Ultrastructural study of satellite lymph nodes in syphilitic rabbits

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SUMMARY In an electron microscopy study of lymph nodes from syphilitic rabbits plasmablasts and plasma cells were unequivocally identified in germinal centres. Up to 20% of the plasma cells possessed unusual cytological features. Paracortical hypoplasia was shown to be associated with actively phagocytic histiocytes. These findings may reflect morphological correlates of the aberrant immune regulation which has been observed in infection with *Treponema pallidum*.

Introduction

Lymphadenopathy is a characteristic of early syphilis. Light microscopy examination of syphilitic human lymph nodes has shown cortical hyperplasia with prominent germinal centres (Harstock *et al.*, 1970; Turner and Wright, 1972) and atrophy of paracortical (thymus-dependent) areas (Festenstein *et al.*, 1967; Levene *et al.*, 1969; Turner and Wright, 1972). Unusual findings have included histiocytic infiltration of the paracortex and the presence of what appear to be plasma cells in germinal centres; in normal lymph nodes or in other infections these cells are usually confined to medullary areas (Turner and Wright, 1972). Infection with *Treponema pallidum* is accompanied by alterations of immune regulation affecting antibody production and cell-mediated immunity (Wright and Grimble, 1974; Musher *et al.*, 1977); these include production of non-treponemal antibody (Turner and Hollander, 1957; Turner, 1970), suppression of the IgG response to a thymus-dependent antigen such as sheep red blood cells (Baughn and Musher, 1978), delayed production of lymphokines (Wicher and Wicher, 1977), and suppressed lymphocyte transformation to a variety of antigens and mitogens (Levene *et al.*, 1969; Musher *et al.*, 1974; Friedman and Turk, 1975;

Musher *et al.*, 1975, 1977; Pavia *et al.*, 1977). In the light of these abnormal immune responses we have undertaken an electron microscopy examination of lymph nodes from syphilitic rabbits. Our study unequivocally identified plasma cells in germinal centres and showed them to have several unusual morphological abnormalities. Ultrastructural examination also showed the paracortical histiocytes to be actively phagocytic. These abnormalities may be related to the changes in immune regulation which have been observed in syphilis.

Material and methods

RABBITS

Outbred New Zealand white male rabbits weighing 2-3 kg were housed individually in stainless-steel cages at an ambient temperature of about 18°C. Rabbits with a positive Venereal Disease Research Laboratory (VDRL) test reaction were excluded because of the possibility that this resulted from a subclinical infection with *Treponema cuniculi*.

ORGANISMS

T. pallidum (Nichols strain) was maintained by intratesticular passage in rabbits. Inflamed testes were removed aseptically, minced in sterile saline, and ground with sand with a mortar and pestle. After centrifugation at 270 × g for 30 minutes to remove sand and cellular debris, the number of treponemes in the supernatant was determined by darkfield microscopy. Rabbits used in this study were shaved

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on the back and infected intradermally with 10⁷ organisms per site to a total of six to eight sites per rabbit.

OBTAINING, PROCESSING, AND EXAMINING LYMPH NODES

Lymph nodes are virtually never palpable in normal, uninfected rabbits. Early in the course of syphilis, however, satellite lymph nodes may be found draining the chancres in the fore and hind regions. These nodes were sampled at one, two, four, eight, 10, and 16 weeks after intradermal inoculation. At least two rabbits were studied for each time interval.

Rabbits were killed by an intravenous injection of air. Lymph nodes were excised rapidly and immediately placed in 1% phosphate-buffered glutaraldehyde or paraformaldehyde-glutaraldehyde (Karnovsky, 1965) and then in 1% phosphate-buffered osmium tetroxide; they were all stained together with uranyl acetate, dehydrated in a graded series of alcohols followed by propylene oxide, and embedded in Araldite-Epon (Araldite 506 and Epon 812, E. F. Fullham, Inc., Schnectady, N.Y. 12301, USA). Lymph nodes from these same rabbits were processed for conventional histology by fixing in Bouin's solution, embedding in paraffin, sectioning at 5 μ m, and staining with haematoxylin-eosin.

Sections were cut on a Porter Blum MT II ultramicrotome. Semi-thin (1 μ m) sections were stained with methylene blue (Reynolds, 1963) and used for orientation. Thin sections were stained with uranyl acetate (Richardson *et al.*, 1960) then lead citrate (Reynolds, 1963) and examined with a Phillips 200 electron microscope.

Plastic 1 μ m sections were studied for each time interval. To estimate the degree of cortical hyperplasia 500 cells from the centre of well-developed germinal centres were examined and the percentage of cells which contained mitotic figures was calculated (Table). For each time interval observations on five slides from five different samples were averaged.

Results

LYMPH NODE CORTEX

Light microscopy examination of 1 μ m sections showed cortical hyperplasia with prominent germinal centres; lymphoblasts predominated, appearing as large cells with centrally placed nuclei, well-defined nucleoli, and extensive euchromatin. The cytoplasm contained many polyribosomes, rough endoplasmic reticulum, and mitochondria (Fig. 1). Cells ranging in maturational stages from large to small lymphocytes constituted the remainder of lymphoid

Table Results of sampling of satellite lymph nodes from syphilitic rabbits

Week	Specimen no. *	% Mitotic cells†
1	117	4.0
	139	4.5
	176	3.2
2	124	2.9
	145	2.6
	182	2.7
4	127	0.8
	163	0.5
8	145	1.7
10	146	4.3
16	152	3.9

*Each number represents a different rabbit lymph node; these were minced and five samples were studied from each specimen.

†Each value represents the average of mitoses in five samples.

