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Intercellular Substances in Hodgkin's Lymphomas *

Ultrastructural Investigations

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Summary. 20 biopsy specimens, selected from lymph node biopsies of 100 untreated patients with Hodgkin's disease, were investigated electron microscopically to examine the formation of extracellular material at ultrastructural level.

In the lymphocyte predominance type (2 cases) intercellular substance consists of a moderate amount of fine granular material. In nodular sclerosis (9 cases) different types of fibrillar structures can be distinguished: Typical collagen fibers including "spiny collagen", fibrils of 80–100 A thickness without periodicity, and peculiar cross banded structures (dense bands: 400–600 A, periodicity of 1 000–1 300 A). In areas rich in fibrillar intercellular substance activated macrophage-like cells predominate. The mixed cellularity type (6 cases) shows small amounts of intercellular material. In places there is a peculiar rhythmic arrangement of this material, with a resemblance to the cross banded structures. Lymphocyte depleted cases (3 examples) also reveal homogenous, flocculent and cross banded intercellular material. The latter structures are rather abundant, esp. in areas of so-called hyalinosis.

These different structural organizations of fibrillar and banded intercellular substances are considered to be the expression of a varying arrangement and deposition of collagen fibers and their precursors. The development of cross banded structures may be related to altered synthetic processes. The role of macrophage-like cells in the production of the fibrous material is discussed.

Key words: Hodgkin's lymphomas – Intercellular substance – Cross-banded structures – Spiny collagen – Macrophage-like cells.

Introduction

Since the fundamental studies of Lukes and coworkers (Lukes and Butler 1966, Lukes et al. 1966, Lukes 1972) the prognostic significance of certain histological

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features in untreated Hodgkin's lymphomas has been generally accepted. On the basis of various light microscopical criteria 4 histologic types were defined at the Rye conference. The typing of Hodgkin's lymphoma is determined by 3 histological variables: firstly by the amount of lymphocytes, secondly by the frequency of the diagnostic Reed-Sternberg cells and the occurrence of other giant cell types and thirdly by the nature of the intercellular substance.

In contrast to the cytology of Hodgkin lymphomas, which has been intensively studied by various morphological methods in the last ten years (Mori and Lennert 1969 and Dorfman et al. 1973 have used electron microscopy) less attention has been paid to the organization and synthesis of the extracellular material. That is surprising, since peculiar forms of intercellular substance as recognized by polarization microscopy and reticulin fiber staining, are decisive for the histologic characterization of Hodgkin's lymphoma.

We have investigated the morphological structure of intercellular substance in Hodgkin's lymphoma with the aid of the electron microscope. Following the recognition of several types of intercellular structures and their neighboring cells, it was a further aim of the study to correlate fibrillary structures with the probable "producer" cells.

Material and Methods

We have investigated 20 biopsy specimens prepared for electron microscopic examination, selected from lymph node biopsies of 100 untreated patients with Hodgkin's disease. Immediately after removal the tissue was fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for 2 h, postfixed 1 h in osmium tetroxide and embedded in Epon. The ultrathin sections were contrasted with uranyl acetate and lead citrate.

From this material additional sections were made for light microscopic examination. They were stained by routine methods in lymph node diagnosis: H&E, Giemsa stain, Gömöri's silver impregnation and PAS method. In every case a polarization microscopic examination was performed. Occasionally imprints of the material were available.

Results

1. Lymphocyte Predominance Type (2 Cases). Proliferation of lymphocytes is the diagnostic feature. Histiocytic cells (epitheloid cells – cp. Lennert, 1972) are present in varying amounts. Diagnostic Reed-Sternberg cells are scanty, but more frequently a special variant of giant cells, the so-called L&H cells (Lukes and Butler, 1962), can be seen. Other cell types, such as eosinophils and plasma cells, are uncommon or absent. Light microscopically, neither intercellular material nor fibrils were seen.

Electron microscopically the intercellular substance consists of only a moderate amount fo fine granular material. The cells generally resemble lymphocytes and are in close contact with each other. They appear slightly activated. Singular lymphocytes display features of necrosis with a dark cytoplasm. Occasionally phagocytic phenomena are seen which resemble apoptosis. Only a few macrophage-like cells are discernible (Fig. 1).

