fixed in 10% buffered formalin and embedded in paraffin.

Adult spleens ($n=15$) were surgically excised for trauma (7 cases), for staging procedures in Hodgkin's disease (4 cases) or during gastrectomy (4 cases). Parallel samples of each spleen were also frozen and fixed in formalin or in methacarn and embedded in paraffin.

Fifteen lymph nodes showing focal micrometastatic deposits from gastric (4 lymph nodes), mammary (8 lymph nodes) and colonic (3 lymph nodes) carcinomas were obtained at surgery from 6 patients.

The remaining 61 lymph nodes were all surgically excised for diagnostic purposes from different patients; the 13 spleens were also obtained at surgery. The histopathological diagnoses of all

Table 2. Monoclonal antibodies used in the current study

Reagent	Specificity	Dilution	Source	References
lu-5	Broad range of cytokeratins (CKs), including CK 8 and 18	1/ 5	Boehringer	(von Overbeck et al. 1985; Franke et al. 1987)
KL 1	Broad range of CKs, including CK 8 and 18	1/150	Immunotech	(Viac et al. 1983)
PKK 1	CK 8, 18, 19	1/ 50	Labsystem	(Holthöfer et al. 1983)
AE3	CK 1–8	1/100	CRL	(Cooper et al. 1985)
MAK 6	CK 8, 14, 15, 16, 18, 19	1/ 10	Triton	(Nagle et al. 1986; Chan et al. 1988)
CAM 5.2	CK 8, 18, 19	1/ 20	Becton-Dickinson	(Makin et al. 1984)
NCL-5D3	CK 8, 18, 19	1/ 20	Eurodiàgnostic	(Angus et al. 1987)
RCK 102	CK 5, 8	1/ 20	Eurodiagnostic	(Broers et al. 1986)
35βH11	CK 8	1/200	Enzo Biochem	(Gown and Vogel 1984)
AE1	CK 10, 14, 15, 16, 19	1/200	CRL	(Cooper et al. 1985)
34βE12	CK 1, 5, 10, 14	1/200	Enzo Biochem	(Gown and Vogel 1984)
V9	Vimentin	1/ 20	Dakopatts	(Osborn et al. 1984)
HHF35	Muscle-actin	1/500	Enzo Biochem	(Tsukuda et al. 1987)
1A4	α-Smooth muscle actin	1/500	Sigma	(Skalli et al. 1986)
DeR 11	Desmin	1/ 20	Dakopatts	(Debus et al. 1983)
D 33	Desmin	1/ 10	Sanbio	(van Mujien et al. 1987)
R4/23	Dendritic cells	1/ 10	Dakopatts	(Naiem et al. 1983)

these cases are listed in Table 1; the non-Hodgkin's lymphomas were classified according to the updated Kiel classification (Stansfeld et al. 1988) and immunophenotyped on frozen tissue sections.

For all the samples frozen and formalin- or methacarn-fixed, paraffin-embedded material was available.

For the immunocytochemical staining, serial sections of frozen tissues and paraffin-embedded sections were immunostained for CKs, vimentin, desmin, muscle-specific actin isoforms, S-100 protein, and for a dendritic reticulum cell-associated antigen (immunodetectable in frozen and methacarn-fixed samples only) using the ABC staining procedure (Hsu et al. 1981) as detailed previously (Doglioni et al. 1987).

The specificity, working dilutions and sources of the primary monoclonal antibodies are reported in Table 2. The polyclonal antiserum to the S-100 protein was obtained from Dakopatts (Copenhagen, Denmark), and used at 1/1000 dilution. The remaining reagents for the ABC staining technique were purchased in kit form from Vector (Burlingame, Calif.).

Proteolytic digestion [0.025% trypsin (Sigma, St. Louis, Mo.) in Tris-HCl 0.05 M, pH 7.8, for 30 min at 37° C] of the formalin-fixed tissue sections was performed prior to immunostaining for CKs with all but the KL1 mAbs, and for desmin with the DeR 11 mAb.

Control sections for specificity were incubated with Tris-buffered saline or normal mouse or rabbit serum in place of the specific antibodies, and constantly remained unstained. Frozen and formalin- or methacarn-fixed sections of a breast carcinoma with the overlying epidermis were also immunostained as known positive controls for all the cytoskeletal antigens tested. They consistently displayed strong and specific immunoreactivity.

The cytological specimens were immunostained for CKs only, using CAM 5.2 mAb.

Selected cases (frozen and formalin- or methacarn-fixed sections of 4 normal lymph nodes and cytological smears from 3 fine-needle aspirates) were also immunostained for CKs according to the alkaline phosphatase anti-alkaline phosphatase (APAAP) staining procedure (Cordell et al. 1984), using commercially available reagents in kit form (Dakopatts).

Results

A preliminary investigation on a limited series of normal lymphoid tissues demonstrated that the number of immunoreactive cells was highly variable in the different samples, mainly due to the diverse mAbs used and to the staining of frozen or fixed and embedded tissue sections. Therefore, after immunostaining with the different mAbs we compared the number of CK-immunoreactive cells/10 hpf (400 ×) detectable in adjacent serial sections of the diverse tissue samples, as well as in corresponding areas of the mirror sections of the 30 lymph nodes which had been partly frozen and partly fixed in formalin or in methacarn.

In all these 30 cases, CK-immunoreactive cells were identified with all except the AE1 and 34βE12 mAbs to CKs. The highest number of immunoreactive cells was observed in frozen tissue sections immunostained with CAM 5.2, MAK 6 and NCL-5D3 mAbs. The immunostaining results obtained with the same mAbs on the fixed samples and with the remaining mAbs both on frozen and on fixed samples were then compared with the above figure, which was arbitrarily given the value of 100% immunoreactivity. The number of immunoreactive cells of a given section was therefore expressed as a percentage of the cells decorated by the CAM 5.2, MAK 6 and NCL-5D3 mAbs on the corresponding frozen sections of the same lymph node (Table 3).

