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ORIGINAL ARTICLE

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Increased numbers of cytokeratin-positive interstitial reticulum cells (CIRC) in reactive, inflammatory and neoplastic lymphadenopathies: hyperplasia or induced expression?

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Abstract A total of 291 enlarged lymph nodes showing a range of reactive-inflammatory processes, primary and metastatic neoplasms were studied to determine the distribution and immunoprofile of their cytokeratin-positive interstitial reticulum cells (CIRC) in comparison with normal nodes. In 258/291 nodes (89%), CIRC numbers were distinctly increased in the subcapsular, paracortical and, occasionally, in the medullary zones; often, these increased CIRC formed networks around follicles, sinuses and vessels. CIRC had comparatively small, irregularly shaped bodies and dendritic processes; occasionally, giant forms were noted. CIRC contained cytokeratins (CK) 8 and 18 but not 19, as shown by immunohistochemistry, and by gel electrophoresis with subsequent immunoblotting. They co-expressed vimentin consistently, alpha-smooth-muscle actin frequently, and desmin less frequently. They did not contain desmoplakins, Factor VIII, S-100, LCA, B and T lymphocyte- and macrophage-associated antigens, chromogranin A, synaptophysin or the A-80 glycoprotein. We found no clear correlation between the increased CIRC and given nodal disease processes. However, CIRC were most abundant in nodes free of but draining malignant tumours; bizarre CIRC assemblies were noted in HIV lymphadenopathy. CIRC appear to represent a subset of the so-called "fibroblastic reticulum cells" of lymph nodes. Their function remains undetermined; their increase in diverse lymphadenopathies suggests that they partake in nodal reactions to injury. It remains unclear whether the increase in CIRC relative number is due to proliferation or to CK gene induction processes but their presence and potential capability to undergo hyperplasia with dysplastic forms should alert pathologists to possible diagnostic pitfalls. In addition, we discuss that CIRC may undergo transformation and represent the "cell of origin" of certain CK-positive tumours restricted to lymph nodes.

Key words Lymph nodes · Cytokeratin-positive cells
Extrafollicular cells · Reticulum cells

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Introduction

In 1990, we described three cases of what appeared as carcinoma limited to thoracic lymph nodes in which extensive and repeated clinico-pathological studies and surgical procedures including excision of pulmonary tissues failed to reveal a visceral primary tumour. Further studies of these cases including follow-up periods of up to 12 years disclosed no evidence of a conventional primary carcinoma [14]. By immunohistochemistry, the tumour cells showed a complex array of cytokeratin (CK) polypeptides, mostly coexisting with desmoplakins and desmosomal structures and with vimentin, and also with alpha-smooth-muscle actin and/or desmin occasionally. In addition, one case had a neuroendocrine cell subpopulation [14]. Since then, we have studied additional cases of carcinoma of various phenotypes involving thoracic, cervical and axillary lymph nodes in which, again, extensive clinical investigations and surgical procedures did not disclose a visceral primary. Independent observers reported eight additional cases of carcinoma with neuroendocrine differentiation limited to lymph nodes of various chains in which no convincing primary carcinoma was found [9].

In our earlier report, we discussed the possibility that the carcinoma-like cell groups may have arisen in the lymph nodes themselves [14], specifically, from the CK-positive extrafollicular reticulum cells indigenous to lymphoid organs originally described by Franke and

Moll and subsequently confirmed by Coggi et al. [6] and other observers [8, 11, 17a, 44]. This intriguing notion was also raised by Eusebi et al. [9] with regard to their neuroendocrine carcinomas restricted to nodes.

One of the previous studies outlining the presence of CK-positive cells in lymphoid organs included lymph nodes regarded as "normal" and additional nodes displaying various pathological changes [8]. It remained unclear to what extent the number, appearance, distribution and immunoprofile of the CK-positive cell populations varied among those samples. Also, their patterns of co-expression with other significant molecules and their relationships with other nodal cells were not defined. We therefore undertook a systematic immunohistochemical study with a broad panel of antibodies applied to 61 cases of lymphadenopathy of diverse aetiologies comprising a total of 291 lymph nodes, and compared the findings with those made in 17 non-enlarged nodes regarded as histologically normal by several independent observers. We noted that 258/291 enlarged lymph nodes showed variably increased number of CK-positive cells as compared with their normal counterparts. These characteristic cells were irregularly distributed in the subcapsular, interfollicular, perisinusoidal and perivascular interstitium, extending at times into the medulla and forming a conspicuous, multilayered network amidst preserved follicles. They co-expressed vimentin consistently, alpha-smooth-muscle actin frequently, and desmin less frequently.

Materials and methods

The enlarged lymph nodes were removed either because primary pathology was suspected or as part of therapeutic or prophylactic protocols for concurrently or previously excised and documented malignant tumours including breast, thyroid, tongue, oral mucosa, stomach, colon and prostate carcinomas, and malignant mesotheliomas and melanomas. Most of these nodes showed non-specific reactive changes including variably prominent follicular hyperplasia and/or sinus histiocytosis, and focal fibrosis. In a number of nodes, besides the non-specific changes, areas of metastatic tumour were present. Nodes totally obliterated by tumour were not included in this study. The cases of non-neoplastic, primary lymph node pathology included some with a well-defined aetiology or associated condition (toxoplasmosis, sarcoidosis, etc, and HIV-lymphadenopathy without superimposed processes) while the rest were reactive lymphadenopathies of undetermined aetiology. Standard references were followed for the designation of the nodal compartments and for the classification of nodal abnormalities [22, 33, 37, 43]. Four cases of non-Hodgkin, B cell lymphoma were also studied. The basic histological diagnoses and pertinent number of lymph nodes are summarized in Table 1. The normal control nodes were obtained in the course of reparative or exploratory procedures.

Relatively thin tissue samples were fixed in 10% formalin for periods of approximately 4 h and rarely exceeding 8 h; the temperature of the paraffin (Surgipath) during embedding did not exceed 56° C. Paraffin sections were prepared 5 μm thick and subjected to protease (0.1% trypsin; Sigma, St. Louis, Mo., USA) treatment prior to exposure to the primary CK antibodies. Selected sections were exposed to microwaving (3 cycles of 5 min each), and some to microwaving and protease (0.001% trypsin) in succession (for details see [32, 38]).

Monoclonal antibodies (Mabs) used were CAM 5.2 to CK 8 (Beckton-Dickinson, San jose, Calif., USA), K_s 19.1 to CK19 (Progen Biotechnik, Heidelberg), AE1/AE3 cocktail recognizing several low and high molecular weight CK polypeptides (Boehringer, Indianapolis, Inn., USA) and various other antibodies outlined in previous publications [10, 32]. Additional Mabs used were: V9 and VIM3B4 to vimentin (Boehringer or Progen), alpha-sm-1 to alpha smooth muscle actin (Progen), DE-B-5 to desmin (Boehringer), PD7-26/2B11 to leukocyte common antigen (Dako, Carpinteria, Calif., USA), A-80 recognizing a mucin-like glycoprotein associated with exocrine differentiation [13, 19, 23, 39] (Mab provided Dr. Gary Gooch, Abbott Laboratories, North Chicago, Ill., USA), DP1-2.17 to desmoplakins (Boehringer or Progen), L26 (Dako) and CD3 (Beckton-Dickinson) recognizing lymphocytes of B and T lineage respectively, CD 68 (Dako) and MAC-387 [4] (Dako) that recognize macrophages, M869 to chromogranin A (Dako) and SY38 to synaptophysin [12, 45] (Boehringer or Progen). The antiserum Z-311 to S-100 protein (Dako) was also used. Single label immunostaining was carried out by the ABC-peroxidase technique using commercial reagents (Vector Laboratories, Burlingame, Calif., USA). Binding sites were visualized with 3,3'-diaminobenzidine (3-DAB, Aldrich Chemicals, Danvers, Mass., USA); sections were briefly counterstained with haematoxylin. We attempted to quantify our findings by rating the relative frequency of CK-positive cells in individual nodes as follows: 0=rare or no cells found, 1+=sporadic cells in perifollicular and subcapsular stroma, 2+=some cells found around most follicles and subcapsular stroma, occasionally in layers, and 3+=abundant cells in the T zone around most follicles and subcapsular stroma, often in multiple layers, occasionally extending into the medulla. The intensity of staining was graded as 1+=weak, 2+=moderate and 3+=strong.

