Short Report

Adrenergic innervation in reactive human lymph nodes

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(*Accepted 13 October 1998*)

ABSTRACT

Several experimental models have demonstrated that the central nervous system is functionally linked to the immune system by means of the autonomic nervous system. Samples of 36 lymph nodes of patients whose ages ranged from 16 to 69 y were studied. In order to demonstrate the existence and distribution of sympathetic nerve fibres, a polyclonal antibody antityrosine hydroxylase (TH), with the streptavidin-biotin system of detection, was used. TH-positive nerve fibres appeared in all reactive patterns of the lymph nodes studied. Thin nerve fascicles ramified at the hilar region and also in the connective tissue septae. Adventitial adrenergic nerve fibres were found following afferent, and to a lesser extent, efferent blood vessels. Another source of incoming nerve fibres was found at capsular level, accompanying blood vessels. On the arterial side, the innervation ceased before reaching the follicular arterioles. Our demonstration of innervation in postcapillary venules could support a regulatory role of adrenergic neurotransmitters in lymphocyte traffic. Occasional nerve fibres were also seen in T areas among parenchymatous cells. These findings confirm the existence of sympathetic innervation in human lymph nodes, and provide indirect evidence that the psychoneuroimmune axis could also exist in humans.

Key words: Sympathetic innervation; tyrosine hydroxylase.

INTRODUCTION

It is known that the central nervous system (CNS) interacts with the immune system through the autonomic nervous system, modifying the immune response (Riley, 1981; Shekelle et al. 1981; Livnat et al. 1985; Felten et al. 1987, 1988). The distribution of nerve fibres in normal lymph nodes has been demonstrated in mice and rats (Felten et al. 1984, 1985; Novotny & Kliche, 1986; Villaro et al. 1987; Novotny et al. 1993, 1994, 1995*a*, *b*), but little information is available about the innervation of human lymph nodes (Volik, 1965; Winckler, 1966). These human studies were performed on fetal lymph nodes. Adrenergic nerves can be revealed immunohistochemically in tissue sections, applying an antibody to tyrosine hydroxylase (TH), considered to be the key enzyme for the rate-limiting step in catecholamine biosynthesis (Weiner, 1970). In this study the adrenergic innervation of human reactive lymph node has been studied by means of antityrosine hydroxylase polyclonal immunolabelling.

MATERIALS AND METHODS

Samples of 36 human lymph nodes were studied. Their sizes varied between 3 and 15 mm in their major axis, derived from 9 patients whose ages ranged from 16 to 69 y. They were obtained in the course of oncological surgical resections (mastectomies, colectomies, prostatectomies, etc.). Other lymph node resections performed for diagnostic purposes were added. Lymph nodes without neoplastic or other evident pathological processes were selected. Two adenopathies were from a patient treated with diphenylhydantoin for 2 y. In accordance with international ethical requirements lymph nodes were not obtained from healthy people. Only reactive lymph nodes were selected and they were classified according to Sternberg (1992) in a pure or mixed

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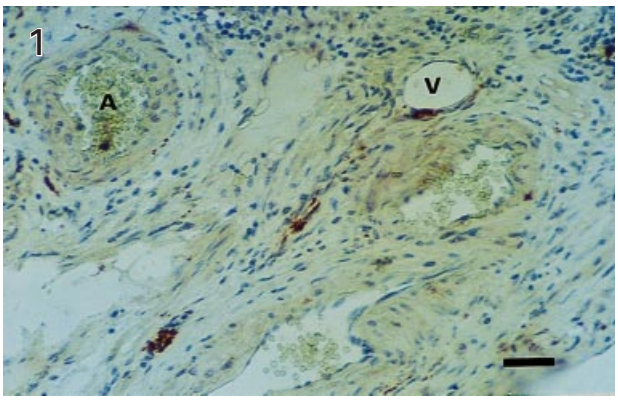
pattern of sinus histiocytosis, follicular or paracortical hyperplasia. Immediately after recovery, half of each lymph node was fixed in formalin and the other half in Bouin fluid, and then embedded in paraffin. Sections (5 μm) were stained following routine techniques (haemalum-eosin, Giemsa, Wilder, PAS). Immunohistochemical detection for polyclonal anti-TH (Chemicon International, USA) was performed, using a working dilution of 1:128 in 0.05 M Tris buffer, pH 7.4 with bovine serum albumin 1%. Endogenous tissue peroxidase activity was blocked with hydrogen peroxide 0.03%, and nonspecific reaction was avoided with Protein Block Serum Free agent (Dako, USA). The slides were treated with the heat-induced epitope retrieval method in order to retrieve antigenicity (Shi et al. 1991). The sections were incubated for 48 h at room temperature with the primary antibody. Streptavidin-biotin polyclonal kit (Immustain, UK) was used, with aminoethylcarbazole as chromogen. The slides were lightly counterstained with haemalum. Negative controls were performed on lymph node sections, omitting either the primary or the secondary antibody. Sections of mouse brain were used as positive controls.

