

IgG in the walls of the post-capillary venules of human lymph nodes

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Summary

IgG immunoglobulin was demonstrated in the wall of the post-capillary venules of human para-aortal lymph nodes by using a direct immunoperoxidase technique. The relationship of this immunoglobulin to the recirculation of the small lymphocytes is discussed.

Introduction

Post-capillary venules are vessels with high cuboidal endothelium normally encountered in the paracortical areas of the lymph nodes and in the interfollicular areas of the tonsils and Peyer's patches (2, 3, 8). During the recent years, considerable interest has been focused on these vessels due to their important role in the recirculation of the small lymphocytes (T-cells) from blood to the lymphatic tissues (2, 3, 4). Despite a considerable number of studies the exact mechanism regulating this recirculation remains obscure (1, 3, 4, 5, 6). Recently, IgG has been detected in the wall of post-capillary venules of human tonsils and its relationship to the T-cell recirculation has been speculated (7). The present report is an approach made with a direct immunoperoxidase technique to detect if such an immunoglobulin is present in the walls of the post-capillary venules of the lymph nodes, too.

Materials and Methods

The present series consists of 40 lymph nodes collected from the para-aortal regions of 10 patients at autopsy. The ages of the patients varied between 47 and 65 years and all of them were free from any kind of malignancy that could influence the immunologic status.

The collected lymph nodes were immediately frozen in liquid nitrogen and stored at -40°C until processed.

For the detection of IgG, a direct immunoperoxidase technique was used, the main

steps of which are briefly summarized below. The frozen sections of 5 micron thickness were fixed in acetone for 2 minutes followed by drying in air for 30 minutes. The sections were washed in PBS for 5 minutes after which they were incubated with peroxidase-conjugated goat-anti-human-IgG (Miles Laboratories, Inc. Elkhart, Indiana) at room temperature for 45 minutes. After three washes with PBS the antibody binding site was localized by incubating the slides in DAB (75 mg of 3,3'-diaminobenzidine tetrahydrochloride in 100 ml of Tris-HCl buffer, pH 7.6, where 1.5 ml of 10% H_2O_2 is added) for 5 minutes at room temperature. All steps were made as duplicates and the control slides were similarly processed except for the incubation with anti-IgG. One section of each node was processed according to the routine histologic methods and stained with Hematoxylin-eosin.

Results

All the lymph nodes studied contained many high endothelium walled post-capillary venules in their paracortical areas (Fig. 1.). In the sections stained with the immunoperoxidase technique brown precipitates of varying intensity were detected in germinal centers of the lymph node cortex and in the plasma cells of the medullary cords. In the paracortical area, the precipitates were localized in the walls of the post-capillary venules. The intensity of the brown precipitates varied considerably, but in generally it was less than that encountered in the germinal centers and plasma cells.

In the post-capillary venules, the brown precipitate was localized in the luminal border of the endothelium, in the intercellular site or along the basement membrane of the venule (Fig. 2).

Fig. 1 This photomicrograph shows a typical post-capillary venule located in the lymph node paracortex. This vessel has an endothelium made up of high cuboidal cells (asterisk), lymphocytes are seen in the lumen and basement membrane is clearly visible (arrow). (H and E, original magnification x1000)

