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Non-lymphoid accessory cells in the cutaneous infiltrate of B cell lymphomas. An immunohistochemical and ultrastructural study

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SUMMARY

We have investigated the occurrence, immunohistochemical profile, ultrastructural features and relationships to lymphocytes of the non-lymphoid accessory cells in the dermal infiltrate of five patients affected by B cell lymphoma with secondary involvement of the skin. Typical non-lymphoid accessory cells were found in all cases. Most of these cells had ultrastructural features which resembled those of the poorly differentiated dendritic reticulum cells described in follicular lymphomas of the lymph nodes. The immunohistochemical findings of DRC-1 +, C3b r+ dendritic cells often arranged in follicular-like structures with neoplastic B cells and only few, scattered OKM1 +, OKM5 + mononuclear phagocytes support the hypothesis that the vast majority of the non-lymphoid cells observed in our cases were poorly differentiated dendritic reticulum cells.

These results and previously published reports indicate that the organization of the dermal infiltrate of B cell lymphomas tends to reproduce the typical arrangement of the B zone of the lymphoid tissue, although with a lesser degree of differentiation, similar to that observed in lymph node follicular lymphomas.

In normal lymphoid tissue, lymphocytes interact with other cells types, stromal cells and accessory cells.[1,2] Stromal cells are regularly associated with collagen bundles and basal membrane-like material; these cells form the reticular framework of the lymphoid tissue, dividing it into compartments and hence, directing migrating lymphocytes and accessory cells. Accessory cells interact with T and B lymphocytes during acquired specific immune responses; they can also mediate non-acquired, 'natural' immunity independently of lymphocytes.

In the germinal centres of secondary lymphoid follicles, which are specific sites of B cell differentiation, typical accessory cells, dendritic reticulum cells, are found.[3–6] Dendritic reticulum cells exhibit 5-nucleotidase activity,[7] bear complement (C_3) receptors on their

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surfaces[8] and specifically react with R4/23 monoclonal antibody.[9] On electron microscopy, these cells characteristically show narrow plasma membrane infoldings coated with electron dense material, presumably representing fixed immunocomplexes; this hypothesis is supported by autoradiographic[3] and immunohistological studies.[8,10] Dendritic reticulum cells thus appear to play a role in trapping antigen within germinal centres. They also form a specific microenvironment that is preserved in low-grade malignant lymphomas, of which follicular lymphoma is the most common example.[11–15]

Dendritic reticulum cells have been identified in the cutaneous infiltrate of B cell lymphomas by enzyme-cytochemistry and immunohistochemistry.[16–20] Little is known about the ultrastructure of non-lymphoid accessory cells in cutaneous B cell lymphomas; some authors have observed, in some cases of cutaneous B cell lymphoma, non-lymphoid cells with the features of either dendritic reticulum cells or fibroblastic reticulum cells.[21–23] Further investigations are necessary to assess the extent of the participation of these cells in the cutaneous infiltrate of B cell lymphomas and to investigate their ultrastructural features and their relationships with infiltrating neoplastic lymphocytes. Better knowledge on these points is necessary before it is possible to speculate on their significance and their possible functional role (if any) in these lymphomas.

We report here the occurrence, immunohistochemical profile, ultrastructural features and relationship to lymphocytes of non-lymphoid accessory cells in the dermal infiltrate of five patients with B cell lymphoma with secondary involvement of the skin.

METHODS

Five Caucasian patients, seen in the Dermatological Clinic, University of Florence, were studied. Before starting treatment, a skin biopsy was taken from each patient and processed for light and electron microscopy and for immunohistochemisty. Clinical details of the patients are summarized in Table 1. The diagnosis was made according to the Kiel classification.[5,24]

Light microscopy

The specimens were fixed in phosphate buffered formol and routinely embedded in paraplast. Histological sections were stained with haematoxylin and eosin, PAS, and reticulin stain.

