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Paracortical Post-capillary Venules of Human Lymph Nodes with Special Reference to the Distribution of Their Endothelial IgG

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Summary

The distribution of IgG in the endothelium of the paracortical post-capillary venules (PCV) of human lymph nodes was studied in frozen sections by using an indirect immunoperoxidase technique. Three different patterns of distribution could be differentiated (luminal site, intraendothelial and basement membrane site). Each of these patterns was characteristically encountered in association with one of three grades of PCV (graded on the basis of the height of their endothelial cells). The significance of this close relationship between the IgG distribution and the changes in PCV endothelium was discussed in regard to the T-lymphocyte recirculation. A hypothesis was introduced describing the possible sequence of events involved in the traversing of T-lymphocyte through the PCV endothelium.

Introduction

The key role played by the post-capillary venules in the recirculation of the T-lymphocytes seems to be well documented (1, 2, 3, 4, 6, 7, 10, 12). The mechanisms regulating the passage of T-cells through the endothelium of the post-capillary venules (PCV) are not known with certainty. IgG found on the endothelial surface of the PCV:s in human tonsils and lymph nodes has been speculated to be one of these regulating factors (9, 11). Recently, structural changes in PCV endothelium correlating with the state of T-lymphocyte depletion have been encountered in the lymph nodes of both cancer patients and experimental animals artificially rendered T-cell depleted with anti-theta-globulin (12, 13, 14).

In the present work an attempt was made to correlate the structure of the PCV endothelium with the distribution of the endothelial IgG in human lymph nodes.

Material and Methods

The present series comprises a total of 37 lymph nodes collected from the abdominal cavity of 15 patients at autopsy. The ages of the patients varied between 40 and 68 years. In order to obtain the largest possible variation of the structure of PCV endothelia, cases with malignancies were also included in the present series (14). The collected lymph nodes were frozen and stored at – 40 C until used.

For the demonstration of IgG, an indirect immunoperoxidase technique was used according to the modification of *Pinkus* et al. (8). Instead of the paraffin sections of the original work (8), frozen sections of five micron thickness were used in the present work. All incubations were made as duplicates, and three controls of each node were processed through the sequence of all steps except one of the following incubations: rabbit-anti-human-IgG, goat anti-rabbit-whole serum or peroxidase-anti-peroxidase soluble complex (PAP). All slides were counterstained with Hematoxylin.

Post-capillary venules were classified into three grades according to the criteria outlined previously (12, 13, 14) (Fig. 1–3). For each lymph node, the post-capillary venule score (PCV-S) was calculated basing on five PCV:s arbitrarily selected from each node (12, 13, 14). Thus, a total of 185 PCV:s were evaluated in the study. The values of the PCV-S fall between the limits of 1.0 and 3.0 (12, 13, 14).

Three types of IgG distribution were differentiated in the study. Type I was characterized by the localization of IgG on the luminal site of the PCV endothelium (Fig. 4). In Type II, IgG was found to be localized inside the endothelium (Fig. 5), and in Type III it was localized inside the endothelium (Fig. 5), and in Type III it was localized along the basement membrane (Fig. 6).

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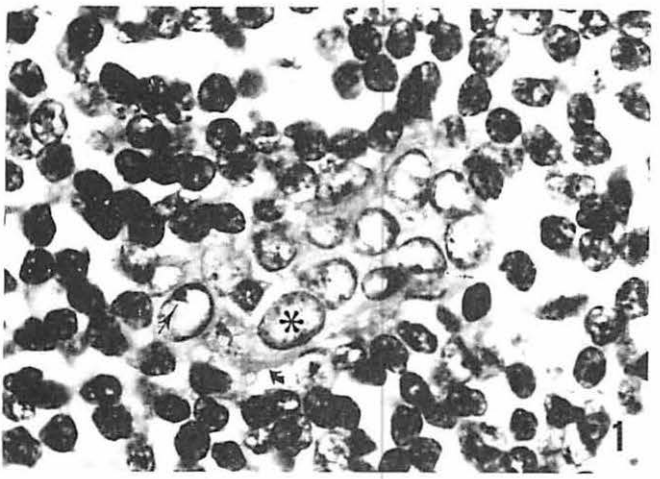

Fig. 1 Grade 3 post-capillary venule in transverse section. The endothelium is made up of high cuboidal cells (asterisk) with prominent nucleoli (thin arrow). Lymphocytes are seen in the lumen as well as passing through the endothelium from blood to the paracortical area of the node. The basement membrane (thick short arrow) of the venule is also clearly visible. (H and E, original magnification x1000).