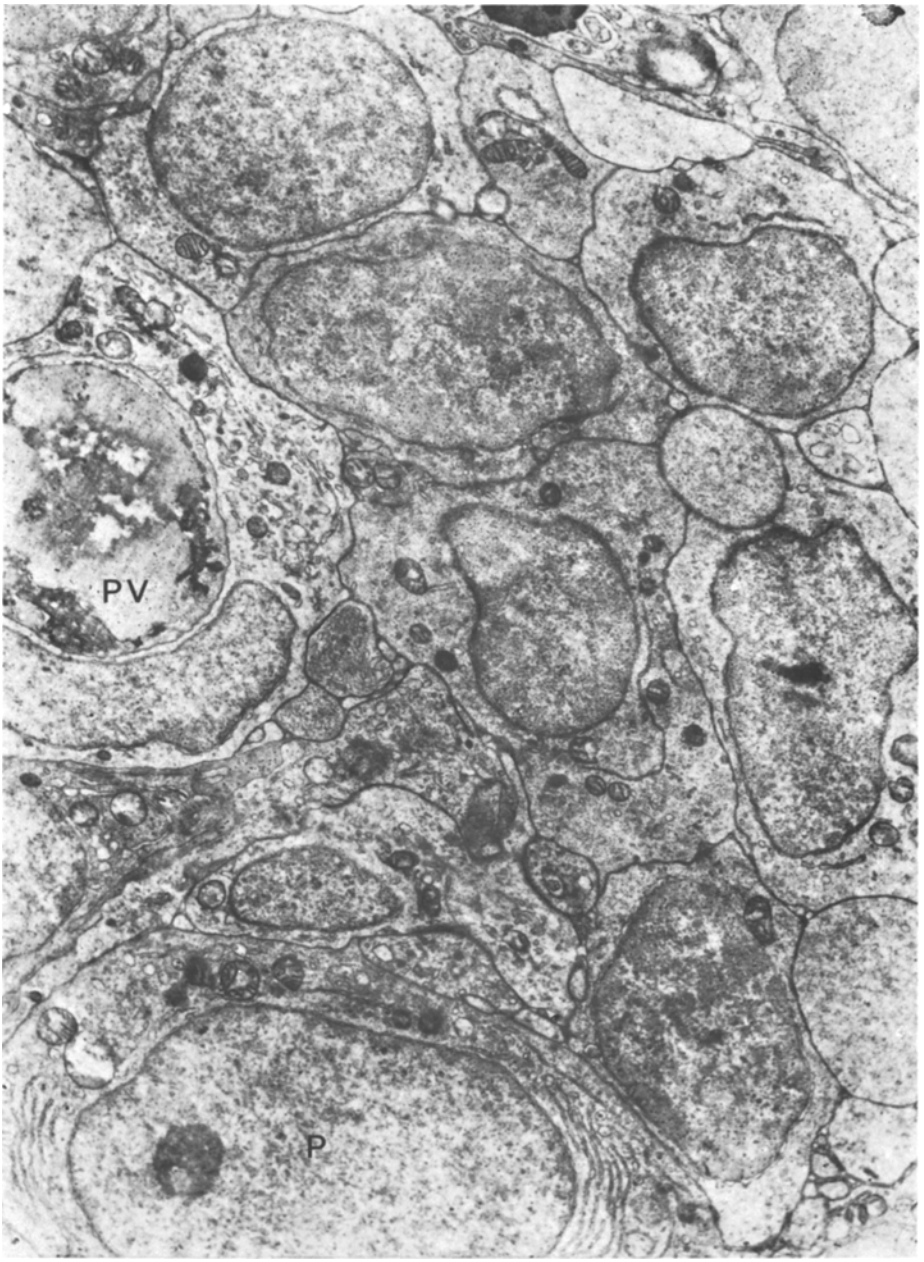

Fig. 1. Lymphocyte predominance type of Hodgkin's disease. Nume ous cl ely packed lymphocytes are seen. A plasmocytoid cell *(P)* with parallel arrays of rough adopl mic reticulum and some mitochondria is also present. Cellular debris within a phagocytot vacuo is marked *(PV)* (×7500)

2. Nodular Sclerosis (9 Cases). Broad bands of collagenous material produce a subdivision of the abnormal lymphoid tissue into partially or completely isolated cellular nodules. Typical lacunar cells are present. Lymphocytic cells predominate but eosinophils and neutrophils, plasma cells and their precursors are also present on occasion.