Immunohistochemistry

The tissue was snap-frozen and stored at −80°C. Cryostat sections (6 μm) were air dried, fixed

TABLE 1. Clinical details of patients studied

Patient no.	Age years	Sex	Cutaneous lesions	Primary localization (lymph nodes)	Diagnosis (type of lymphoma)
1	80	F	Multiple nodules (trunk, abdomen, extremities)	Right axillary	CB, diffuse
2	20	M	Solitary plaque (trunk)	Mediastinal	IB, diffuse
3	68	M	Solitary nodule (scalp)	Right laterocervical	CB/CC, diffuse
4	56	M	Solitary plaque (lower abdomen)	Left inguinal	CC, diffuse
5	22	M	Solitary plaque (trunk)	Left laterocervical	CB, diffuse

CB = centroblastic; CB/CC = centroblastic/centrocytic; CC = centrocytic; IB = immunoblastic.

in chloroform-acetone for 5 min and incubated for 30 min with the primary monoclonal antibody. The monoclonal antibodies used are listed in Table 2. The sections were then incubated with biotin-conjugated sheep anti-mouse serum (Amersham, U.K.) for 30 min and with streptavidin-biotin-peroxidase complex (Amersham, U.K.) for 20 min. The staining reaction was obtained with aminoethylcarbazole and hydrogen peroxide. The sections were finally counterstained with Mayer's haematoxylin and mounted with glycerine. Normal human lymph nodes served as positive controls. Sections incubated without the primary monoclonal antibody or without any antibody were negative controls for the second polyclonal antibody and for the peroxidase enzyme reaction, respectively. For quantitative analysis, the stained cells were counted in five consecutive microscopic fields at high magnification (× 400). The mean number of cells was calculated. Only cells in which the nucleus was in the plane of the section were considered.

Electron microscopy

Skin biopsies were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7·3, postfixed in 1% OsO_4 in 0.1 M phosphate buffer, pH 7·3, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop 1 or 102 electron microscope at 80 kV.

RESULTS

Immunohistochemistry

In all cases, B cells (Leu 12 +, HLA-DR +) made up the majority of the infiltrating cells. In Case 2, a kappa light chain monoclonality was observed; in the other cases the dermal infiltrate did not react with anti-kappa or anti-lambda light chain antibodies.

A variable proportion of infiltrating cells (higher in Cases 3 and 4 than in the others) reacted with OKT11, OKT4 and OKT8 monoclonal antibodies, the T4/T8 ratio ranging between 1:2 and 2:1.

DRC-1 +, C3b r + cells with a dendritic shape were consistently observed in the neoplastic infiltrate. In all cases except one (Case 2), these cells were gathered into follicular-like clusters with Leu 12 + lymphocytes (Figs 1, 2 and 3); the centre of the clusters contained mainly IgM + cells, the peripheral rim contained mainly IgD + cells. These clusters were separated from each

TABLE 2. Monoclonal antibodies used in the study

Antibody	Commercial source	Specificity
Leu 12	Becton and Dickinson, USA	
anti-HLA-DR	"	
anti-kappa	"	
anti-lambda	"	
anti-IgM	"	
anti-IgD	"	
OKT11	Ortho Diagnostic Systems, USA	see Foon & Todd, 1986[25]
OKT4	"	
OKT8	"	
OKM1	"	
OKM5	"	
anti-DRC 1	Dakopatts, Denmark	
anti-C3b receptor	"	

other by areas containing both Leu 12+ cells and reactive T11+ T lymphocytes, but devoid of DRC-1+, C3br+ cells. In Case 2, DRC-1+, C3br+ cells were scattered among the infiltrating neoplastic cells without any follicular-like organization.

Some OKM1+, OKM5+ cells were dispersed among the infiltrating cells.

Electron microscopy

In all the patients, the dermal infiltrate mainly consisted of lymphocytes and typical non-lymphoid cells.

FIGURE 1. DRC-1+ cells collected into a follicular-like structure with B cells (Case 3). (Original × 250).