For double label studies, single sections were deparaffinized and processed for immunofluorescence microscopy using commercial reagents (Medac or Dianova, Hamburg, Germany) combining either one of the aforementioned Mabs to specific CK with the antiserum A82 to Factor VIII-related antigen (Dako), or, alternatively, combining any given Mab with a well-characterized guinea pig antiserum that recognizes CK polypeptides 8 and 18 (courtesy of Dr. Gerda Bruder, Progen, Heidelberg).

Selected lymph node samples known to contain hyperplastic CK-positive cells were snap-frozen. Sections 20 μm thick were processed for a high salt-detergent-insoluble cytoskeletal preparation by microdissection and sequential extractions as previously described [1, 29]. As controls, microdissected samples of human gastric mucosa were similarly processed. Cytoskeletal proteins

Table 1 Increased numbers of cytokeratin-positive interstitial reticulum cells (CIRC) in diverse lymphadenopathies

Basic diagnosis	No. of Positive[a] nodes	% of Positive nodes
Associated with but not involved by previously or concurrently removed malignant tumours	173/190	91%
Partly replaced by metastatic tumour	33/37	89%
Mostly/-totally replaced by malignant lymphoma	1/4	25%
Reactive lymphadenopathies (follicular, sinusoidal and mixed) of undetermined etiology	19/24	79%
Dermatopathic lymphadenitis	6/8	75%
Toxoplasmosis	2/2	100%
Sarcoidosis	8/10	80%
HIV-associated	16/16	100%
Total	258/291	89%

[a] Positive is defined as 2+ to 3+ as described in the Materials and methods section

were then separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose paper and examined by immunoblotting (for details of method see [1, 31]). After transfer, total proteins were visualized using Ponceau-S-red staining. Mabs to CK polypeptides 8, 18 and 19 were used (M20 to CK 8, Eurodiagnostics, Appeldoorn, The Netherlands, K_s 18.174 to CK 18 and K_s 19.2 to CK 19, Progen). Bound antibodies were detected using peroxidase-conjugated rabbit antibodies to mouse immunoglobulins (Dako). Staining was performed with 3-DAB (see above), hydrogen peroxide and nickel-II-sulphate.

Results

Normal lymph nodes

In these samples, occasional CK-positive cells were found in the paracortex beneath the subcapsular sinus as well as in the perifollicular stroma. Convincing CK-positive cells were not found within the follicles or deep in the medullary stroma. In many instances, thin, delicate processes were seen; less frequently, the main cell body and its dendritic processes were simultaneously evident (Fig. 1a). Without exception, more cells were decorated

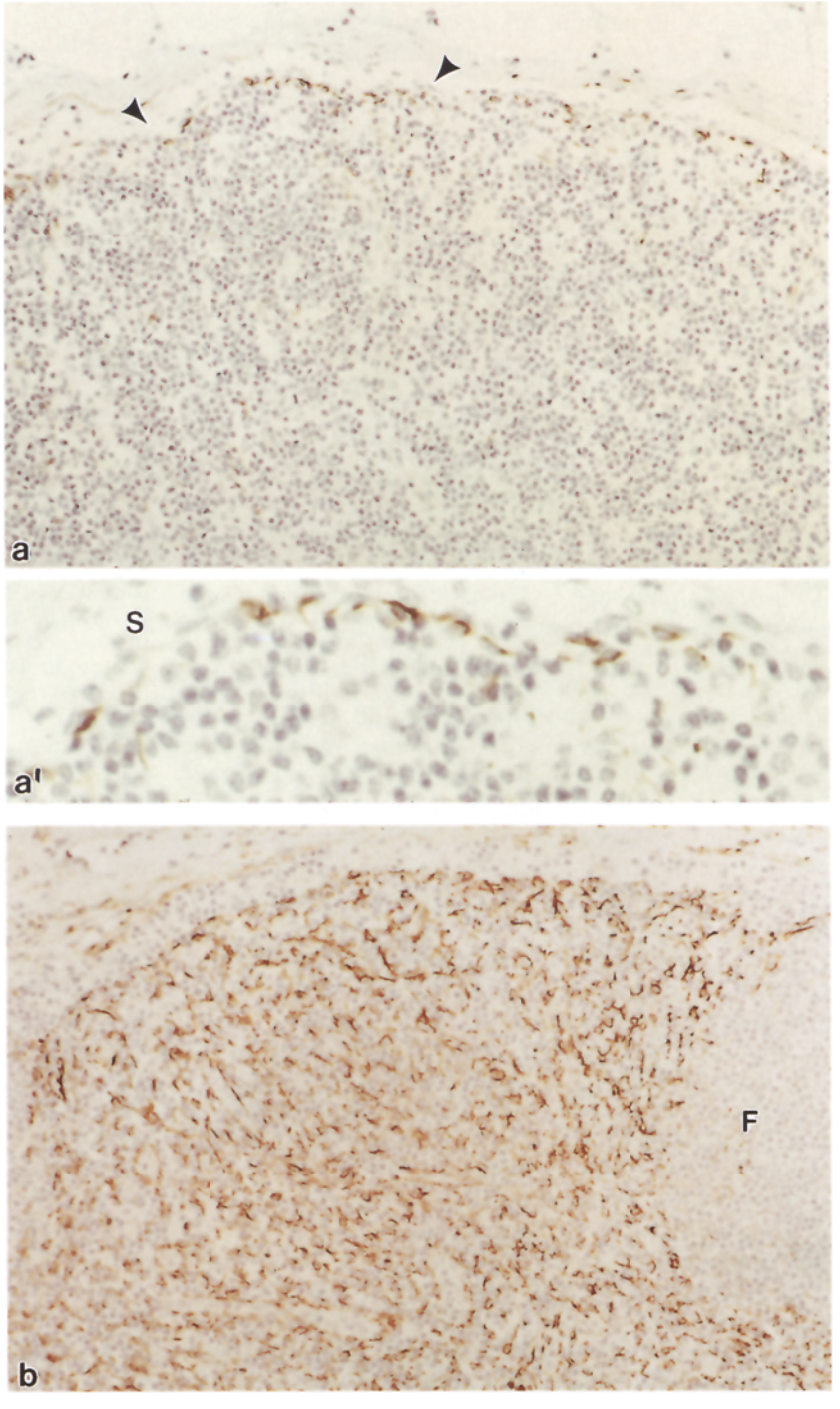

Fig. 1 **a** Normal lymph node immunostained with Mab CAM 5.2 to cytokeratin 8 (after treatment with microwaves and trypsin); note scanty positive cells in subcapsular stroma; ×180, **a′**) higher magnification of area outlined by *arrowheads* in 1a shows several cytokeratin-positive cells and their delicate processes (*S*, marginal sinus); ×560. **b** Reactive lymphadenopathy immunostained with Mab to cytokeratin 8 (same technique as in 1a); note massive increase in cytokeratin-positive interstitial reticulum cells (CIRC) in paracortex and surrounding preserved follicle (*F*), ×180

with Mab CAM 5.2 to CK 8 and the guinea pig antiserum to CKs 8 and 18 than with the Mab AE1/AE3 cocktail. In three normal appearing lymph nodes, no CK-positive cells were found in the initial sections. However, some cells were identified in subsequent deeper sections thus suggesting a certain heterogeneity in their distribution. According to our quantitative scheme, normal lymph nodes would fit into category 1+ with regard to the frequency of CK-positive cells and between 1+/2+ with regard to intensity of staining. The majority of the CK-positive cells were also recognized by vimentin antibodies; a considerable proportion of them was also stained for alpha-smooth-muscle actin while fewer cells were stained for desmin. All other antibodies and antisera failed to react with the cells in question.