The number of CK-expressing cells showed great variability in all the lymph nodes from different patients (from less than 1 to more than 100 immunostained cells/hpf in the tissue compartments with the highest number of immunoreactive cells, as evaluated in formalin-fixed sections stained with CAM 5.2 mAb) without any definite relationship to the patient's sex, age and underlying disease or to the site from which the lymph nodes had been excised. A similar heterogeneity in immunostaining was also noted in different lymph nodes of the same patients.

The immunoreactive cells were morphologically identifiable as fibroblastic reticulum cells (Tykocinski et al. 1983) and were mainly located beneath the capsule, along the inner side of the subcapsular sinuses, in the paracortical areas and in the medulla, particularly along the sinuses and the small blood vessels. CK-expressing cells were notably absent from the germinal centers and their mantle zones (Fig. 1a, b). In frozen or methacarn-fixed sections, however, NCL-5D3 mAb consistently decorated part of the network of dendritic reticulum cells in the lymphoid follicles.

The CK-immunoreactive extrafollicular reticulum cells did not display any immunoreactivity for the S-100 protein. The dendritic reticulum cells of the lymphoid follicles, however, did react with both the NCL-5D3 and the R4/23 mAbs. These mAbs produced a similar decoration of the network of dendritic cells, though the staining pattern of the NCL-5D3 mAb was coarser and less extensive than that of the R4/23 mAb (Fig. 2).

Fetal and neonatal lymph nodes were particularly rich in CK-immunoreactive cells. The immunoreactivity for CKs was also fully retained by the reticulum cells in the cytological smears of lymph node fine-needle aspirates (Fig. 3). The results of CK localisation were not

Table 3. CK immunoreactivity of reticulum cells

Type of tissue	CAM 5.2	MAK 6	NCL-5D3	KL1	RCK102	lu-5	AE3	PKK1	35βH11	AE1	34βE12
Frozen	++++	++++	++++	+++	++	+	+/−	+/−	+/−	−	−
Methacarn-fixed	++++	+++	+++	+++	+/−	+/−	+/−	+/−	+/−	−	−
Formalin-fixed	+++	++	++	++	+/−	+/−	−	−	−	−	−

++++, Highest number of immunoreactive reticulum cells (100% immunoreactivity); +++, 75–99% immunoreactivity; ++, 50–74% immunoreactivity; +, 25–49% immunoreactivity; +/−, 1–24% immunoreactivity; −, no immunoreactive cells

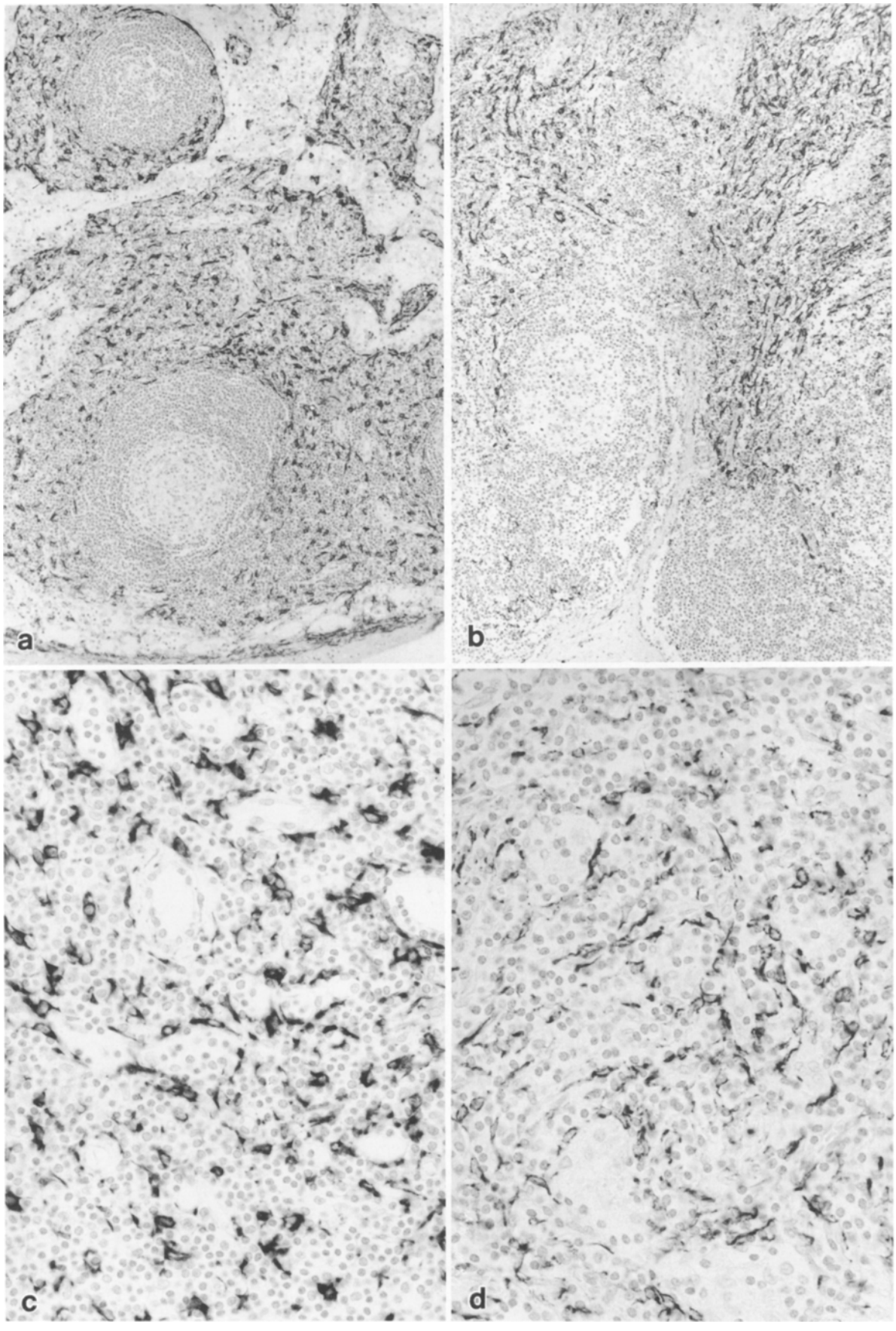

Fig. 1. Cytokeratin (CK)-immunoreactive reticulum cells in normal adult lymph nodes, fixed in methacarn (**a, c**) or in formalin (**b, d**). Immunoperoxidase staining with CAM 5.2 monoclonal antibody (mAb) ×100 (**a, b**), ×400 (**b, d**)