Fig. 1 An electron micrograph showing a typical area from a germinal centre of a syphilitic lymph node draining a seven-day chancre. Note the mitotic cell (M), a lymphoblast (L) with modest endoplasmic reticulum (ER) and mitochondria, and a plasmablast (Pb) with dilated endoplasmic reticulum (magnification $\times 7000$).

cells that were present. A striking finding was the presence of plasmablasts and plasma cells in germinal centres (Figs. 1 and 2), these cells usually being found

Fig. 2 An electron micrograph showing a characteristic plasma cell (PC) in a germinal centre. The cell is identified by its eccentric nucleus (N) with extensive marginated heterochromatin and abundant well-developed dilated endoplasmic reticulum. Sections of cytoplasm of two adjacent plasma cells can be noted (magnification $\times 7200$).

only in the medulla. Typical plasma cells contained eccentric nuclei with heterochromatin dispersed along the periphery. They had large amounts of dilated rough endoplasmic reticulum and the Golgi apparatus was often prominent. Up to 20% of plasma cells had cytological abnormalities, including polymorphic nuclei, multiple nucleoli or morphological alterations of the Golgi apparatus or both (Figs. 3 and 4). Immature plasma cells, the plasmablasts, had moderate amounts of endoplasmic reticulum, centrally placed nuclei, and some heterochromatin. Hyperplasia of germinal centres, as determined by the percentage of mitotic cells, was prominent in the first two weeks, declined by the fourth week, but again was present at the tenth and sixteenth week (Table).

LYMPH NODE PARACORTX

Light microscopy examination of 1 μm sections

Fig. 3 The ultrastructure of an anomalous plasma cell. Note three distinct nucleoli (nu) in a cell with well-developed cytoplasmic endoplasmic reticulum (magnification $\times 4300$).

Fig. 4 Highly magnified ($\times 20\,000$) view of the Golgi region (G) of a plasma cell shows that normally well-defined lamellae have lost their parallel orientation, and large vacuoles (V) appear irregularly dilated. That this is not an artefact of fixation can be readily appreciated by the well-preserved endoplasmic reticulum and whirls of polyribosomes (P).

showed hypoplasia coupled with histiocytic infiltration (Fig. 5). Electron microscopy examination showed the histiocytes to be large, relatively electron-dense, phagocytic cells with modest amounts of isolated ribosomes. These cells were actively phagocytic and contained debris consisting of dense inclusions and myelin figures (Fig. 6).

CONTROL LYMPH NODES FROM RABBITS INFECTED WITH BCG OR STAPHYLOCOCCUS AUREUS

The lymph nodes of rabbits challenged at multiple sites at the back with *Staph. aureus* or *Mycobacterium tuberculosis* var. *bovis* (BCG) were different from those of syphilitic animals; neither contained the well-developed germinal centres with plasma cells that so typically characterised syphilitic lymph nodes. Nodes from animals infected with *Staph. aureus* showed modest primary nodules; BCG nodes were essentially normal. Lymph nodes infected with both *Staph. aureus* and BCG showed modest paracortical hyperplasia in direct contrast to the hypoplasia seen in rabbits infected with *T. pallidum*.

Discussion

Plasma cells have been shown to be present in germinal centres of lymph nodes from syphilitic patients based on identification of pyroninophilic cells by light microscopy examination (Turner and Wright, 1972). In the present study plasma cells were definitively identified as a prominent component of germinal centres in experimental syphilis in the rabbit. Electron microscopy examination also

Fig. 6 Electron micrograph of a section through one portion of a histiocyte. The nucleus (N) is seen at the upper left-hand margin. Areas from two lymphocytes are seen in the lower right-hand corner. The histiocyte is typically phagocytic with many dense inclusions (I) and myelin figures (m) (magnification $\times 4800$).

Fig. 5 Light micrograph showing histiocytic infiltration of the hypoplastic paracortex (magnification $\times 950$).

showed a variety of abnormalities in these cells, including lobulated nuclei, multiple nucleoli, and peculiarities in the Golgi region. These changes are most unusual. Since multiple nucleoli are seen only in immature precursors their persistence in plasma cells which have well-developed cytoplasmic endoplasmic reticulum may reflect asynchrony in cellular development. Abnormalities of the Golgi region were similar to those which have recently been shown to accompany an arrest of immunoglobulin synthesis (Tartakoff and Vassalli, 1977). The presence of these unusual cytoarchitectural features is of great interest in the light of the recent demonstration that the IgG response to a thymus-dependent heterologous antigen is nearly obliterated in early syphilis (Baughn and Musher, 1978).

The changes in the paracortex are in agreement with several previous reports which have demonstrated paracortical hypoplasia in syphilis (Festenstein *et al.*, 1967; Levene *et al.*, 1971; Turner and Wright, 1972). In addition to confirming the presence of histiocytes the present study shows them to be actively phagocytic. It is interesting to speculate as to whether phagocytic histiocytes are responsible for paracortical atrophy or whether they appear as a result of other factors which have brought about this atrophy. Treponemes were not detected in these histiocytes; however, treponemal antigen could also be responsible for histiocytic infiltration. In any case, hypoplasia of the paracortex, a thymus-dependent area, may be the anatomical correlate of suppressed cellular immunity which occurs during early syphilis (Levene *et al.*, 1969; Musher *et al.*, 1974; Friedman and Turk, 1975; Musher *et al.*, 1975, 1977; Pavia *et al.*, 1977). The presence of hypoplasia together with histiocytic infiltration is not unlike the changes observed in lymph nodes of patients with lepromatous leprosy (Turk, 1976), a disease which is also characterised by suppression of thymus-mediated immune function.

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