Reactive and inflammatory lymphadenopathies

In 258/291 enlarged lymph nodes, CK-positive cells were clearly, and in some samples, massively increased (Fig. 1b). In most instances, their numerical increase was associated with moderately enhanced immunoreactivity, and, to variable degrees, with more conspicuous cell bodies and processes (see below). According to our quantitative scoring, the positive nodes would fall in the 2+/3+ categories in relation to both extent and intensity of the reactions. Interestingly, in cases in which multiple nodes from the same chain were removed, units with enormous increases in CK-positive cells coexisted with others showing little if any increase. Yet, even in nodes in which the number of CK-positive cells was only modestly or not visibly increased, the intensity of the CK reactions was enhanced. As noted in normal nodes, the most intense and extensive reactions in the increased CK-positive cells were obtained with the antibodies to CK 8 and 18; consistently, less extensive and intense reactions were noted with Mabs AE1/AE3. Sections subjected to successive microwaving and mild protease treatment prior to immunostaining were more extensively and intensely immunostained than those subjected to conventional protocols without microwave treatment (Fig. 2a, b). Notably, even in the case of most conspicuously increased CIRC, mitotic activity was rarely detected.

The number of lymph nodes showing increased numbers of CIRC in given lymphadenopathies, and the corresponding percentages are summarized in Table 1.

Immunostaining of step sections with antibodies to CK 8 and desmoplakins showed a distinct distribution of the pertinent molecules parallelling that in normal nodes [10, 35]. The cell bodies and processes of the CK-positive cells predominated in the paracortical and extrafollicular regions whereas the characteristically extensive desmoplakin reactions were noted in the "retothelial" cell meshwork of the subcapsular (Fig. 3a, b) and other sinus corresponding to the recently defined complexus adhaerentes [35], and in the follicular dendritic reticulum cells where they showed the normal punctate desmosomal appearance (Fig. 3c, d).

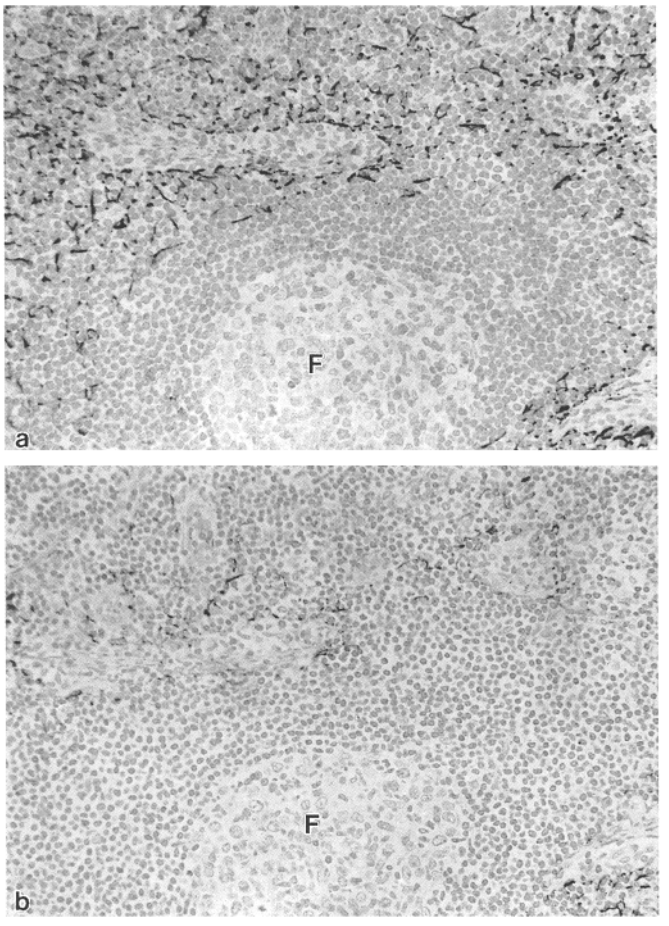

Fig. 2 Step sections of reactive lymphadenitis immunostained with Mab CAM 5.2 to cytokeratin 8. **a** section exposed to microwaving and mild protease treatment (0.001% trypsin) prior to staining shows large number of CIRC; **b** section exposed only to protease treatment (0.1% trypsin) shows far fewer CIRC; the same follicle (*F*) is noted in both sections, ×220

Step sections immunostained for CKs and alpha-smooth-muscle actin showed a parallel paracortical distribution. As was the case in normal lymph nodes, somewhat more cells were CK-than alpha-smooth-muscle actin-positive (Fig. 4a, b). Step sections stained for CKs and desmin showed again a similar paracortical distribution for both filamentous proteins although only a small minority of the CK-positive cells appeared also to react for desmin (Fig. 4c; compare with 4a and 4b). CK-positive cells could be readily distinguished from macrophages in step sections stained with Mabs CAM 5.2 and with MAC 387 or CD68 respectively. Macrophages tended to show larger bodies and were present within sinus and in the interstitium as well as in follicles whereas the CK-positive cells had for the most part slender bodies and processes, predominated in the stroma, were uncommon within follicles and mostly restricted to the outermost part of the mantle zone, and were not found within sinus (Fig. 4d, e). Step sections labelled with the antiserum Z-311 to S-100 protein and Mab CAM 5.2 showed similar contrasts between S-100- and CK-positive cells.