With the electron microscope different fibrillar structures can be found:

1. Collagen fibers with the typical periodicity of 600–700 A. These fibers measure nearly 700–800 A in diameter (Fig. 2a).

2. In some cases and varying in different locations, this collagen is modified by irregular granules of a relatively dense material adhering to its outer surface. As a rule the granules are of 200 A in diameter and tend to be associated with the dark bands of the fibers, producing the "spiny" collagen appearance. Such collagen formations have already been described by Banfiled et al. (1973) (Fig. 2b).

3. Fibers measuring 200–400 A in diameter with and without the typical periodicity of collagen.

4. Fibrils without periodicity having a diameter of 80–100 A (Fig. 2c).

5. Scanty peculiar cross banded structures can also be observed. They consist of dense bands with a thickness of about 400–600 A which are arranged in a repeating periodicity with a distance of approximately 600–700 A between them. From this a total periodicity of 1 000–1 300 A results. Amidst such cross banded structures 80–100 A thick filaments are to be detected. They run at a right angle to the axis of the dense bands. This can clearly be demonstrated by staining after periodic acid oxidation and silver impregnation. These structures were not infrequently found in proximity of small vessels (Fig. 2d).

Ultrastructurally lymphocytes with "dark" or "light" cytoplasm in different stages of activation are visible, as are plasma cell precursors and plasma cells. Apart from eosinophilic and neutrophilic granulocytes numerous macrophage-like cells can be seen.

The most fascinating observation is the finding that in areas rich in fibrillar intercellular substance macrophage-like cells prevail over all other cell types, including fibroblast-like cells. The appearance of the macrophage-like cells is very variable: it extends from typical oval blood monocyte-like cells with microvilli and numerous lysosomes to cells with abundant microfibrils in their cytoplasm. As transitional forms, cells with reduced lysosomes and an increased number of mitochondria, smooth surfaced vesicles, slender profiles of rough endoplasmic reticulum and a prominent Golgi apparatus together with villous projections on their surfaces forming partially interdigitating processes, may be seen. Furthermore, several macrophage-like cells are partially destroyed and are visible only as remnants. In this context it is noteworthy that 2–3 cells often form little cell complexes. However, specialized cell contacts between these cells are lacking (Fig. 3). Apart from the clearly macrophage-like cells a further cell type can be identified in the fibril-rich areas. It corresponds completely to the lacunar cells in nuclear configuration and cytoplasmic organelle content. This cell type possesses a hyperlobated nucleus with marginally condensed chromatin, a prominent multicentric Golgi apparatus, numerous mitochondria and smooth surfaced vesicles, a moderate or small content of rough