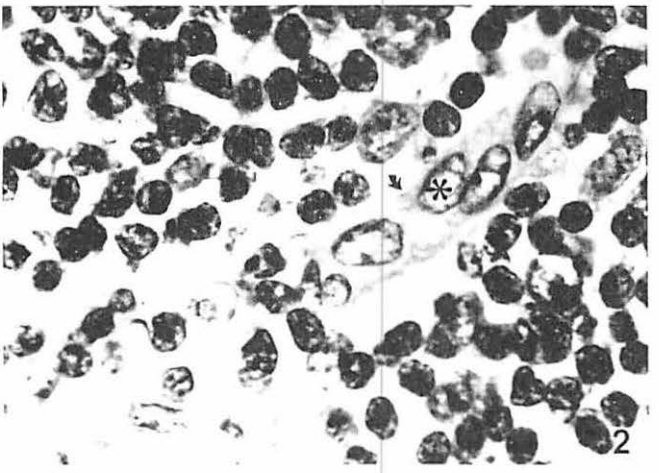

Fig. 2 A post-capillary venule of Grade 2. The endothelium of this vessel is composed of low cuboidal cells (asterisk). Small lymphocytes are not present in the lumen or in the endothelium. The basement membrane (arrow) as well as the nucleoli of the endothelial cells are clearly visible. (H and E, original magnification x1000).

The staining reactions in the small arteries of the nodes were also recorded for the controls sake, only.

Results

The mean PCV-S value of 2.102 was obtained for the series, the SD (standard deviation) and SEM (standard error of mean) being 0.338 and 0.055, respectively.

Table 1 summarizes the Types of IgG distribution in relation to the PCV grades. The figures indicate that Type I seems to be char-

Table 1 The types of IgG distribution in relation to the grade of post-capillary venules

PCV Grade	Type of IgG distribution I		II		III	Total N:o of PCV:s
1	0	***	8	***	42	50
	***		**		***	
2	22	*	28	**	16	66
	***		*		***	
3	50	***	18	***	1	69
Total N:o of PCV:s	72		54		59	185

Explanation of the symbols: PCV denotes for post-capillary venule; Asterisks denote the level of significance calculated with the chi-square test as follows: * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

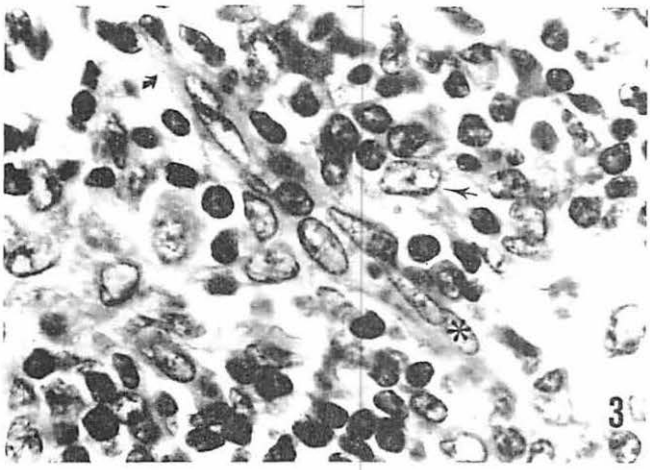

Fig. 3 This photomicrograph demonstrates a post-capillary venule of Grade 1. The lumen of this venule is flattened, the endothelium is made up of flattened cells (asterisk) and the basement membrane (thick short arrow) is conspicuous. The surrounding paracortical area shows a paucity of small lymphocytes and consequently the reticulum cells (thin arrow) forming the basic framework of the lymph node are seen more strikingly than usually. (H and E, original magnification x1000).