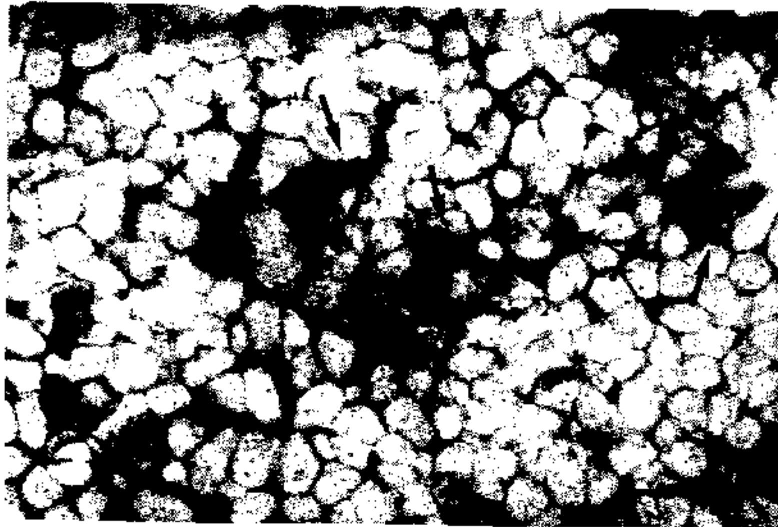

FIGURE 2. The dendritic shape of some DRC-1+ cells (arrows) is clearly shown (Case 3). (Original × 700).

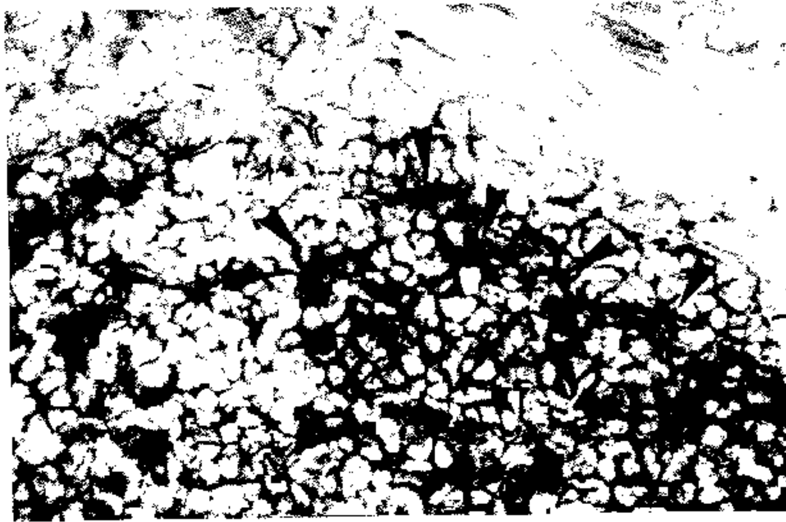

FIGURE 3. C3br− dendritic cells (arrowheads) with weakly stained B cells (Case 4). (Original × 450).

Most of the non-lymphoid cells had an irregular shape, with cytoplasmic projections which varied in number and length in different patients. The plasma membrane formed a small number of narrow invaginations (Fig. 4), which were not coated with electron dense material. Desmosome-like junctions between these cells were rare. The cytoplasm of these cells contained