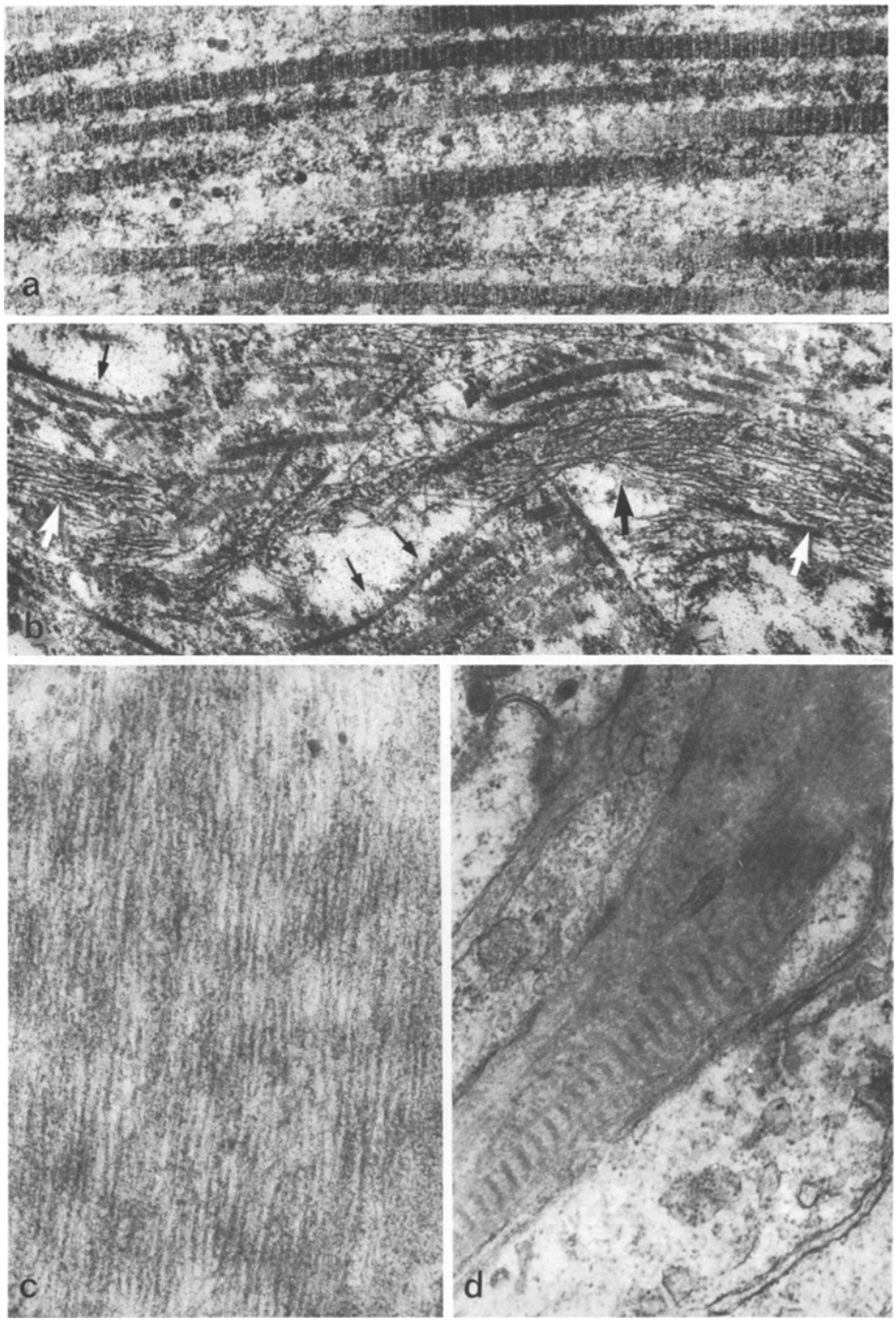

Fig. 2a–d. Examples of intercellular substance in nodular sclerosis. **a** Typical collagen fibers with a clear periodicity. The fibers have a diameter of about 700 A (×55,000). **b** Apart from typical collagen fibers so-called spiny collagen (→) can be detected. Numerous fibrils with a diameter of about 200 A are visible (→) (×37,500). **c** The fibrils arranged in parallel measure 100 A in diameter and are without clear periodicity (×66,700). **d** Cross banded structures with a periodicity of 1100 A. Thin fibers running in a right angle to the axis of the dense bands can also be seen. This intercellular material is deposited between tumor cells with "light" cytoplasm (×35,000)