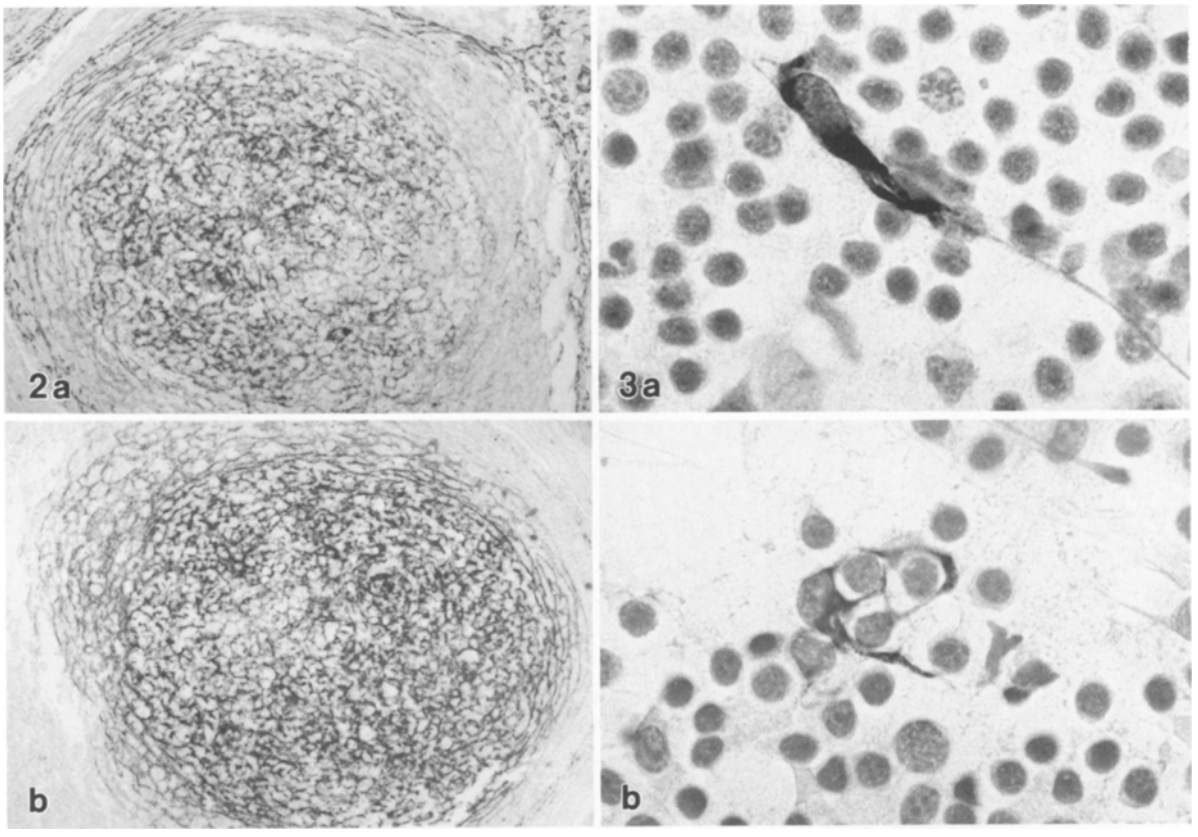

Fig. 2. In adjacent sections, the network of dendritic reticulum cells of the lymphoid follicles is decorated by the NCL-5D3 mAb to CKS (**a**) and by the R4/23 mAb (**b**). Immunoperoxidase staining of methacarn-fixed tissue without counterstain, ×250

Fig. 3a, b. CK-immunoreactive reticulum cells in fine-needle aspirates of hyperplastic lymph nodes. The immunostained cells have long cytoplasmic processes which sometimes encircle small lymphocytes (**b**). Immunoperoxidase staining with CAM 5.2 mAb, ×400

affected by the use of either the ABC or the APAAP staining procedures either in cytological or histological preparations.

In neonatal and adult spleens, CK-expressing reticulum cells were mainly found in the marginal zone of the white pulp (Fig. 4a) and in periarteriolar lymphocyte sheaths, while they were only occasionally seen in the red pulp. In the spleens of 11- to 13-week fetuses, the CK-expressing cells were very prominent and were characterised by long, thin cytoplasmic processes (Fig. 4b, c). In the 17-week fetus, CK-immunoreactive cells made up a homogeneous network throughout the spleen, which was particularly evident in frozen (Fig. 4d) and methacarn-fixed sections; in older fetuses, when the distinction between white and red pulp was sharper, the number of immunostained cells in the red pulp was reduced and the network discontinuous.

In all the pathological cases, CK-expressing reticulum cells were consistently identified, though their number was even more variable than in normal samples. Therefore, in the following paragraphs, only the most singular distribution patterns of CK-immunoreactive cells in different diseases will be presented.