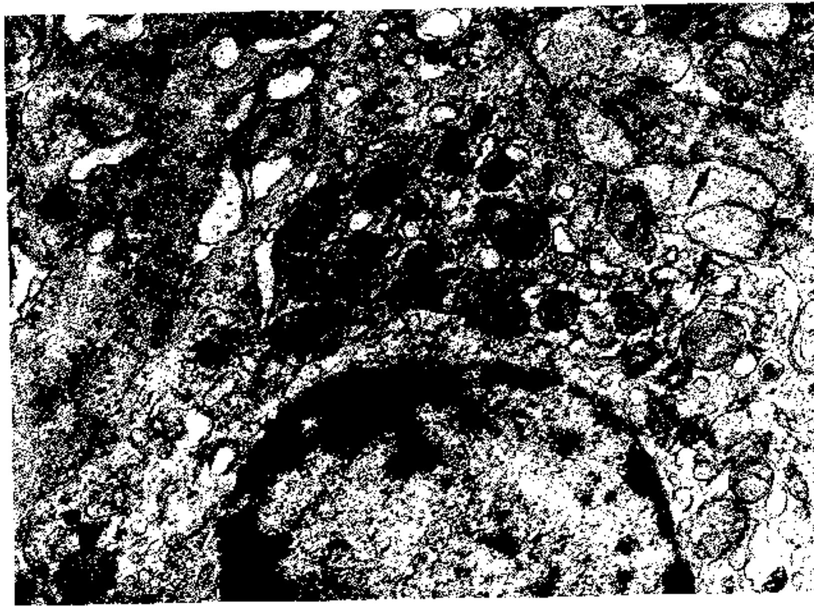

FIGURE 4. Non lymphoid cell in the dermal infiltrate of Case 1. Many narrow invaginations of the plasma membrane are shown (arrows). The cytoplasm contains small mitochondria, few cisternae of rough endoplasmic reticulum and many smooth surfaced vesicles and tubules. A rim of microfilaments is located beneath the plasma membrane (original × 27,000).

flat cisternae of rough endoplasmic reticulum, a well developed Golgi apparatus (Fig. 5), smooth vesicles and tubules (Fig. 6), some coated vesicles close to the plasma membrane (Fig. 5) and a variable number of round, membrane bound bodies, presumably primary lysosomes (Fig. 6). Secondary lysosomes or residual bodies were rare. The nucleus (Fig. 5) had a frequently indented outline, with a peripheral rim of condensed chromatin, a usually well recognizable fibrous lamina and a small nucleolus. Few of these cells were binucleated, or perhaps multinucleated (Fig. 7). These non-lymphoid cells and their cytoplasmic projections always contacted several lymphocytes (Figs 5 and 6).

Some non-lymphoid cells had few, slender cytoplasmic processes which extended among collagen bundles. The cytoplasm of these cells (Fig. 8) contained several cisternae of rough endoplasmic reticulum, a Golgi apparatus less developed than in the above mentioned cells, few Golgi vesicles and no smooth vesicles or tubules outside the Golgi region; coated vesicles and round, homogeneously dense, membrane bound bodies were virtually absent. These cells always had a single nucleus, with a more condensed chromatin pattern than in the cells mentioned above.

FIGURE 5. Non lymphoid cell in the dermal infiltrate of Case 5. The nucleus (N) has a slightly, but definitely, indented outline with a thin peripheral rim of condensed chromatin and a well recognizable fibrous lamina. In the cytoplasm, the Golgi apparatus (Go) is well developed. A cross-sectioned invagination of the plasma membrane (arrow), with two coated pits (arrowheads), is also shown. This cell is in contact with an infiltrating lymphoid cell (Ly), identification of which was made at low magnification. (Original × 27,000).