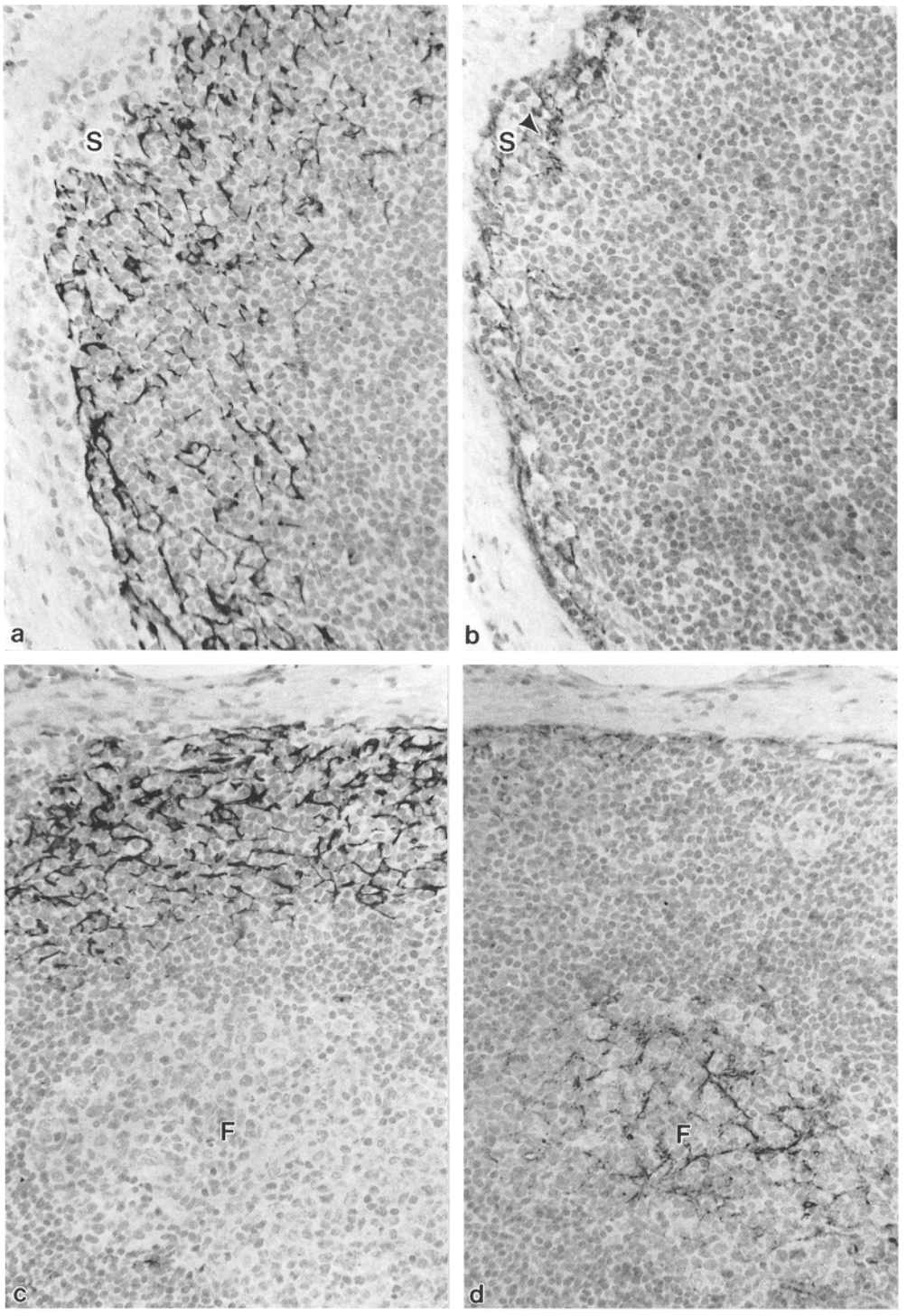

Fig. 3 Step sections of subcapsular paracortex immunostained after treatment with microwave and trypsin with Mabs to cytokeratin 8 (**a** and **c**) and desmoplakin (**b** and **d**); note prominent and rather abundant CIRC in subcapsular stroma (**a** and **c**) whereas convincing desmoplakin positivity is noted only in sinus (*S*) retothelium (*arrowhead*) and within follicle (*F*), ×280

The latter were almost invariably slender, and smaller than classical dendritic cells. Moreover, CK-positive cells were predominantly paracortical, with only occasional elements present in the follicular mantle zones, whereas the S-100 positive dendritic cells were consistently intrafollicular as well as paracortical but not intrasinusoidal, thus contrasting with macrophages (Fig. 4f). In nodes showing prominent sinus histiocytosis, the CK-positive cells were separate and distinct from the histiocytes (see below). CIRC were not seen to react with antibodies to chromogranin A or synaptophysin (not shown).

The distribution pattern of the increased CK-positive cells was predominantly paracortical as in normal nodes. However, in several cases of 3+ hyperplasia, multiple albeit irregular layers of cells formed a conspicuous network in the superficial and deep paracortical zones surrounding preserved follicles (Fig. 5a). Differing again

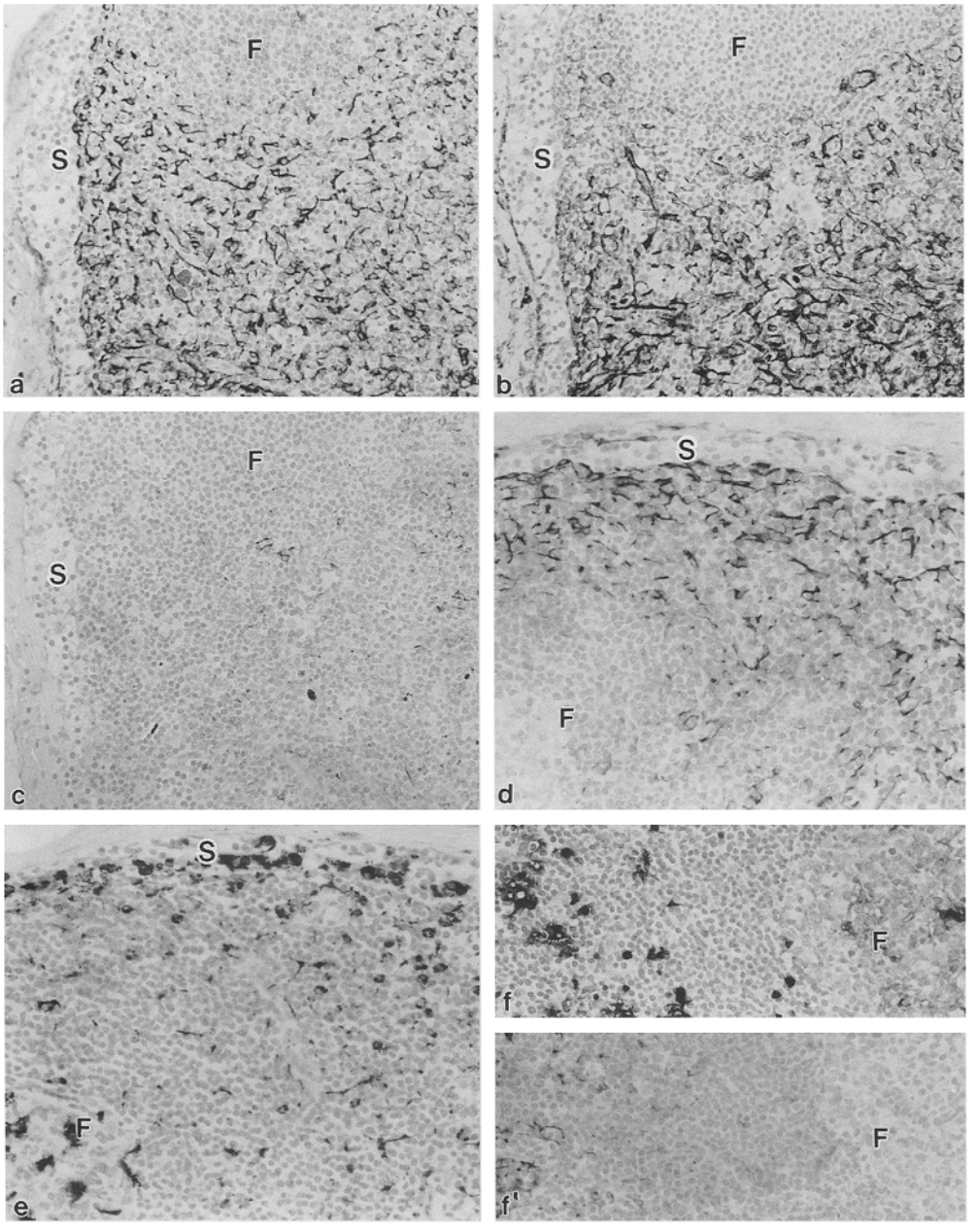

Fig. 4 Step sections displaying immunostaining of paracortex including subcapsular sinus (*S*) and part of a follicle (*F*); **a**) immunostaining for cytokeratin 8 shows abundant subsinusoidal CIRC that do not extend into follicle; **b**) staining for alpha-smooth-muscle actin shows abundant positive cells in a similar distribution; note strong reaction in the capsule itself; and, **c**) desmin staining depicts comparatively few and scattered but similarly distributed cells, ×180. Step sections of paracortex immunostained for cytokeratin 8 (**d**) and CD68 (**e**, 0.1 trypsin); note CIRC in subsinusoidal paracortex but neither in sinus (*S*) nor in follicle (*F*) whereas CD68 reactive macrophages can be seen in all these locations, ×240. Step sections of paracortex immunostained with antiserum to S-100 protein (**f**, 0,1 trypsin) and Mab to cytokeratin 8 (**f'**); S-100 reactive dendritic cells are seen in paracortex and well as within follicle (*F*) whereas cytokeratin-positive elements are restricted to the paracortex, ×220

from normal nodes, intricate networks of CK-positive cells extended into the superficial and deep medullary stroma where they surrounded foci of sinus histiocytosis (Fig. 5b, c), were prominent amidst active appearing connective tissue, and were also intimately associated with but nevertheless distinct from conspicuous vascular channels (Fig. 5d, and see below).