In micrometastases-bearing lymph nodes, the reticulum cells of the uninvolved areas showed a pattern of CK immunoreactivity similar to that of the normal lymph nodes. The staining pattern of reticulum cells, however, was strikingly different from that of the neoplastic cells, which exhibited a strong and globoid cytoplasmic reactivity, whereas the former mostly showed a delicate decoration of their cytoplasmic processes (Fig. 5a). In rare circumstances, however, it was impossible to distinguish the reticulum from the metastatic cells solely on the grounds of their pattern of CK immunoreactivity. However, the staining of micrometastases-bearing lymph nodes with the AE1 mAb allowed clear-cut identification of the metastatic cells, which were strongly labelled, as opposed to the unstained reticulum cells (Fig. 5b).

In sarcoidosis, the CK-immunoreactive reticulum cells were numerous beneath the capsule and around the epithelioid granulomas, which frequently appeared almost entirely encircled by these cells (Fig. 6). Similarly, in toxoplasmic lymphadenitis, the CK-expressing cells were numerous and often surrounded the epithelioid micronodules. In Hodgkin's disease (HD), immunostained reticulum cells were quite abundant, especially in the mixed cellularity and in nodular sclerosis types; in the latter, however, the sclerotic areas were completely devoid of these cells. CK-immunoreactive cells were occasionally seen encircling diagnostic Reed-Sternberg cells (Fig. 7).

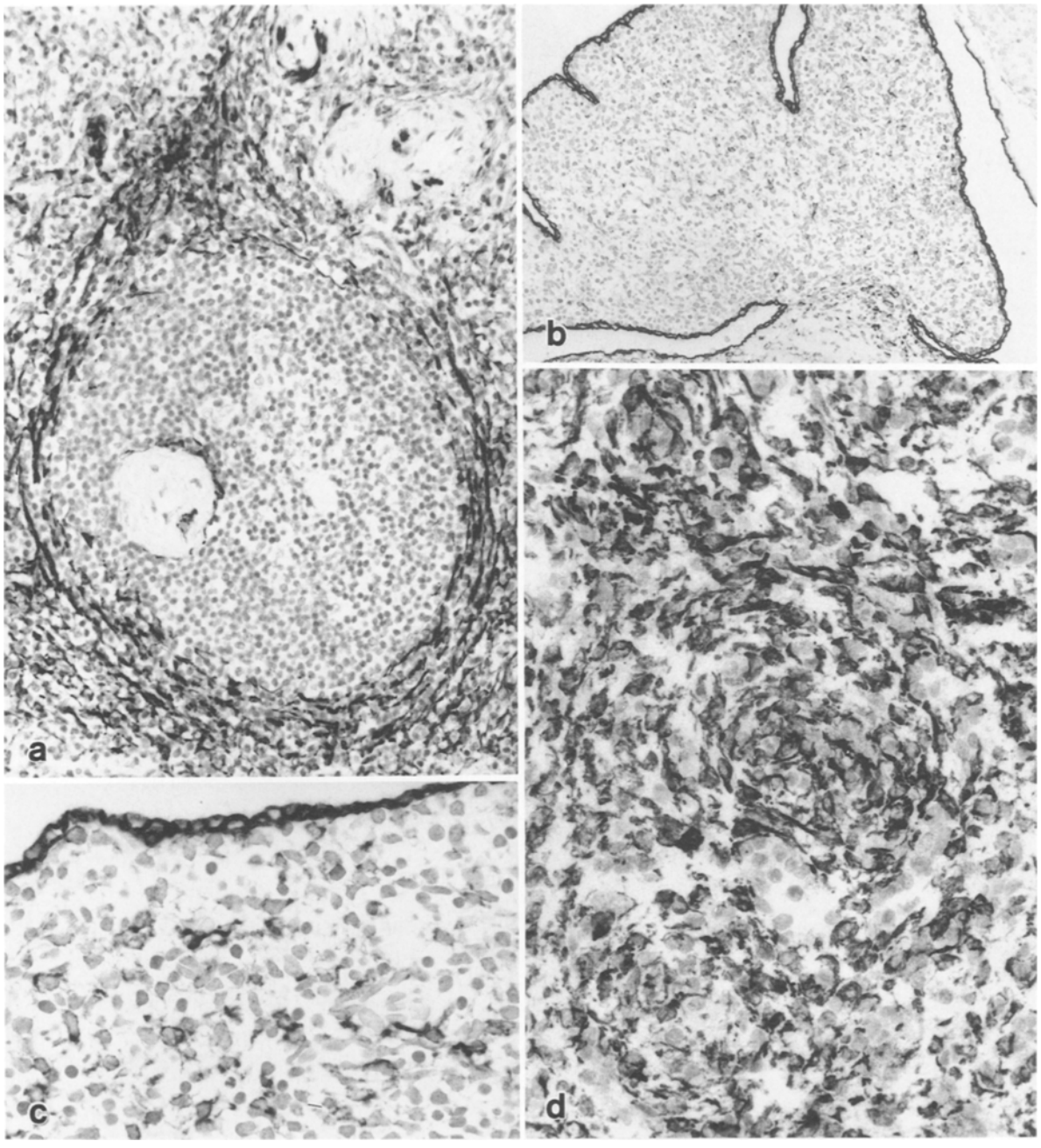

Fig. 4a–d. CK-immunoreactive reticulum cells of the human spleen. In the adult spleen (**a**, ×250) the immunostained cells are mainly located at the periphery of the white pulp. In fetal spleens, immunoreactive cells are already detectable at the 11th week of gestation (**b**, ×100; **c**, ×400) and they are particularly prominent at the 17th week (**d**, ×400). Immunoperoxidase staining of methacarn-fixed (**a–c**) and of frozen (**d**) tissue sections with CAM 5.2 mAb

In follicular lymphomas, CK-expressing cells surrounded the neoplastic nodules (Fig. 8). Furthermore, NCL-5D3 mAb also decorated a subpopulation of the R4/23-positive dendritic reticulum cells of the neoplastic follicles. In one case of lymphoplasmacytic immunocytoma with marked plasmacytic differentiation, a perinuclear immunoreactivity for CKs was also showed by some neoplastic cells.