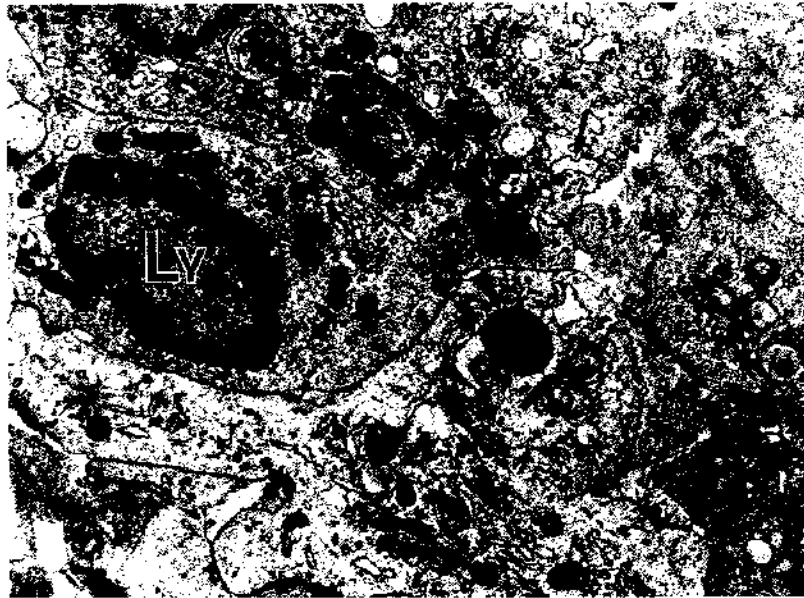

FIGURE 6. Dermal infiltrate from Case 4. A lymphoid cell (Ly) is in contact with two non lymphoid cells. The cytoplasm of the latter cells contains some mitochondria and cisternae of rough endoplasmic reticulum, numerous smooth surfaced vesicles (V) and tubules (arrows) and few, round, homogeneously dense bodies of different size (arrowheads), presumably primary lysosomes. (Original × 11,500).

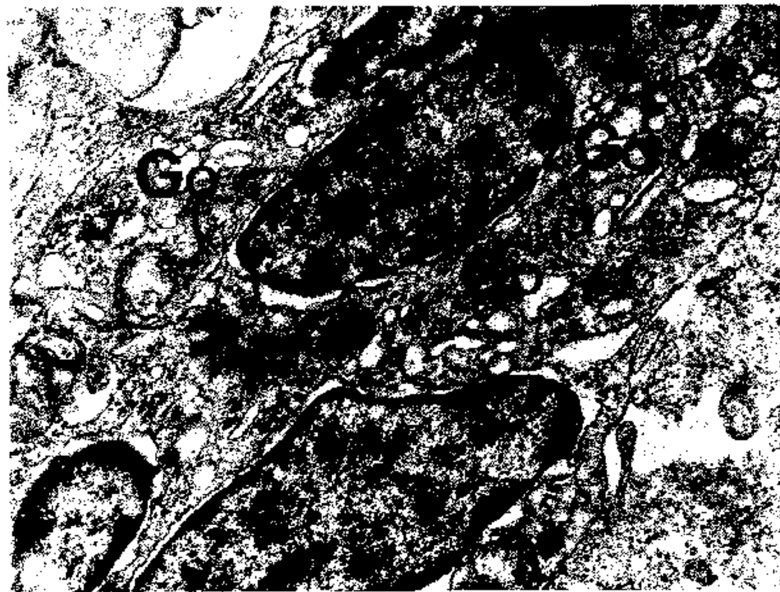

FIGURE 7. Dermal infiltrate from Case 3. A non lymphoid cell with extensive Golgi apparatus (Go) and scanty cisternae of rough endoplasmic reticulum contains four nuclear profiles, possibly pertaining to 2–4 nuclei. (Original × 16,000).