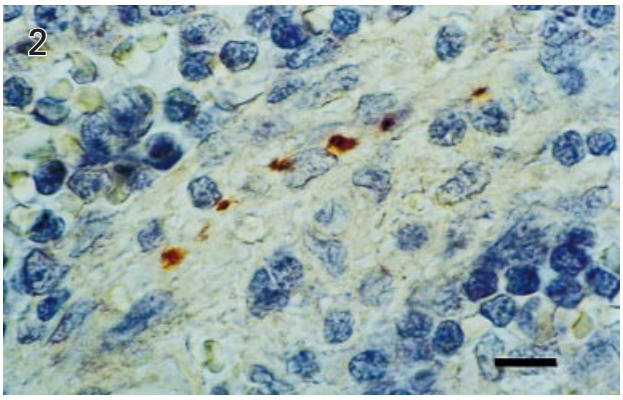
RESULTS

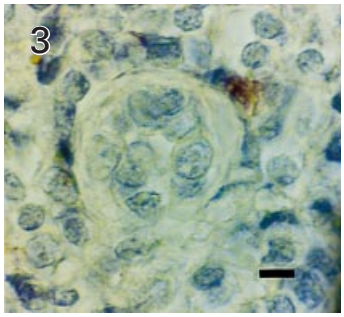
In a total of 36 lymph nodes studied, samples of the following reactive patterns were found: 9 simple follicular hyperplasia, 2 predominantly sinus histiocytosis, 9 with a mixed pattern of follicular hyperplasia and sinus histiocytosis, 10 with paracortical hyperplasia and follicular hyperplasia. While 5 lymph nodes presented the 3 basic patterns mixed, only 1 lymph node showed paracortical hyperplasia and sinus histiocytosis. All reactive patterns studied showed the existence of TH-positive nerve fibres. Small nerve bundles appeared at the hilus (Fig. 1) and individual nerve fibres were seen around arterial and venous vessels, arterial branches being more extensively innervated than venous branches. The fibres were predominantly located in the vascular adventitia or in the most external layer of smooth muscle cells. An unramified thin nerve trunk was frequently found adjacent to the wall of a hilar vein. No fibres were detected in vascular structures considered to be intranodal lymphatic vessels. Thin TH-positive nerve was observed in the capsular connective tissue and in its intranodal septal extensions (Fig. 2). Some isolated nerve fibres were found in the same location while others travelled around arterial and venous vessels. Immunopositive nerve fibres varied in length, ranging from short and delicate fibres to longer and thicker extensions with multiple varicosities (Fig. 2). The arterioles of the connective tissue septae, as well as the small arteries of the medullary cords, also displayed adventitial fibres. We did not find fibres within the follicles, in the mantle or in centrofollicular arterioles. No pericapillary nerve fibres were found in the samples studied. Nerve fibres could be seen, but in smaller numbers, in large venous vessels, venules and postcapillary venules (Figs 1, 3). Longitudinal nerve fibres in T areas between lymphoid cells, not related to vascular structures were also demonstrated infrequently (Fig. 4). In these areas we observed rounded positively labelled structures that are discussed later. A marked increase of innervation was detected in 2 adenopathies with a mixed pattern of paracortical and follicular hyperplasia (Fig. 5). Long nerve tracts appeared between parenchymatous cells (Fig. 6). These adenopathies were from the patient treated with diphenylhydantoin.