As stated above, CIRC found in lymphadenopathies were often larger and more intensely reactive than those in normal nodes. Numerous and bizarre CIRC with coarse and ramified, curvilinear processes were seen in a reactive node draining a malignant melanoma but free of tumour cells (Fig. 6a, b). The most conspicuous variants of CK-positive cells were seen in cases of advanced HIV-associated lymphadenopathy in which bizarre, giant and occasionally multinucleated cells were present in addition to the customary smaller and slender elements (Fig. 6c, d and inset).

Neoplastic lymphadenopathies

Samples of non-Hodgkin's lymphoma showed only occasional and focal increases of CIRC. In contrast, most lymph nodes partially involved by various metastatic carcinomas, mesothelioma and melanoma showed at least moderately increased numbers of CK-positive cells. Interestingly, in cases from which multiple nodes were obtained, and where some contained metastatic carcinoma whereas others did not, the number of CK-positive cells was generally greater in the latter than in the former.

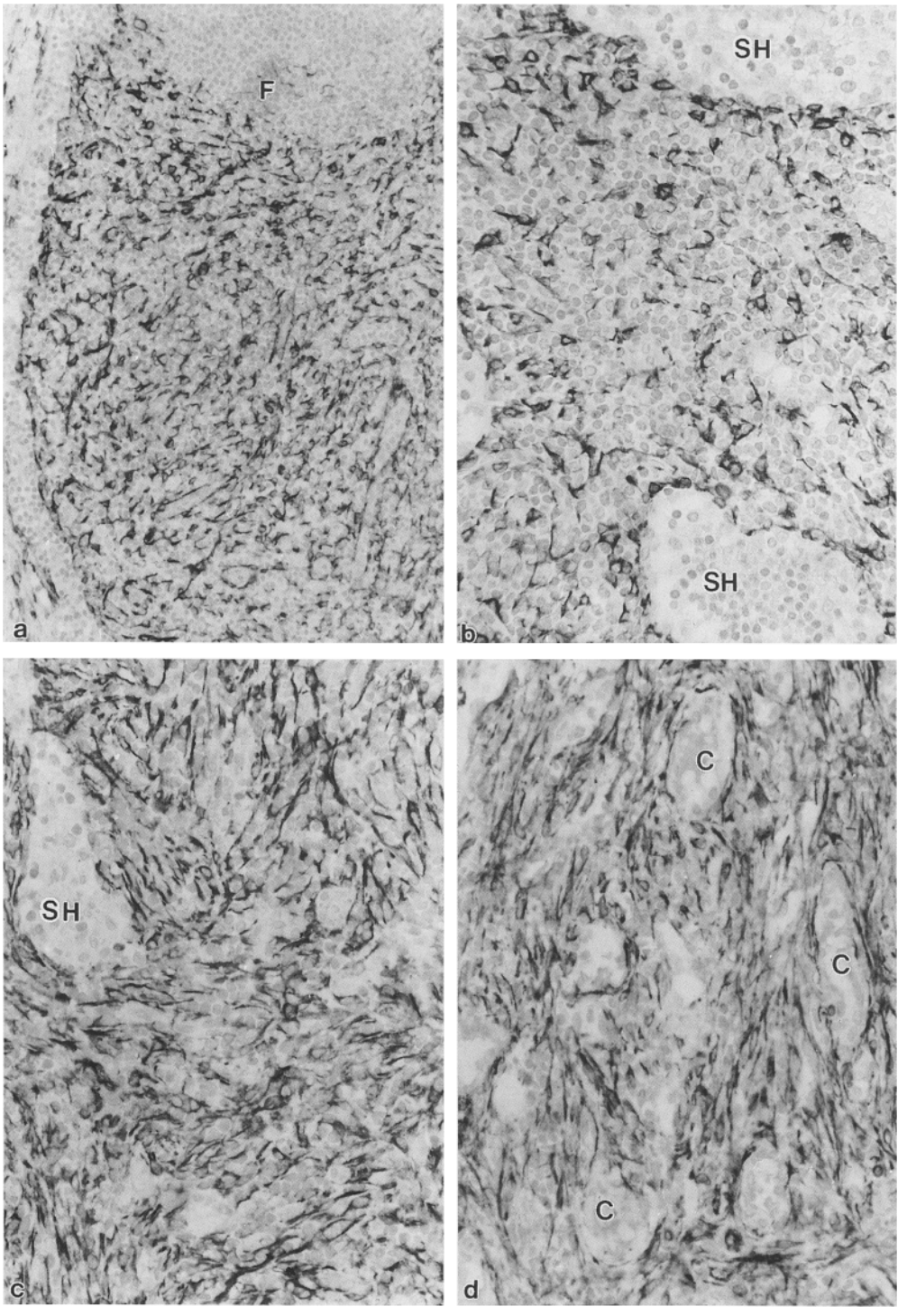

Fig. 5 Sections from enlarged lymph node with 3+ CIRC reaction immunostained with Mab to cytokeratin 8; **a**) subcapsular paracortex showing network of CIRC that do not involve follicle (*F*), ×280; **b**) slightly higher magnification of deeper paracortex depicting abundant CIRC that do not involve foci of sinus histiocytosis (*SH*); **c**) superficial and deep (**d**) medullary stroma showing prominent CIRC around areas of sinus histiocytosis (*SH*) and encompassing prominent capillaries (*C*) in field showing fibrosis, ×340

For the most part, the increased CIRC were distinct and separate from the metastatic malignant cells. In the areas in which metastatic carcinoma had totally obliterated the pre-existing nodal architecture, CK-positive cells could not be identified with certainty. However, step sections immunostained for cytokeratin and desmin or alpha-smooth-muscle actin showed that some slender cells positive for desmin and/or alpha-smooth-muscle actin persisted within the tumour suggesting that at least some CK-positive cells could occur within metastatic neoplasms (Fig. 7a, b).