Immunoreactive reticulum cells were particularly numerous and evenly distributed among the neoplastic cells in the cases of T-cell pleomorphic lymphoma, of AILD-type T-cell lymphoma (Fig. 9), and of T-zone lymphoma.

In congestive splenomegaly and myeloproliferative disorders of the spleen, immunoreactive cells were prominent not only in the marginal zone but also in the red pulp cords, where they were mainly located in a subendothelial position (Fig. 10). In splenic lymphomas, CK-immunoreactive cells surrounded neoplastic lymphoid nodules (Fig. 11) and extended into the adjacent red pulp. In all the remaining pathological cases, the number of immunostained reticulum cells was similar to those

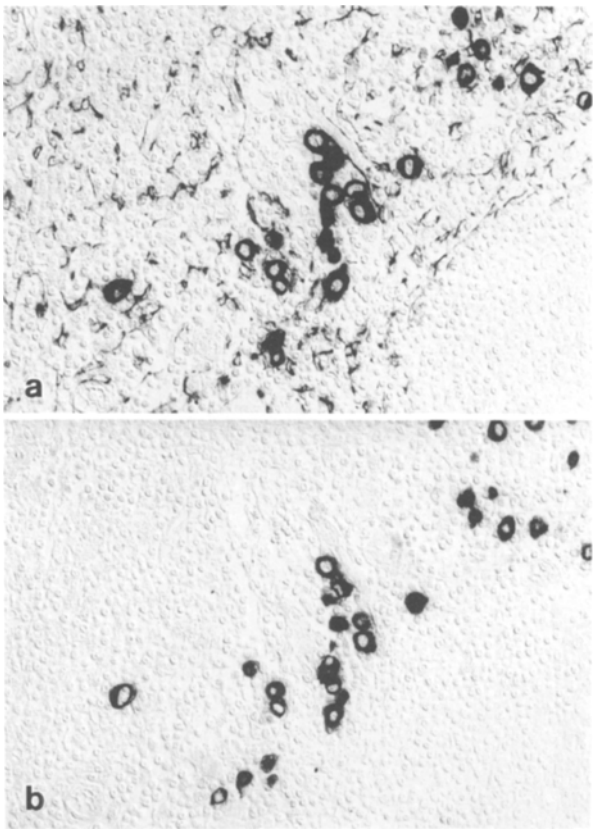

Fig. 5. In adjacent sections of a micrometastase-bearing lymph node, MAK 6 mAb immunostains both the neoplastic cells and the reticulum cells (**a**), whereas AE1 mAb decorates the former cells only (**b**). Immunoperoxidase staining of formalin-fixed tissue without counterstain; differential interference contrast optics, ×250

of the normal tissues or slightly decreased (especially in AIDS-related complex lymphadenopathy and in high-grade B-cell lymphomas).

All the CK-immunoreactive reticulum cells invariably co-expressed vimentin in serial sections; moreover, some of these cells were also immunoreactive for desmin and muscle-specific actin isoforms (m-actin) in all the cases, except for the initial stages of spleen (11 to 12-week fetuses) and lymph node (17-week fetus) development, in which only CK- and vimentin-expressing reticulum cells were observed.

The number of reticulum cells showing desmin immunoreactivity in the normal and in most of the diseased lymph nodes and spleens was variable but quite low, ranging from 1% to 10% of the cells which expressed CKs in adjacent serial sections. In B-cell low-grade lymphomas, in T-cell lymphomas (Fig. 12a, b) and in HD, the number of desmin-expressing cells was higher (approximately 30%). At least some reticulum cells appeared to simultaneously express the three distinct IF proteins.

Finally, m-actin immunoreactivity also was consistently documented in several reticulum cells (20–60% of the CK-immunoreactive cells) and showed a distribution strikingly similar to that of the latter cells, both in normal and pathological (Fig. 12c) samples. In most cases, however, a few extrafollicular reticulum cells expressed m-actin and vimentin but consistently failed to immunostain for either CKs or desmin.

Discussion

The expression of CKs is highly characteristic of, but not invariably restricted to, human epithelial and mesothelial cells, because these IF proteins, in addition to desmin and/or vimentin, have also been immunolocalised, in cells other than epithelial (Czernobilsky et al. 1985; Miettinen et al. 1985; Jahn et al. 1987; van Mujien et al. 1987; Gown et al. 1988; Turley et al. 1988).

The current investigation confirms and extends previous findings on the expression of CK polypeptides in extrafollicular reticulum cells of human lymphoid organs (Franke and Moll 1987). Indeed we have documented the occurrence of CK-immunoreactive fibroblastic reticulum cells not only in a large series of normal adult tissues (85 lymph nodes and 15 spleens), but also in fetal lymph nodes and spleens, as well as in different pathological conditions of these organs. Moreover, our immunostaining results demonstrate that CK-expressing cells are easily identifiable also in formalin- or methacarn-fixed, paraffin-embedded lymphoid tissues.

The CK-immunoreactive reticulum cells do not include the interdigitating reticulum cells, because they lack S-100 protein immunoreactivity (Franke and Moll 1987), as confirmed by our findings, and they do not express the antigen defined by the R4/23 mAb, which is a marker of the dendritic reticulum cells. The occasional immunoreactivity for NCL-5D3 mAb of some R4/23-positive dendritic reticulum cells of the lymphoid follicles in frozen or methacarn-fixed tissues is at variance with the immunostaining results obtained with all the other mAbs to CKs used in the current and in the previous (Franke and Moll 1987) studies. This finding could be related to an unwanted cross-reactivity of this particular mAb to CKs or, if sustained by further investigations including gel-electrophoretic analysis of tissue extracts, it might lead to the identification of another subset of reticulum cells with a unique pattern of CK expression.