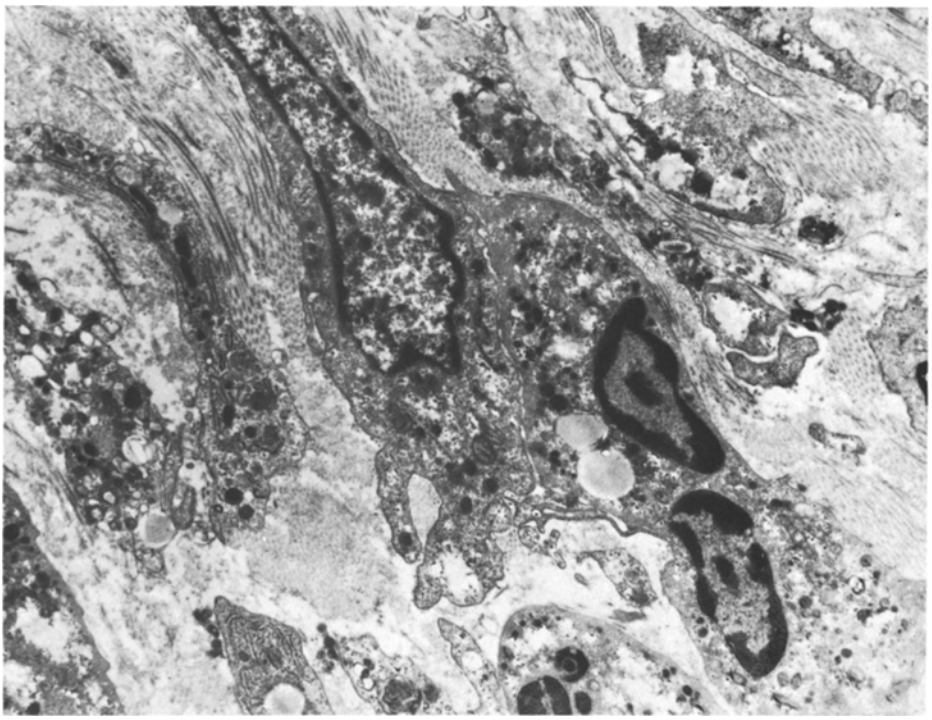

Fig. 3. Macrophage-like cells in nodular sclerosis. These cells are found in areas rich in fibrillar intercellular substance. Note the numerous lysosome-like dense bodies within the cytoplasm. Two closely apposed tumor cells in the center of the microphotograph have only a little gap between them, but specialized cell junctions are lacking (×9,300)

endoplasmic reticulum and numerous microvilli-like projections on the cell surface. Subplasmalemmal 50–80 A thick filaments forming a dense network may be found.

Finally, around activated capillary vessels a multiplied basement membrane can sometimes be seen which seems to merge, in its outer parts, with the surrounding fibrillar substance. Continuous transformations of vascular wall cells into cells of the surrounding lymphoma tissue were not seen.

3. Mixed Cellularity Type (6 Cases). In this type the cellularity is heterogenous. Lymphocytes with different degrees of activation, blast-like cells with pronounced nucleoli, plasma cells and their precursors, eosinophils and neutrophils, together with numerous macrophage-like cells with varying organelle composition and partially prominent interdigitating processes were found. The diagnostic Reed-Sternberg cells revealed typical huge, rounded or oblong shaped nucleoli. Moreover, varying cytoplasmic density of lymphoma cells is noticed throughout and some necrotic cells appear scattered in the tissue. Atypical giant cells may be encountered.