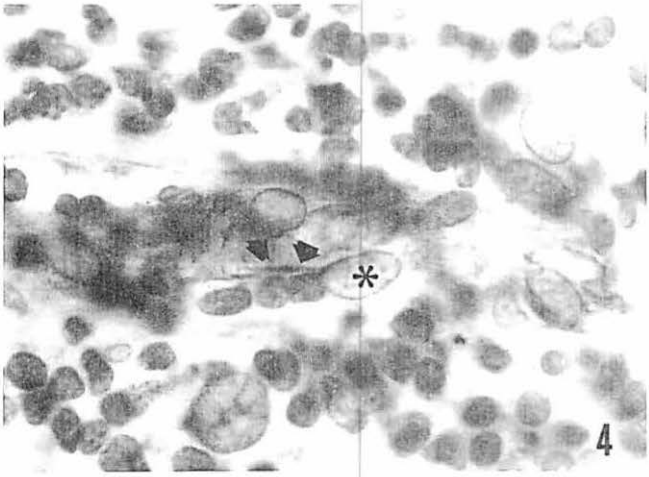

Fig. 4 Grade 2 post-capillary venule stained with the indirect immunoperoxidase technique for the localization of IgG. The endothelial cells (asterisk) are of the low cuboidal variety. The positive staining reaction for IgG is localized in the luminal site of the endothelium (arrows). This kind of staining pattern was called Type I distribution of IgG. (Indirect immunoperoxidase technique, counterstained with Hematoxylin, original magnification x1000).

acteristic to grade 3 PCV, Type II to grade 2 PCV and Type III to grade 1 PCV. The differences seems to be of statistical significance, too. The control slides, not incubated with one of the immunoglobulins or with PAP, were always negative in regard to the staining reaction for IgG. On the other hand, sixteen out of the thirtyseven nodes contained small arteries which showed a weak positive reaction for IgG. This reaction was always limited to the intimal surface of the vessel and it was never encountered in the media or adventitia of the artery.

Discussion

Ultrastructural studies have provided evidence that the PCV serves as a route of passage from blood to the lymphatic tissue for the small circulating T-lymphocytes (1, 3, 4, 6, 7). The factors regulating this passage are largely unknown. Recently, morphological alterations in the PCV endothelium have been detected in conditions thought to be associated with T-lymphocyte depletion (12, 13, 14). The venules of the lymph nodes and Peyer's patches but not those of the thymus loose their high

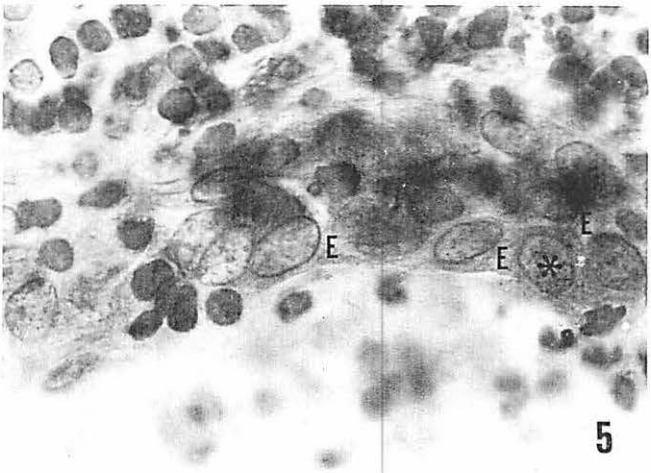

Fig. 5 Grade 3 post-capillary venule showing Type II distribution of IgG. The endothelial cells (asterisk) are of the high cuboidal variety. The light brown granular precipitate indicating the presence of IgG was localized throughout the endothelium (E) and was called an intraendothelial or Type II distribution of IgG. (Indirect immunoperoxidase technique, counterstained with Hematoxylin, original magnification x1000).