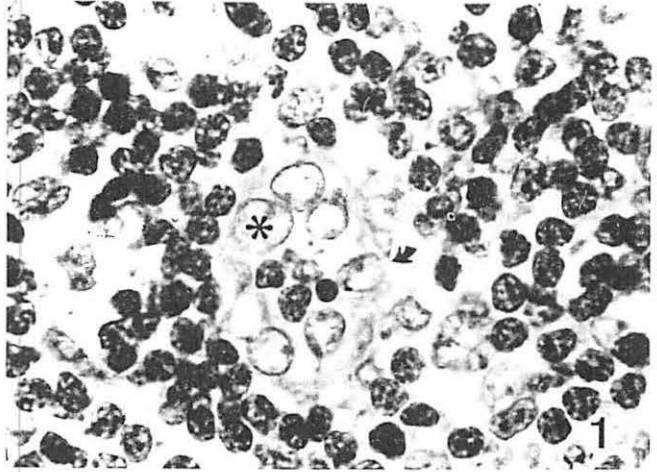
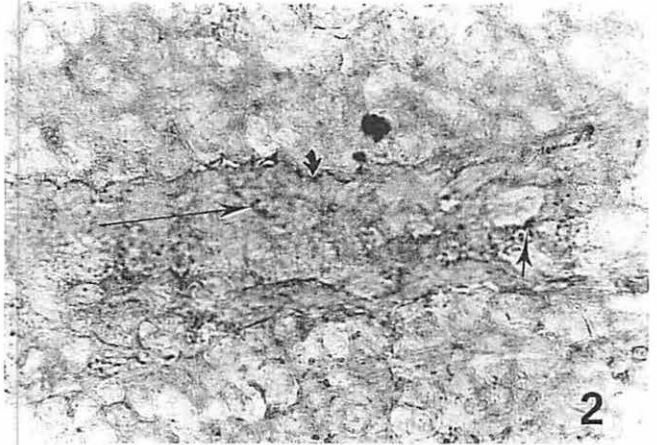


Fig. 2 In this section, stained with direct immunoperoxidase technique, a longitudinal section of a post-capillary venule is seen. The dark precipitate shows the distribution of IgG which is mainly located along the basement membrane (thick, short arrow) but traces of it are also encountered in the luminal border of the endothelium (thin, short arrow) and in the intercellular site of the endothelium (thin, long arrow). (Direct immunoperoxidase, no counterstain, original magnification x1000)



In the control sections, not treated with the anti-immunoglobulins, only those elements containing an endogenous peroxidase activity, i. e. the neutrophilic granulocytes and tissue mast cells, showed a faint positive reaction when developed with DAB.

Discussion

An important role in the recirculation of the small lymphocytes has been attributed to the high endothelium walled post -capillary venules of the lymphoid organs (1, 2, 3, 4,). Some controversy seems to exist concerning the route which the lymphocytes use in passing through the post-capillary venule endothelium. There are workers who believe

that this passage takes place through the cytoplasm of the endothelial cells (4) and others who suggest that the lymphocytes use an intercellular route (1, 6). The documentation for the last mentioned alternative seems to be more convincing (1, 6).

Recently, immunofluorescent studies have shown IgG localized in the wall of the post-capillary venules of human tonsils (7). The origin of that immunoglobulin was discussed and its possible relationship to the recirculation of the lymphocytes was speculated (7). After that, an electronmicroscopic study revealed membrane-bound secretory granules in the cytoplasm of the endothelial cells of the lymph node post-capillary venules (1).

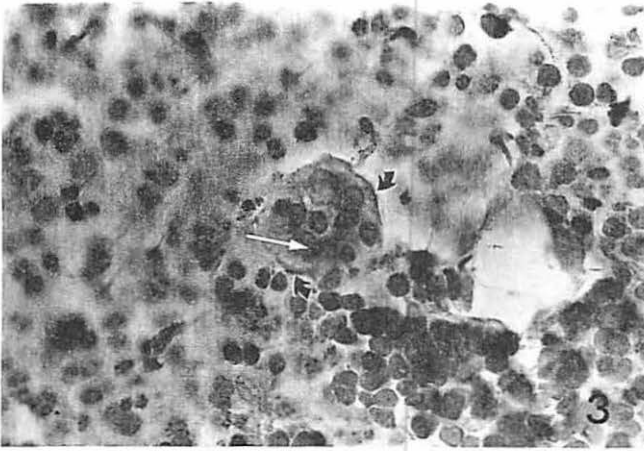


Fig. 3 This photomicrograph demonstrates a high endothelium walled post-capillary venule in transverse section. In this case the most intense reaction for IgG is seen along the basement membrane (thick, short arrows) and only traces are seen in the luminal and intercellular sites of the venule endothelium (thin arrow). (Direct immunoperoxidase, counterstained with Hematoxylin, original magnification x1000)