Moreover, our immunocytochemical data substantiate the selective expression of CK polypeptides 8 and 18 in reticulum cells of lymphoid organs. Indeed, these cells were only decorated by mAbs whose spectrum of immunoreactivity includes CK polypeptides 8 and/or 18, whereas they were consistently unreactive to mAbs against CK 1, 5, 10, 14, 15, 16 and 19.

The results of the current investigation indicate that the CK-immunoreactive cells of human lymph nodes and spleen represent a subpopulation of reticulum cells which is normally present in the lymphoid organs, and that CK expression in these cells is not induced by specific pathological conditions. This conclusion can be drawn because we have also investigated truly normal lymphoid tissues obtained from 8 human fetuses of different gestational ages. These tissues are particularly rich in CK-expressing reticulum cells which form a tight and diffuse

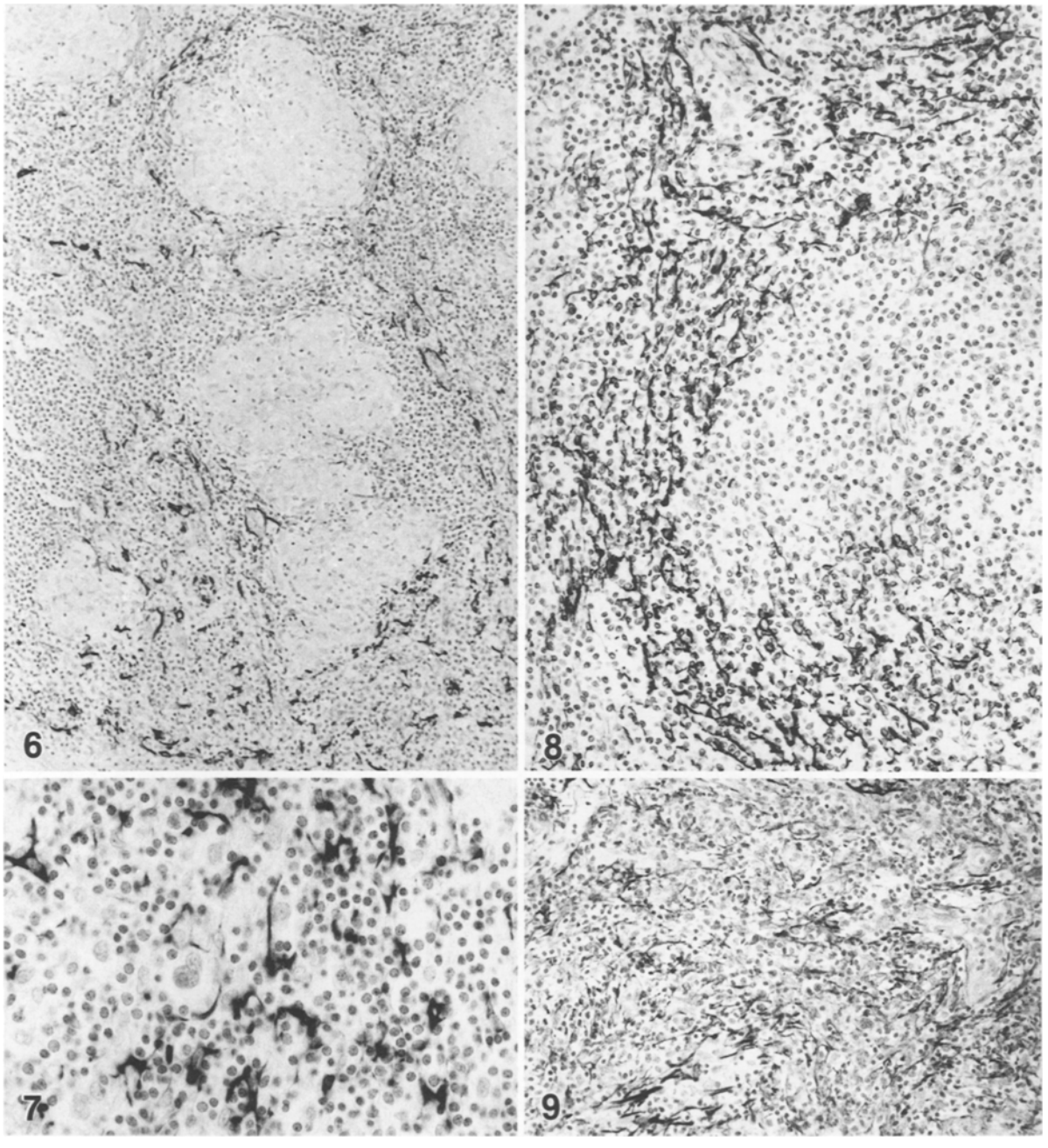

Fig. 6. In sarcoid lymph nodes, CK-immunoreactive cells are found mainly around the epithelioid granulomas. Immunoperoxidase staining of formalin-fixed tissue with CAM 5.2 mAb, ×250

Fig. 7. Immunoreactive reticulum cells in a case of mixed cellularity Hodgkin's disease. A diagnostic Reed-Sternberg cell is almost entirely encircled by the immunostained cells. Immunoperoxidase staining of formalin-fixed tissue with CAM 5.2 mAb, ×400

Fig. 8. In follicular centroblastic-centrocytic lymphomas, numerous CK-immunoreactive cells are present at the edge of the neoplastic follicles. Immunoperoxidase staining of methacarn-fixed tissue with MAK 6 mAb, ×250

Fig. 9. Numerous elongated immunoreactive cells are interspersed among the neoplastic cells of the AILD-type T-cell lymphomas. Immunoperoxidase staining of formalin-fixed tissue with MAK 6 mAb, ×250

stromal meshwork in the initial stages of lymph node and spleen development. Subsequently, they are outnumbered by the lymphocyte population and apparently confined to specific compartments of the lymphoid organs.