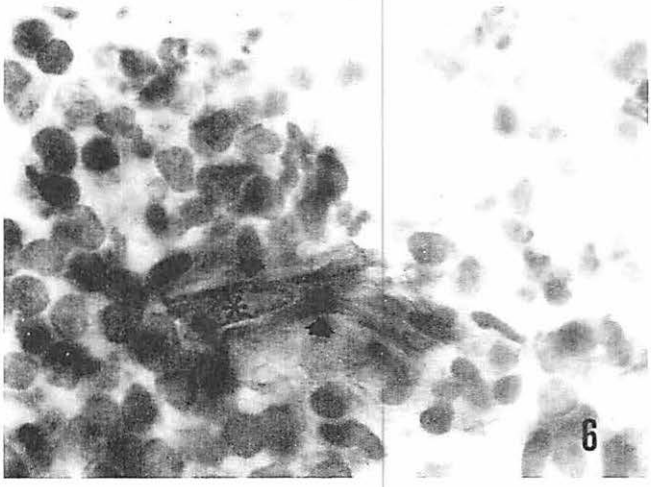

Fig. 6 Grade 1 post-capillary venule exhibiting Type III distribution of IgG. The endothelium of this vessel is made up of flattened cells (asterisk). The dark brown precipitate representing the localization of IgG is found exclusively on the basement membrane site of the endothelium (arrows). (Indirect immunoperoxidase technique, counterstained with Hematoxylin, original magnification x1000).

cuboidal endothelium in cases with depleted T-cell populations in the respective thymus-dependent areas of these organs (paracortex of the lymph node and interfollicular area of Peyer's patch) (12, 13, 14). Like the relationship of IgG, found in the PCV endothelium, to the recirculation of T-cells has been discussed (9, 11), the possible significance of the above mentioned structural changes in this respect has been reasoned, too (12, 13, 14). The present work was undertaken to clarify if these two factors, endothelial IgG and the endothelial alterations, could in some way be related to each other.

The PCV-S of the present series (2.102) was slightly below the value of the larger human control material of a previous study (14). This is undoubtedly due to the inclusion of a few cases with malignancies in the present series, known to have a lower mean PCV-S on the basis of a previous work (14). Thus, the present material, although small in number, seems to be a representative one and suitable for the present purpose.

The results of the present work indicate that there is a relationship between the endothelial changes and the distribution pattern of the endothelial IgG. In PCV:s with high cuboidal endothelium, IgG seems to localize mainly on the luminal surface of the cells, compared with the basement membrane site of localization in PCV:s with flattened endothelium. In the PCV:s with low cuboidal endothelium, the most common type of IgG localization is the intraendothelial one. Thus, the changes in the structure of PCV endothelium, previously found to be correlated with the activity of T-cell population (12, 13, 14) seem to be associated with changes in the localization of endothelial IgG. This observation could favor the concept that this endothelial IgG is involved in the regulation of T-cell passage through the PCV endothelium. The sequence of events in this passage procedure can be hypothesized as follows: In the PCV:s with high cuboidal endothelium, normally encountered in the nodes with active T-cell recirculation, the IgG on the luminal surface of the endothelium serves as a detector for the T-lymphocyte surface antigens (theta for example) thus recognizing these cells from the blood flow. A loose, reversible binding of these antigens to the endothelial IgG could happen facilitating the movement of these lymphocytes through the endothelium between two adjacent endothelial cells. In fact, there is ultrastructural evidence for such an intimate contact between the endothelial cells and the traversing lymphocytes (1) furnished by a fusion of the glycocalyces of these two cells. This binding undoubtedly will be broken at the moment the lymphocyte penetrates the basement membrane thus leaving the IgG at the basement membrane site of the endothelium, probably for use anew by the endothelial cells. The intraendothelial localization (Type II) of IgG could represent the stage where IgG is moving to ward the cell surface. The accumulation of all IgG on the basement membrane site of the flattened endothelium is associated with intracytoplasmic changes of the endothelial cells, as has been observed previously (1). In this situation, the traffic of lymphocytes through the endothelium has ceased.

It was also observed that some of the small arteries and arterioles of the lymph nodes gave a faint positive staining reaction for IgG, an observation contradictory to the previous findings (11). This discrepancy is most probably due to the fact that the indirect immunoperoxidase technique used in the present work is much more sensitive than the direct one previously used (11). The significance of such an intimal immunoglobulin found in the renal arterioles has been recently discussed by *Lapenas* et al. (5) who state that this positive reaction for IgG must be due to the antibody trapping rather than specific binding. This concept is supported by the findings of the present study where IgG was observed to be inconstantly present on the intimal surface of the arterioles, only.

It can be concluded on the basis of the present results that the distribution of the endothelial IgG is closely related to the structural changes of the PCV endothelium, known to bear a close relationship to the state of T-lymphocyte recirculation. Futher studies with the refined immunohistochemical techniques at ultrastructural level are indicated to assess the final role of IgG in the recirculation of T-lymphocytes.

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