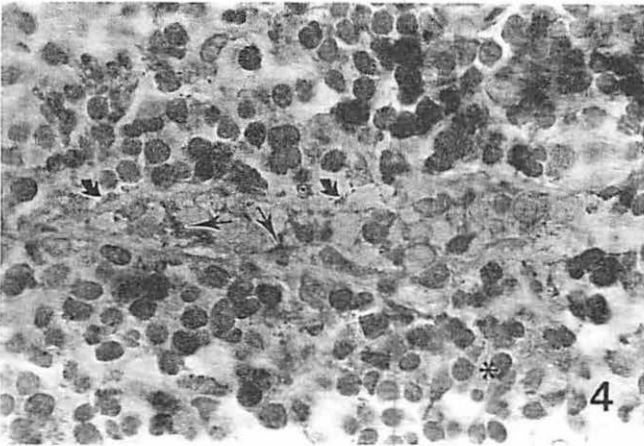


Fig. 4 A longitudinal section of a high endothelium walled post-capillary venule is presented in this figure. The positive brown precipitate indicating the distribution of IgG is localized along the basement membrane (thick short arrows), in the luminal as well as in the intercellular sites (thin arrows) of the vessel endothelium. Some of the lymphocytes show a dark circular precipitate for their membrane-bound IgG (asterisk) indicating that these cells belong to the B-lymphocyte population. (Direct immunoperoxidase, counterstained with Hematoxylin, original magnification x1000)

These granules were seen in the areas of close contact between the lymphocytes and the endothelial cell membranes (1). These granules were suggested to be receptor-immunoglobulins facilitating mutual recognition as well as passage of lymphocytes through the wall of the post-capillary venules.

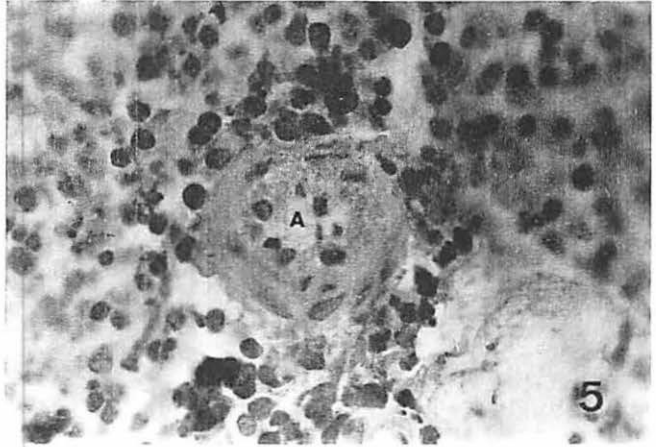
The present results verify the presence of IgG in the walls of the post-capillary venules in the lymph nodes. These nodes were localized in the region which is not subject to such an intense antigen stimulation as the palatine tonsils. This could suggest that the IgG found in the wall of the post-capillary venules is not due to antigenic stimulation but represents an integral component of the endothelium of the

venules. A thought is near that this IgG could be the factor responsible for the regulation of the lymphocyte passage through the endothelium of these vessels. The work is on progress to gain further evidence for this concept.

References

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Fig. 5 This photomicrograph presents a small artery (A) in the medullary region of a human lymph node. No positive reaction for IgG can be demonstrated in the wall of this artery stained with a direct immunoperoxidase method. Many of the surrounding lymphocytes show a strong positive reaction for their membrane-bound IgG and they thus belong to the B-lymphocyte population normally encountered in the medulla. (Direct immunoperoxidase, counterstained with Hematoxylin, original magnification x1000)



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