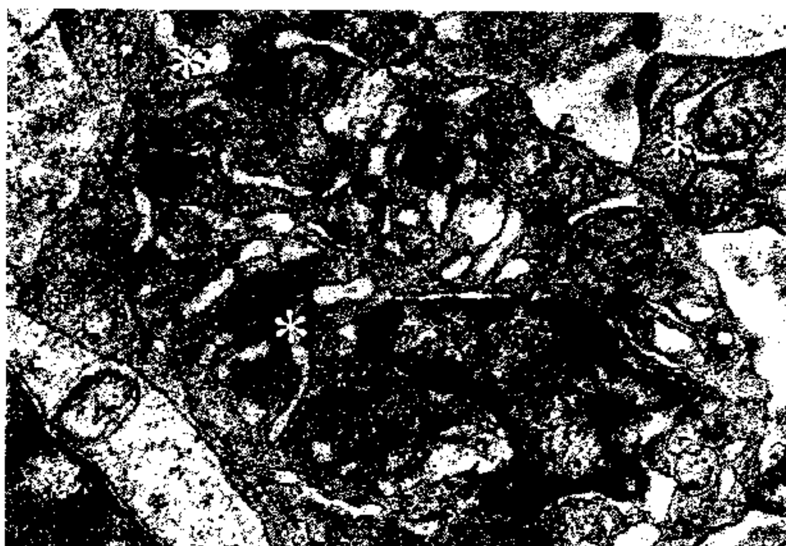

FIGURE 8. Dermal infiltrate from Case 4. A non lymphoid cell is shown, which contains several cisternae of rough endoplasmic reticulum (asterisks). This cell type is different from that shown in Figures 4–7, and resembles fibroblasts. The Golgi apparatus and smooth vesicles and tubules are inconspicuous in this cell type (Original × 13,000).

DISCUSSION

Typical non-lymphoid cells were found in all of five cases of secondary cutaneous B cell lymphoma.

Relatively few of these cells resembled the fibroblastic reticulum cells (stromal cells) described in normal lymphoid tissue[1,2,6] and in cutaneous B cell lymphomas.[21–23] They had few, slender cytoplasmic processes extending among collagen bundles; the cytoplasm contained several cisternae of rough endoplasmic reticulum, a poorly developed Golgi apparatus, few Golgi vesicles and no smooth vesicles or tubules outside the Golgi region; coated vesicles and round, homogeneously dense, membrane bound bodies were not seen; these cells always had a single nucleus, with a moderately condensed chromatin pattern.

Most of the non-lymphoid cells observed in our cases had an irregular shape, more or less dendritic, and shared some ultrastructural features with dendritic reticulum cells described in the germinal centres of secondary lymphoid follicles.[1–6] More precisely, these cells had plasma membrane invaginations, cytoplasmic organization, nuclear outline, chromatin pattern and relationships with lymphocytes similar to dendritic reticulum cells, albeit the invaginations of their surfaces were seldom extensively labyrinthine, electron dense material did not coat the plasma membrane and, as a rule, desmosome-like junctions between these cells and hemidesmosome-like junctions between them and the intercellular matrix were absent. These ultrastructural features suggest that these cells are poorly differentiated dendritic reticulum cells, also on account of the ultrastructure of dendritic reticulum cells described in follicular lymphomas of lymph nodes.[14,15] In follicular lymphoma of lymph nodes, in comparison with reactive follicular hyperplasia, dendritic reticulum cells usually have broader cell processes and sometimes less developed cytoplasmic organelles. Also, in follicular lymphomas of lymph

nodes, binucleated dendritic reticulum cells are rare, desmosome-like junctions between adjoining cells are relatively uncommon and labyrinthine plasma membrane infoldings are never coated with electron dense material. However, in follicular lymphomas of lymph nodes, histiocytic cells are significantly less numerous than in reactive hyperplasia and show no pronounced phagocytic activity, either on light[5] or electron microscopic examination,[14,26] so that the ultrastructural differentiation between histiocytic and dendritic reticulum cells may be a very difficult task.

Comparing our ultrastructural findings with the immunohistochemical finding of DRC-1 +, C3br + dendritic cells often arranged with neoplastic B cells in follicular-like structures and few, dispersed, OKM1 +, OKM5 + cells, which have also been found previously in B cell lymphoma, both in lymph nodes[13] and in the skin[18–20] we interpret the vast majority of the non-lymphoid cells observed in our cases as poorly differentiated dendritic reticulum cells. The follicular-like arrangement of lymphoid and non-lymphoid cells, which was also found in areas without any morphological evidence of lymphoid follicles, reinforces the interpretation of most of the non-lymphoid cells as dendritic reticulum cells.

Our present results and reports in the literature,[16–23] suggest that skin infiltrating neoplastic B cells induce the development of a specialized microenvironment consisting of non-lymphoid B zone-specific accessory cells, albeit with a limited degree of differentiation, in a similar way to that observed in lymph node follicular lymphomas.[5,11–15]

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