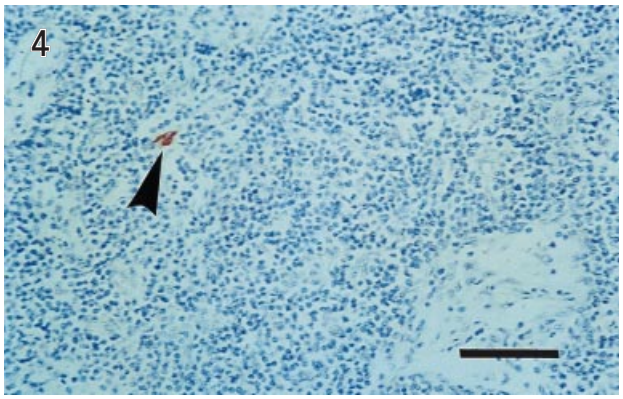
DISCUSSION

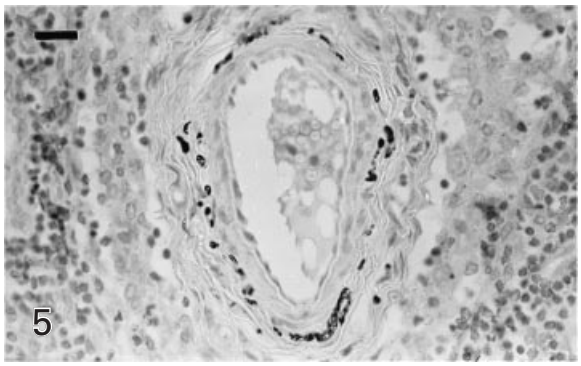
Although several morphological studies demonstrating the presence of sympathetic innervation in lymph nodes of rodents have been carried out (Felten et al. 1984, 1985, 1987, 1988; Novotny & Kliche, 1986; Villaro et al. 1987; Novotny et al. 1993, 1994, 1995*a*, *b*), sympathetic innervation in the adult human lymph node has not so far been explored. This study has demonstrated tyrosine hydroxylase activity in human lymph nodes by means of immunohistochemical reactivity. Patterns of simple follicular hyperplasia, or follicular hyperplasia combined either with sinus histiocytosis or paracortical hyperplasia, have been found in the same ratio. No case of pure paracortical hyperplasia was observed. A basic pattern in the distribution of TH-positive nerve fibres was found in the different types of reactive lymph nodes studied. We have demonstrated that adrenergic fibres enter human lymph nodes along a double path, accompanying branches of either arterial or venous vessels. Most fibres enter the lymph node accompanying the arteries. The number of nerve fibres decreases as these vessels become thinner. Fibres in centrofollicular, mantle arterioles, capillary and hilar lymphatic vessels were not detected. Thin branches of nerve fibres were seen in postcapillary venules. There is no reference in the literature reviewed as to the existence of nerve fibres in postcapillary venules in lymph nodes. In these vessels, adrenergic neurotransmitters and neuropeptides could act on lymphocyte traffic, regulating either the luminal diameter or the lymphocyte-endothelial cell adhesiveness (Tang











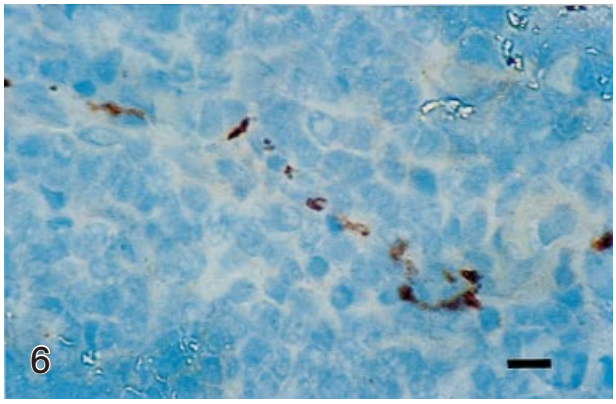


Fig. 1. Hilar region of a peri-ileal adenopathy. TH-positive fibres can be observed around arterial (A), and venous (V) vessels, as well as a thin nerve. Bar, 50μm.

Fig. 2. Nerve in septal connective tissue of a peri-ileal adenopathy. Bar, 10 μm.

Fig. 3. Postcapillary venule with TH-positive fibre in a peri-ileal adenopathy. Bar, 10 μm.

Fig. 4. Nerve fascicle in paracortex (arrowhead) in an inguinal adenopathy. Bar, 100 μm.

Fig. 5. Increased innervation in an arterial vessel of a cervical adenopathy from a patient treated with diphenylhydantoin. Bar, 20 μm.

Fig. 6. Long nerve tract between parenchymatous cells. Cervical adenopathy from the patient treated with diphenylhydantoin. Bar, 10 μm

et al. 1993). Occasional nerve fibres independent of vessels appeared between parenchymatous cells in T areas of reactive lymph nodes. It is difficult to interpret the reactivity for TH among parenchymatous cells. Some of these positive structures could correspond to nonaxonal elements, since TH activity in nonneural cells has been demonstrated (Stewart & Kirby, 1985) as well as the existence of intralymphocytic TH (Bergquist et al. 1994). Further observations including ultrastructural studies will be necessary in order to demonstrate the fine relationship between nerve fibres and parenchymal cells in the human.

The greatest number of nerve fibres in all regions studied was seen in the sample from the patient treated with diphenylhydantoin. Probably the increment of the innervation can be attributed to the drug. It is well known that chronically administered diphenylhydantoin causes architectural alter-

ations of lymph nodes (Jaffe, 1995). Our findings agree with those of other authors (Felten et al. 1984; Novotny & Kiche, 1986; Villaro et al. 1987; Novotny et al. 1993, 1994). However, we did not find a subcapsular plexus as Felten et al. (1984) had observed in rodents. As documented by other authors in fetal lymph nodes (Winckler, 1966, Volik, 1965), we have demonstrated in the adult a capsular source of incoming adrenergic innervation, although we did not observe encapsulated endings in the samples studied as they had described. It has been stated that neurotransmitters and cotransmitted peptides could have a paracrine action on surrounding cells. The immune response could be regulated, controlling cellular differentiation and proliferation (Besedovsky et al. 1979; del Rey et al. 1981; Burnstock, 1983; Livnat et al. 1985).

The present study provides morphological evidence of the relationship between the autonomic nervous system and the lymphoid system in human lymph nodes. This supplies an anatomical basis for the hypothesis that a psychoneuroimmune axis also exists in the human.

ACKNOWLEDGEMENTS

The authors thank Mrs Silvana Podestá and Mrs Gloria Riaño for their excellent technical assistance. This study was partially supported by the CSIC, Uruguay.

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