In the different pathological conditions, the number and distribution of CK-immunoreactive reticulum cells is highly variable, though we could not define any specific relationship between these changes and the underlying disease. Our findings, however, corroborate the impres-

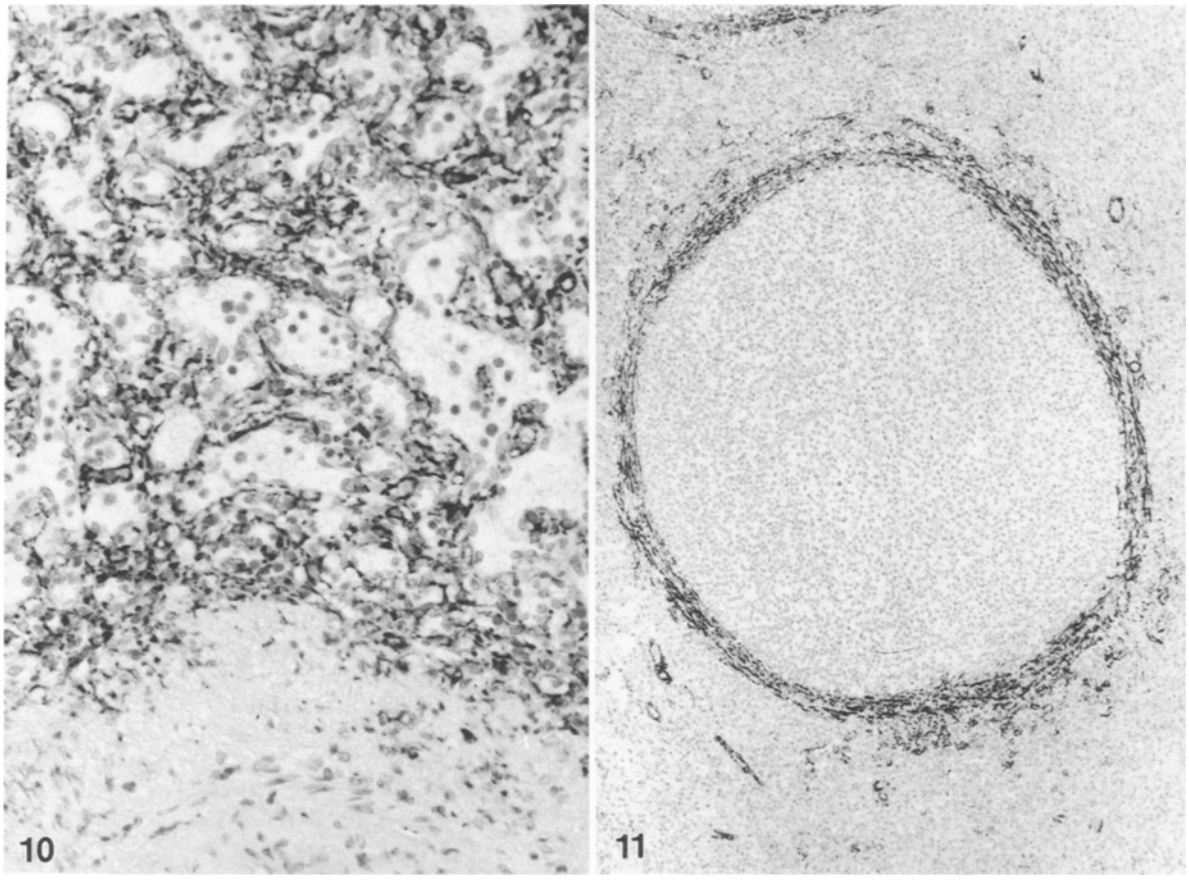

Fig. 10. In congestive splenomegaly, the number of CK-immunoreactive cells in the red pulp is remarkably increased. Immunoperoxidase staining of formalin-fixed tissue with CAM 5.2 mAb, ×400

Fig. 11. The neoplastic nodules of splenic follicular lymphomas are entirely encircled by immunostained reticulum cells. Immunoperoxidase staining of methacarn-fixed tissue with CAM 5.2 mAb, ×100

sion that the number of CK-expressing reticulum cells might be particularly increased in cases of T-lymphocyte activation and, especially, neoplasia (Franke and Moll 1987).

From the comparative evaluation of the staining pattern of extrafollicular reticulum cells for the different IF proteins and muscle actin isoforms, we can also confirm the already reported heterogeneity of these cells (Franke and Moll 1987; Toccanier-Pelte et al. 1987). Indeed, some of them express vimentin only, while others show a much more complex pattern of IF expression, including immunoreactivity for CKs and desmin, and also synthesize smooth muscle-specific actin isoforms. The reticulum cells expressing muscle-specific markers could well correspond to the myoid stromal cells of the lymphoid organs already documented both by ultrastructural and by immunocytochemical studies (Pinkus et al. 1986; Toccanier-Pelte et al. 1987).

CK-immunoreactive reticulum cells in human lymph nodes have been reported only recently (Franke and Moll 1987; Cattoretti et al. 1988; Viale 1988; Iuzzolino et al. 1989; Jack et al. 1989), having escaped detection in all previous studies. The occurrence of CK-expressing reticulum cells in normal and diseased human lymphoid tissues has some relevant implications in the practice of diagnostic immunocytochemistry. Indeed, most of the mAbs to CKs currently used for the identification of metastatic deposits in lymph node sections or fine-needle aspirates react with CK polypeptides 8 and/or 18, which are also expressed by the reticulum cells. The use of these mAbs to CKs is still advocated as a useful adjunct for the detection of even single metastatic cells in lymph nodes (Battifora 1988a, b; Ramaekers et al. 1988), without any mention of the possible false-positive identification of CK-immunoreactive reticulum cells as metastatic cells. This also holds true with respect to the application of CK immunocytochemistry for the diagnosis of lymph node metastases in cytological smears of fine-needle aspirates. A single study reported CK immunoreactivity in non-metastatic cells incidentally (Ramaekers et al. 1988), but this finding was tentatively attributed to a possible endogenous peroxidase activity of the stained cells. We can rule out this hypothesis, since unlike the quoted authors, we have demonstrated CK-immunoreactive reticulum cells on tissue sections and on cytological smears with both the immunoperoxidase and the immunoalkaline phosphatase staining procedures.