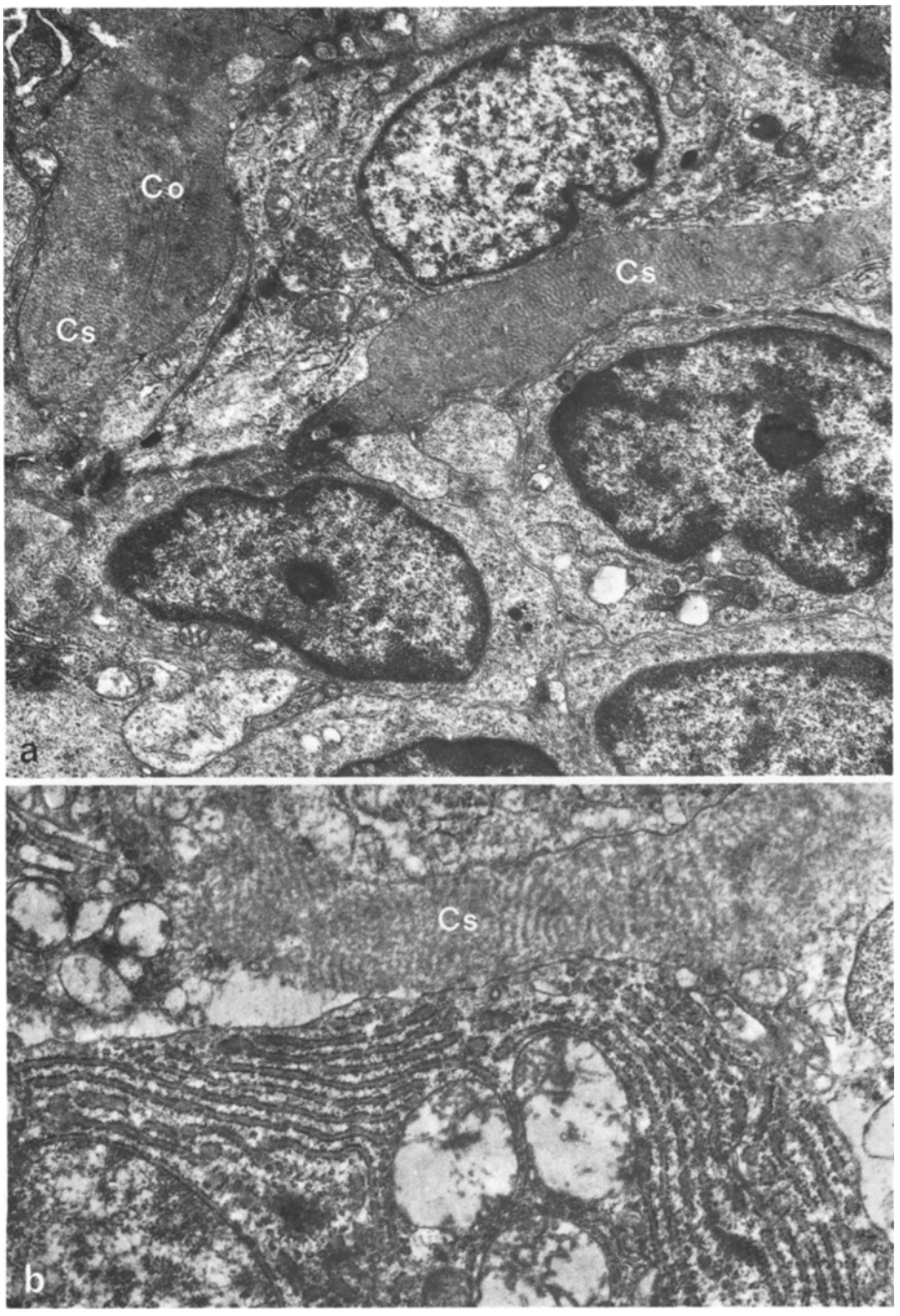

Fig. 4a and b. Mixed cellularity type of Hodgkin's disease. **a** Activated lymphocytes with an increased amount of cytoplasm and enlarged nucleoli. In the intercellular space cross banded structures *(CS)* and some collagen fibers *(CO)* are demonstrable (×9,300). **b** In the neighbourhood of a plasma cell with abundant rough endoplasmic reticulum cross banded structures with a periodicity of 1300 A *(CS)* are visible (×12,800)

The intercellular substance found in small amounts consists of homogenous or flocculent material of low to moderate electron density. Now and then, in small areas, a peculiar rhythmic arrangement of this material can be observed. The periodicity of 1000–1300 A in these structures makes comparable to the cross banded structures described in nodular sclerosis. Collagen fibers are only sporadically recognizable (Fig. 4).

4. Lymphocyte Depletion Types (3 Cases). This Hodgkin type shows a varying histological picture. The common trait is the remarkable paucity of lymphocytes. The quantity of the intercellular substance is not uniform. In some cases a network of Gömöri positive fibers is visible, in others a strong increase of a homogenous eosinophilic material is produced.