Morphologically, the overwhelming majority of CIRC differed significantly from the similarly CK-positive metastatic carcinoma cells. The former were individually arranged and had delicate cytoplasmic processes whereas the latter were individually larger, were organized in clusters and/or glandular aggregates and tended to stain far more strongly. Moreover all carcinomas immuno-

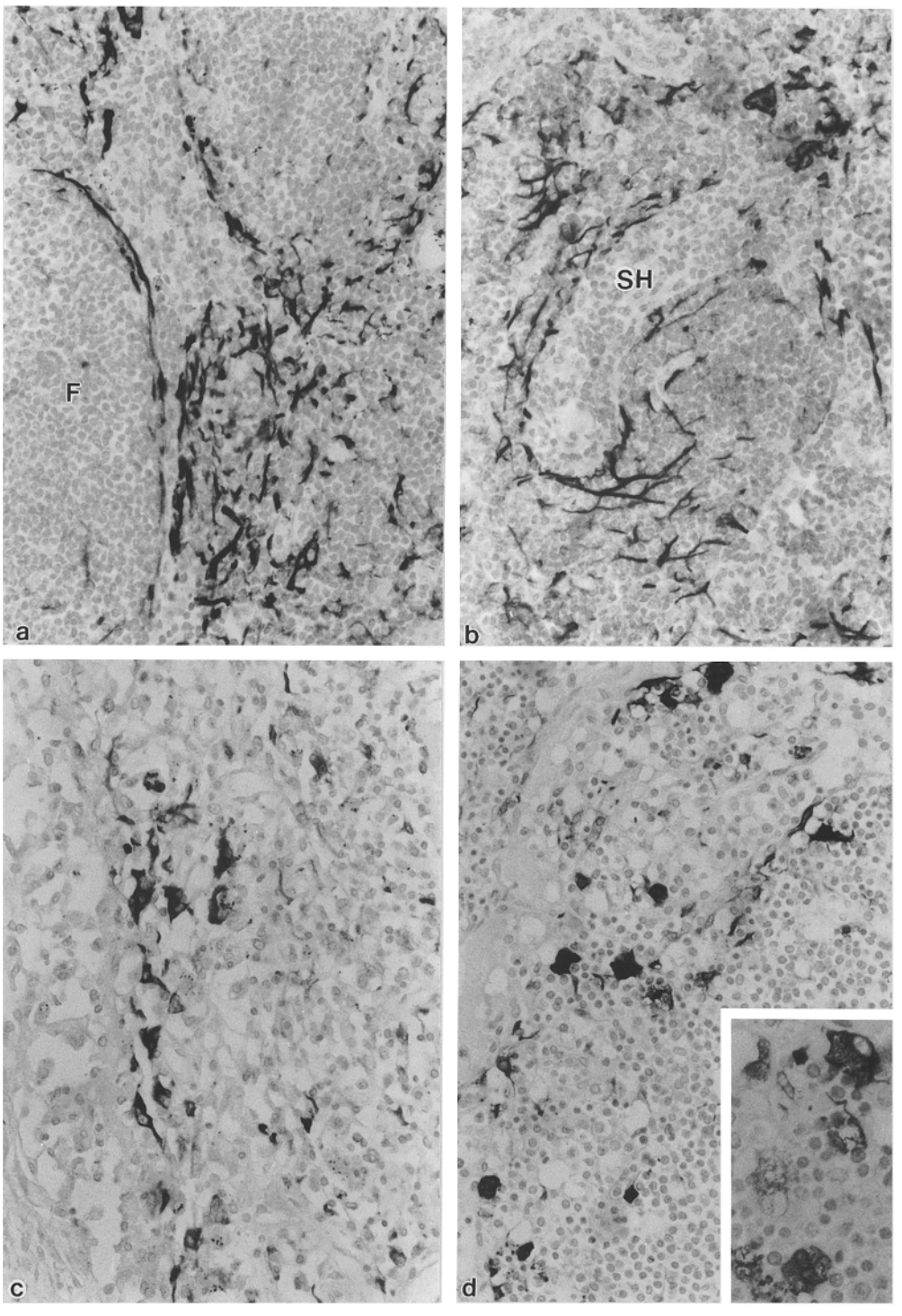

Fig. 6 Reactive lymphadenopathy stained with Mab to cytokeratin 8; node draining but not involved by malignant melanoma; **a**) prominent CIRC with thick, focally branching processes surround uninvolved follicles (*F*); similar cells are noted in deeper stroma around foci of sinus histiocytosis, ×280; **c, d** and *inset*) HIV-associated late stage lymphadenopathy showing the usual slender CIRC but also some bizarre stellate, giant and multinucleated forms, ×280 and (*inset*) ×450 (samples treated with microwave and mild trypsin)

stained for desmoplakin whereas CIRC did not; and, while Mab CAM 5.2 stained some interstitial as well as the carcinoma cells, the Mab to CK19 stained only the neoplastic elements (Fig. 7c, d). Also, breast, stomach, colon and prostate carcinoma cells reacted with the Mab to the A-80 glycoprotein whereas CK-positive cells did not.

Double immunolabels and immunoblot studies

As originally described in normal lymph nodes [10], double immunolabelling for CK and vimentin showed that the increased CK-positive cells were also reactive for vimentin (not shown). Many CK-positive cells were noted to contain alpha-smooth-muscle actin (Fig. 8a, b), and fewer displayed CK together with desmin. Double staining for CK and Factor VIII-related antigen showed that the CK-positive cells were distinct from the promi-