The awareness of a possible false-positive identifica-

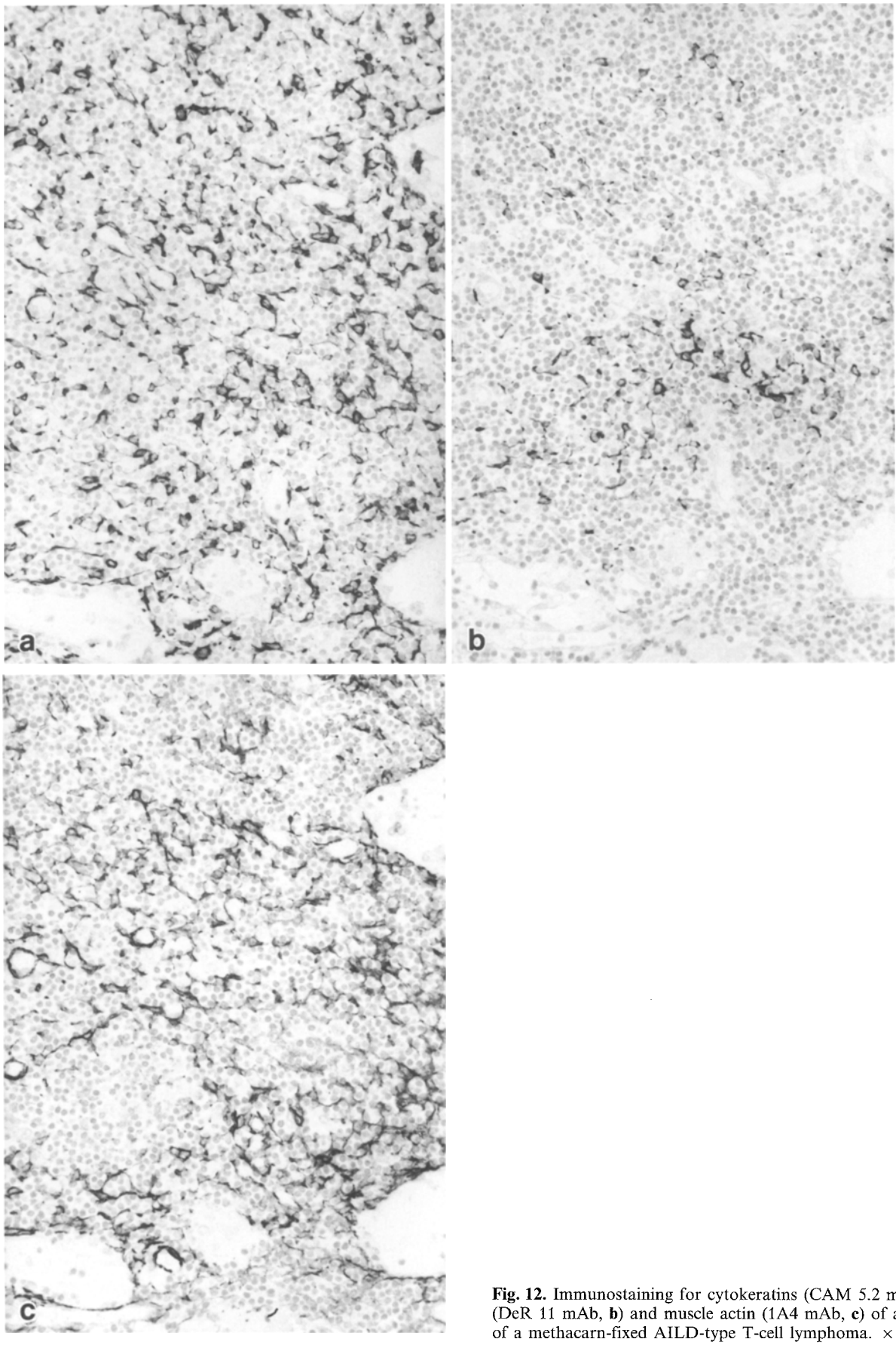

Fig. 12. Immunostaining for cytokeratins (CAM 5.2 mAb, **a**), desmin (DeR 11 mAb, **b**) and muscle actin (1A4 mAb, **c**) of adjacent sections of a methacarn-fixed AILD-type T-cell lymphoma. ×400

tion of metastatic cells should therefore always alert the pathologists, who avoid problems by using mAbs to CKs lacking specificity for polypeptides 8 and 18 or mAbs which do not stain the reticulum cells in formalin-fixed samples, though their spectrum of immunoreactivity includes CK 8 and/or 18. Finally, additional immunoreactions for the detection of epithelial-associated markers which are not expressed by the extrafollicular reticulum cells, such as EMA, desmoplakins and other antigens including the mucin-like glycoprotein recognised by mAb A-80 (Gould et al. 1988), can also be performed to avoid misleading conclusions.

The need for an accurate interpretation of the results of CK immunolocalisation in human lymphoid tissues cannot be overemphasised. Indeed, it is worth stressing that CK immunoreactivity has been also documented in the neoplastic cells of some malignant lymphomas, including lymphoplasmacytic immunocytomas, Ki-1 lymphomas (Delsol et al. 1988), large cell lymphomas (De Mascarel et al. 1989) and plasmacytomas (Sewell et al. 1986; Wotherspoon et al. 1989).

mAbs to CK 8 and 18 alone or in association with antibodies to desmin and m-actin can be effectively used to identify this still poorly known subpopulation of reticulum cells in the lymphoid organs and represent a valuable adjunct in investigations on the biological role of these cells and on the morphogenesis of the lymphoreticular system.

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