Apart from the homogenous and flocculent moderately electron dense intercellular material, fibrils and cross banded structures occur. The fibrils have a diameter between 80 and 100 A and often show an irregular arrangement. The cross banded structures have been described above. In the lymphocyte depletion types these structures are abundant in contrast to nodular sclerosis and the mixed cellularity type. Typical collagen fibers are not present.

The cells ultrastructurally resemble macrophage-like cells, fibroblast-like cells and blast cells. Between the two former types morphologically transitional stages exist. Giant cells were relatively numerous and could have been derived from macrophage-like cells using morphological variables as identification. Plasmocytoid differentiation was seldom seen.

Discussion

Electron microscopic examination of different histologic types of Hodgkin's lymphoma showed different intercellular fibrillar structures. Beside atypical fine fibrils two peculiar components can be distinguished:

(1) the typical collagen fibers and an obviously modified form of collagen (so-called spiny collagen), and

(2) the cross banded structures in the extracellular space.

Collagen fibers show a typical periodicity of 600–700 A. The thickness of these fibers is about 800 A, a second fiber population measures 200–400 A in diameter. Thy typical collagen fibers are most clearly seen in nodular sclerosis. In all probability the granules of spiny collagen correspond to proteoglycan complexes (Banfield et al., 1973). We have no convincing explanation for the spiny collagen. It is possible that this modification is the result of a particular degradation phenomenon or of an abnormal synthetic process, but we found spiny collagen only in nodular sclerosis. The uncharacteristic unbanded fibrils of a width of 100 A may be precursors of typical collagen fibers.

The cross banded structures in the extracellular space which we found in all types of Hodgkin's disease except the lymphocyte predominant type were very similar to fibrous long-spacing collagen. This form of collagen, first observed in Schwann cell proliferations (Luse, 1960; Waggener, 1966; Fisher and Vuzevski, 1968) is considered an abnormal end product of collagen synthesis. We believe,

with Mollo and Monga (Mollo et al., 1968; Mollo and Monga, 1971) that the cross banded structures in Hodgkin's lymphoma are related to altered synthetic processes of collagen rather than to degradation of preexisting structures. Our own unpublished observations of cross banded structures in non-Hodgkin lymphomas (immunocytomas, immunoblastic sarcomas) also favor the concept of altered synthesis. In these tumors we assume that plasma cells and their precursors will change the microenvironment by secreting immunoglobulins and in this way influence the synthesis of fiber producing cells. However, some of the fibrillary proteins in Hodgkin and non-Hodgkin lymphomas may be due to breakdown of immunoglobulins. Moreover, there are arguments in favor of a development from breakdown products of collagen (Nemetschek-Gansler et al., 1977). The frequent proximity of cross banded structures to vascular basement membrane could suggest a relation to collagen type IV (cp. Kefalides, 1975; Gay et al., 1976).

If one is looking for hints on the nature of the "producer" cells of the intercellular material in Hodgkin's lymphoma, it is remarkable that there are correlations between the presence of fibrillar material in the extracellular space and macrophage-like cells whilst fibroblast-like reticular cells are few. Enzyme histochemical investigations also suggest the presence of many true macrophages in lymph nodes of Hodgkin's lymphoma (Dorfman, 1961; Young and Bitter, 1973). Our findings suggest that part of the fibrillar material is produced by macrophage-like reticular cells derived from blood monocytes as well as from fibroblast-like reticulum cells as it is now accepted that histiocytes may act as facultative fibroblasts (in soft tissue tumors, Stout and Lattes, 1967). On the other hand, the presence of many macrophage-like cells could indicate the importance of degradative processes in modelling the intercellular substance.

In *summary* we have concluded that the different structural organization of the intercellular substance in Hodgkin's lymphoma is the expression of a varying arrangement and deposition of collagen fibers and/or their precursors. As a consequence of the clear preponderance of macrophage-like cells over other cell types in areas with abundant intercellular fibrillar proteins these cells must be considered to be responsible for part of the synthesis of the intercellular substance. Macrophage-like cells should thus be considered an essential constitutent of the lesion.

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