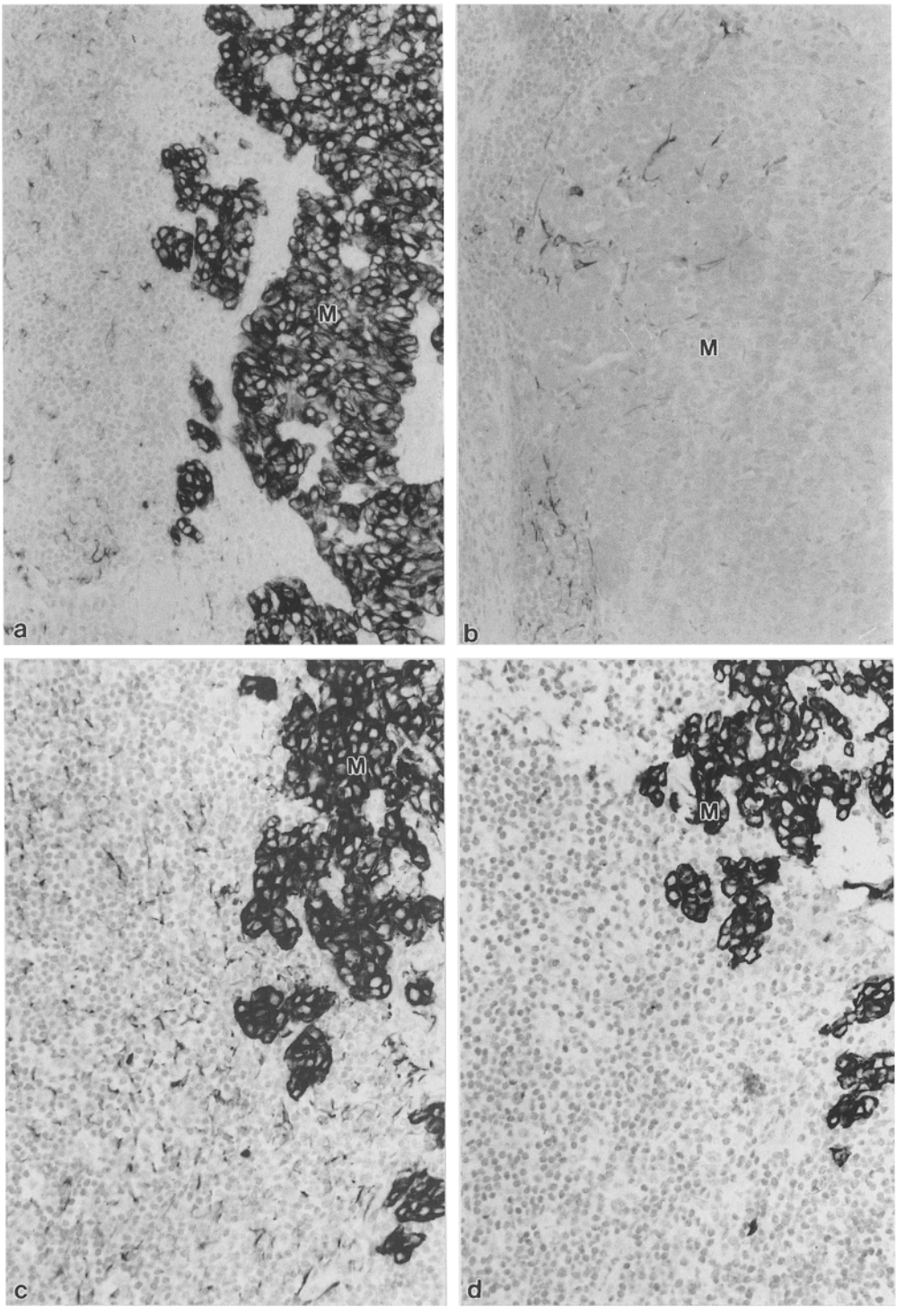

Fig. 7 Step sections of lymphadenopathy containing areas of metastatic breast carcinoma; **a**) immunostaining with Mab to cytokeratin 8 shows rich and extensive reaction of metastatasis (*M*) and scattered, delicate CIRC in uninvolved lymphoid tissue; **b**) staining for desmin depicts scattered, delicate immunoreactive elements without as well as within the now unstained area of metastasis (*M*), ×180; **c**) immunostaining with Mab to cytokeratin 8 shows an increased complement of CIRC and a rich reaction of the metastasis (*M*) contrasting with **d**) immunostained with Mab to cytokeratin 19 which shows staining of the metastatic tumour (*M*) but not of the cells in question, ×280 (same protocol as Fig. 6)

nent as well as intimately associated endothelial and vascular wall elements (Fig. 8e, f, also compare with Fig. 5c). In double immunostaining for CKs and the B-lymphocyte marker L26 and the T-lymphocyte marker CD3, no expression of lymphocyte markers in CIRC was detected. However, these double label experiments did show that the CK-positive cells and their processes were predominantly associated with T-lymphocytes although close vicinity to B-lymphocytes was also occasionally noted.

Immunoblotting of electrophoretically separated cytoskeletal proteins from lymph node samples with increased numbers of CK-positive cells showed modest but readily detectable and significant amounts of CKs 8 and 18 but no CK 19. These findings contrasted with their counterparts in samples from gastric mucosa that showed CK 19 in addition to CKs 8 and 18 (Fig. 9).

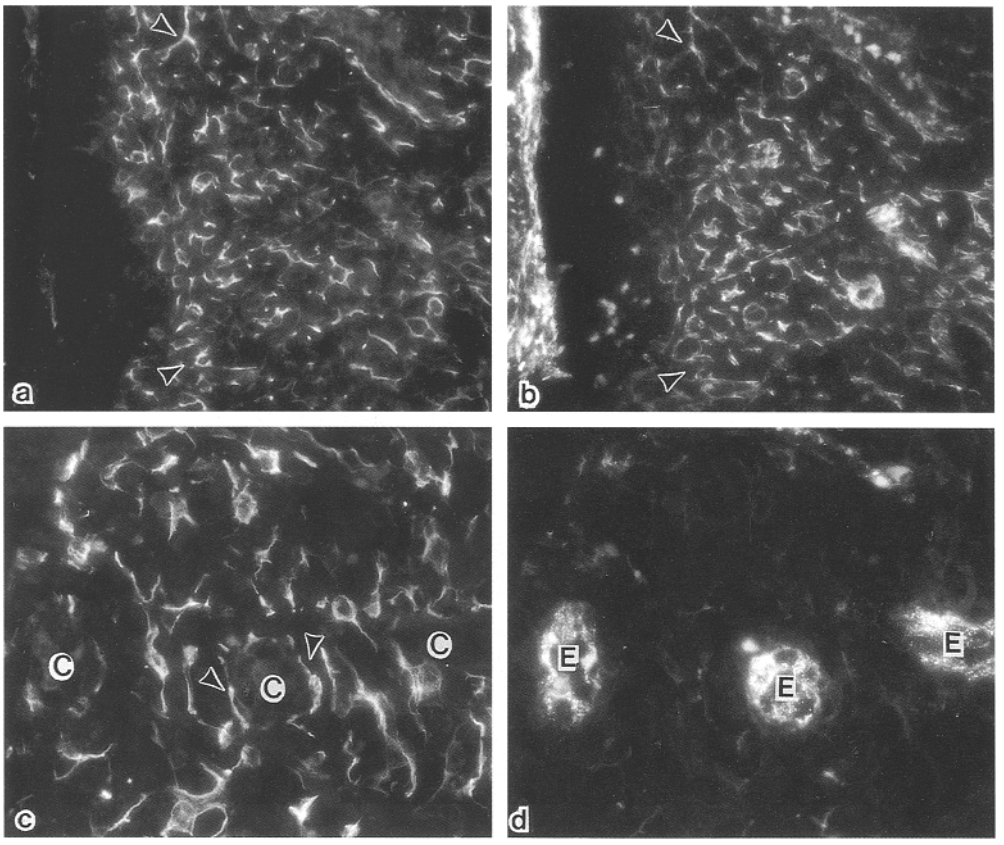

Fig. 8 Double immunofluorescence of reactive lymphadenopathy stained with guinea pig antiserum to cytokeratins 8 and 18 (**a**, Texas red-coupled secondary antibodies) and with Mab to to alpha-smooth-muscle actin (**b**, FITC-coupled secondary antibodies). A number of the comparatively abundant cytokeratin-positive cells (**a**, *arrowheads*) are also reactive for alpha-smooth-muscle actin (**b**, *arrowheads*); cells comprising vascular channels and the lymph node's capsule react strongly for alpha-smooth-muscle actin (**b**) but not for cytokeratin (**a**). Double immunolabel with Mab to cytokeratin 8 (**c**, Texas red-coupled secondary antibodies) and rabbit antibodies to Factor VIII related antigen (**d**, FITC-labelled secondary antibodies); note cytokeratin-positive cell reticulum prominently surrounding capillaries (*C*, and outlined by *arrowheads*) (**c**) contrasting with the strongly Factor VIII-reactive, swollen endothelial cells (*E*) and unstained reticulum (**d**), ×350

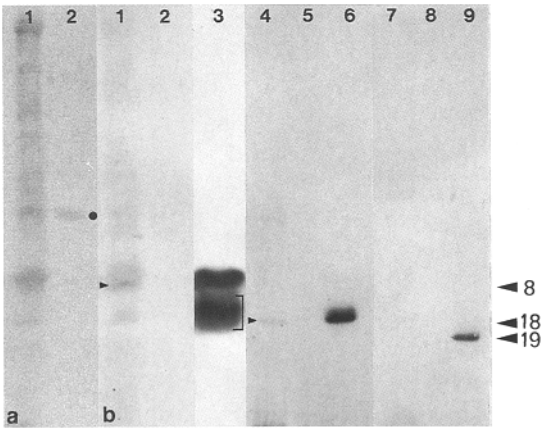

Fig. 9 Immunoblot analysis of a cytoskeletal preparation of a lymph node with abundant CIRC. **a**) Total protein staining (Ponceau-S-red) showing the protein bands of the lymph node (lane 1), and for comparison, bovine serum albumin (BSA, M_r 68000, dot, lane 2); the same nitrocellulose paper strip shown after immunoblotting in b, lanes 1 and 2. **b**) Immunoblot staining; lanes 1–3 stained with Mab M20 to cytokeratin 8 representing reactive lymphadenopathy sample (lane 1), BSA (lane 2) and, as reference, tissue from gastric mucosa (lane 3; bracket denotes degradation products of cytokeratin 8). Lanes 4–6 illustrate reactions with Mab Ks 18. 174 to cytokeratin 18 in lymphadenopathy material (lane 4), BSA (lane 5) and gastric mucosa (lane 6). Lanes 7–9 depict reactions with Mab Ks 19. 2. to cytokeratin 19, representing reactive lymphadenopathy material (lane 7), BSA (lane 8), and gastric mucosa (lane 9). Note in (**b**) lymphadenopathy samples, small but significant amounts of cytokeratin 8 (lane 1 *arrowhead*) and cytokeratin 18 (lane 4, *arrowhead*) while cytokeratin 19 is absent in the lymph node (lane 7) and in BSA (lane 8) but present in the gastric mucosa (lane 9)

Discussion

Our results indicate that the modest complement of CIRC encountered in normal adult lymph nodes frequently undergoes a conspicuous increase in relative number under various reactive and/or inflammatory conditions. The normal distribution of nodal CIRC predominantly in the subcapsular and paracortical interstitium was essentially retained in these reactive lymph nodes. However, reactive CIRC often extended into the medulla and were rarely found in the outermost part of follicular mantle zones thus contrasting with the distribution pattern of CIRC in normal nodes [10]. Also notable in the reactive lymph nodes was the intimate association of CK-positive cells with sinus and venules from which, however, they could be easily discriminated by the application of appropriate immunohistochemical markers. The consistent co-existence of CK and vimentin and the lack of desmoplakin in CIRC of normal lymph nodes were retained in the augmented population of CIRC in reactive nodes. Also, the relative ratios of reactive CIRC containing myoid differentiation markers such as alpha-smooth-muscle actin and desmin remained similar to that originally described in CIRC in normal nodes [10], and in agreement with reports describing stromal cells with myoid differentiation [34, 42].

The present study confirms and extends our and other authors' findings [6, 8, 10], that CIRC are constitutive cells of lymph nodes, and are readily distinguishable from lymphocytes, histiocytes, dendritic and "interdig-

itating" reticulum cells as well as from sinusoidal retothelial cells by a specific set of marker molecules [10, 35, 36]. CIRC might be considered as a distinct subset of the so-called "fibroblastic reticulum cells" originally defined by their intense alkaline phosphatase reactivity (for extensive discussion and earlier literature see [10]); also, CIRC partially overlap but do not entirely coincide with the stromal "myoid cells" as defined by Toccanier-Pelte et al. [42]. The increased CIRC in reactive nodes continued to express the same pattern of CK genes as noted in normal nodes, i.e. those encoding CK8 and CK18 (for immunoblot demonstration see also [10]); apparently, the nodal reactive processes studied to-date do not induce additional genes for other CKs such as those for CK19 or for desmosomal marker proteins.

At present, however, we do not know whether this increase in relative cell numbers of CIRC is the result of a CIRC hyperplasia characterized by proliferation or of the induction of CK8 and CK18 genes in preexisting reticulum cells under the reactive conditions mentioned. Induction of these genes as a response to certain environmental conditions, including drug treatment, has been observed in reactive cells of certain tissues such as serosa [3], vascular endothelia of synovia [17b], vascular smooth muscle cells of or near lesions [18], in Sertoli cells in association with a variety of changes including atrophy and ageing [25, 40], and also in cultures of diverse non-epithelial cells [11, 20, 21]. Detailed studies of possible correlations of CK synthesis with S-phase and mitosis will be required to examine this question.

We have no satisfactory explanation for the observation that prominent CIRC, while remarkably abundant in many reactive nodes, were far less conspicuous in other nodes showing similar histological alterations, and for the notable differences noted among different lymph nodes obtained at the same time from given cases. Moreover, while our samples did not include all known variants of reactive or inflammatory lymphadenopathy, we detected no consistent association between the increased numbers of demonstrable CIRC and the degree of nodal enlargement or with any particular disease process in agreement with an earlier report [8]. Nevertheless the most conspicuous CIRC were found in association with non-specific reactive changes of known or undetermined aetiology, and also in nodes draining malignant neoplasms such as breast carcinoma and malignant melanoma, but not in nodes actually colonized by metastatic tumours or by primary lymphoreticular neoplasms. Bizarre individual CIRC were encountered in nodes with non-specific reactive changes, including non-involved nodes draining a malignant melanoma and in HIV-associated lymphadenopathy. In this condition some of the CK-positive multinucleated, giant cells were somewhat reminiscent of Warthin-Finkeldey cells, recently shown to be non-reactive for various endothelial, histiocytic, B and T lymphocytic, follicular dendritic, and interdigitating reticulum cell markers [5].

In malignant lymphomas, we observed a limited number of CIRC either around neoplastic nodular aggregates or haphazardly interspersed among the neoplastic cells of diffuse lymphomas. This suggests that the reported presence of subpopulations of CK-positive cells in some malignant lymphomas might reflect the presence of normally epithelioid reticulum cells [11] rather than an aberrant CK expression by transformed lymphoreticular elements. The occasional report of LCA-negative lymphomas with diffuse CK-positivity would pose a different problem; in this instance, the distinction must be made among artifactual immunostaining, a truly aberrant CK expression and the possibility of an incorrect diagnosis of lymphoma [7, 41]. An additional differential diagnostic problem has been discussed with regard to the presence of variable numbers of CK18-immunoreactive cells in a minority of large cell anaplastic lymphomas [16]. But, in these lymphomas, the CK-positive neoplastic cells display the usual round forms and lack the conspicuous dendritic processes of the non-neoplastic CIRC.

We found that lymph nodes showing incomplete replacement by metastatic carcinoma generally showed modestly increased complements of CK-positive interstitial reticulum cells. In our experience, given conventional sections of good quality and appropriate immunohistochemistry, the differential diagnosis between carcinoma cells metastatic in lymph nodes and normal or increased CIRC populations should not pose major problems. Where individual or small groups of suspicious cells are seen the problems may become significant. We have been aware of this potentially important pitfall for several years, and noted that the diagnosis of sparse metastatic carcinoma in lymph nodes based exclusively on the identification of some CK-positive cells, could have unfortunate results [13], as also stated by other observers (e.g. Viale 1989). In most instances, even small aggregates of carcinoma cells may be seen to consist of individually larger cells with larger nuclei and generally lacking the prominent cytoplasmic processes characteristic of CIRC. Furthermore, virtually all carcinomas and mesotheliomas of epithelial type will react with desmoplakin antibodies [2, 30] whereas non-transformed CIRC apparently do not. Moreover, most metastatic carcinomas tend to express a broad array of CK polypeptides [15, 26, 27, 28] whereas CIRC in either normal or in increased numbers usually contain only CKs 8 and 18. Finally, most metastatic adenocarcinomas will be recognized by antibodies to various mucin-like glycoproteins (for overviews and additional references see [13, 23, 24, 39] and/or to certain cell specific markers such as thyroglobin in the case of thyroid carcinomas, and prostatic specific antigen (PSA) in the case of prostatic carcinomas. In contrast, none of these probes have been shown to react with CIRC.

The differential diagnosis between prominent and increased CIRC in enlarged lymph nodes and possibly indigeneously arising carcinomas [14] is also soluble. Again in this instance, the neoplastic cells contained a wide array of CKs which consistently occurred together with desmoplakins [14]. Neither of these features was noted in the increased CIRC populations described. Finally, even the most bizarre CIRC simply lacked the convincing features of malignancy evident in the node-restricted

carcinomas described by us [14], and other observers [9]. The fact that CIRC may show a hyperplasia-like response adds credence to earlier speculations that they could also undergo malignant transformation and give rise to tumours with features shared with conventional carcinomas.

The function of the CK-positive cells in normal lymph nodes is unclear, but their increase in association with diverse lymphadenopathies would indicate that they play a role in nodal reactions to injury. Finally, the unique combination of their "epithelioid" immunoprofile, their location and their shape, merits a specific designation. Therefore, we propose that they be termed "cytokeratin-positive interstitial reticulum cells" (CIRC).

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