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# ANTIGEN-INDUCED CHANGES ON HIGH ENDOTHELIAL VENULES IN RAT CERVICAL LYMPH NODES

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*ABSTRACT*

*The effect of antigenic stimulation (paratyphoid vaccine) on high endothelial venules (HEVs) of rat cervical lymph nodes was studied using conventional histological and histometrical techniques. The sequential changes of some morphometric parameters (lymph node weight, HEV diameters) after the antigenic stimulation were studied in rat cervical lymph nodes over a period of five days with daily measurements. Measurements were made on the HEVs in the paracortex especially near the corticomedullary junction. HEVs diameters began to increase two days after the antigenic stimulation ($p<0.001$) and erythrocytes were increased in the lumen of the HEVs. On day four, most of the lymphocytes were detected between the endothelial cells of the HEVs ($p<0.001$). On day five, not only HEVs were increased in number but also endothelial cells were increased in height ($p<0.001$). The weight of the lymph nodes was also highest on this day. The changes of HEVs determined after antigenic stimulation suggest that these specialized vessels have an important regulatory role in the primary immune response.*

Lymphocyte migration from the bloodstream into lymph nodes in most species occurs across postcapillary high endothelial venules (HEVs) (1). Migration of lymphocytes into the lymph nodes is directly controlled by interaction of lymphocytes with the HEVs, located in T cell-dependent areas (paracortex) of the lymph nodes (2).

Since HEVs function as a gate of entry for lymphocytes from the peripheral blood to the lymph node parenchyma, they play a major role in the recirculation, distribution, and homing of lymphocytes in different lymphoid organs (3). HEV endothelial cells (HECs) express on their surface specific lymphocyte binding molecules known as addressins, which allow lymphocytes to bind to the endothelium as the first step of migration into the tissue (4).

Lymph node histology is highly variable and dependent on the different kinds of antigenic challenges (5). In stimulated lymph nodes, activity and the kinetics of HECs may vary due to entry of lymphocytes (3) and during immune response in HEVs (6). Analysis of some observations suggests that recruitment of lymphocytes by HECs for the sake of participating in local specific immune activities is antigen specific, despite the implication of homing receptors of lymphocytes (7).

In this study, we histologically examined the changes of HEVs in the cervical lymph nodes of rats during a primary immune response.

*MATERIALS AND METHODS*

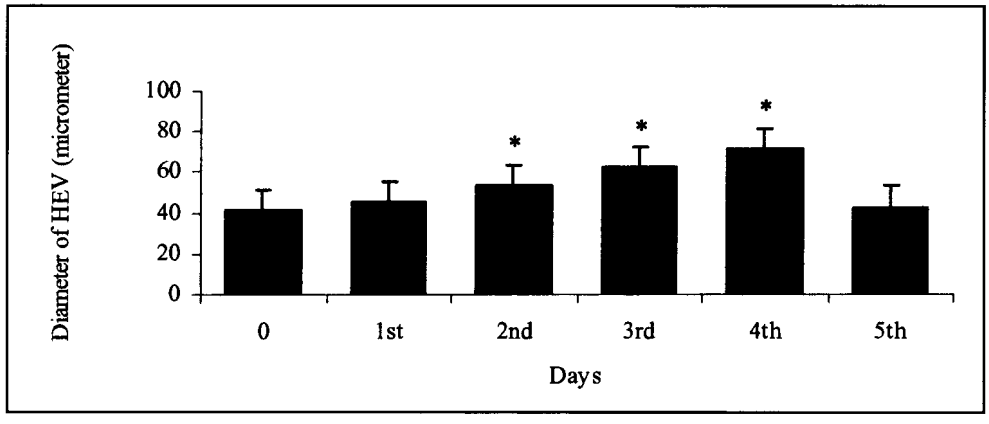


*Fig. 1. Changes in HEV diameter after antigenic stimulation. *: p<0.001.*

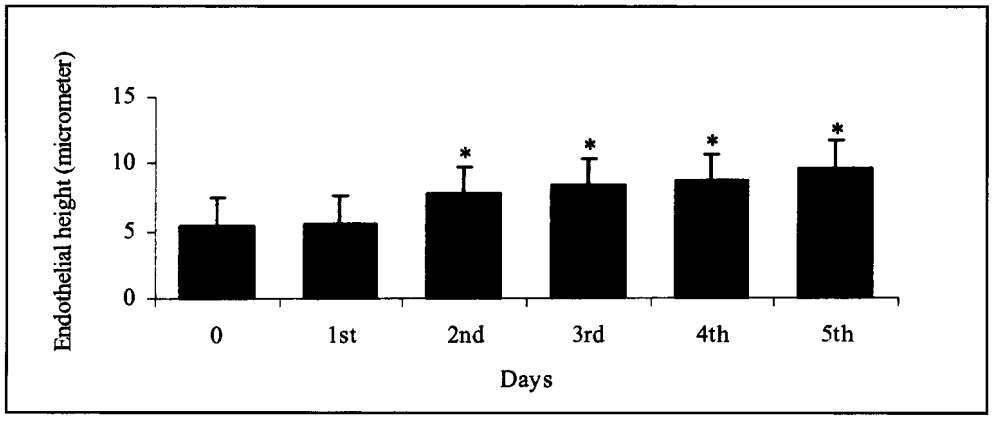


*Fig. 2. The endothelial height of HEV after antigenic stimulation. *: p<0.001.*

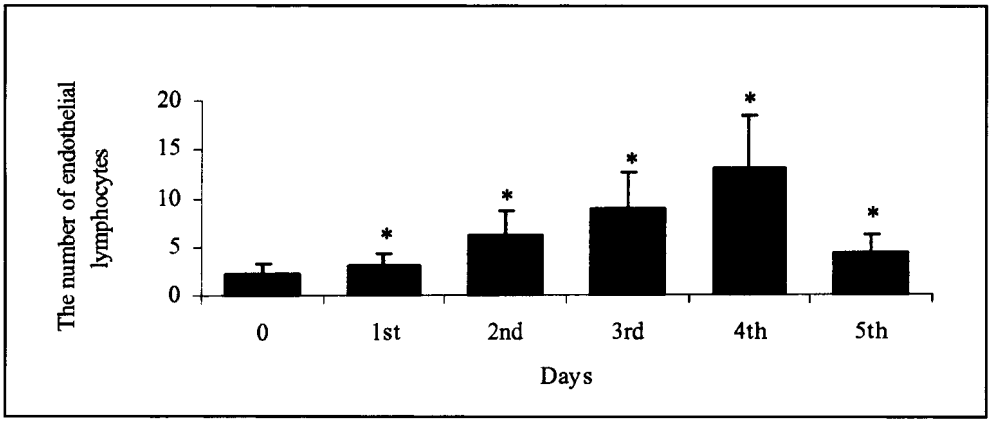


*Fig. 3. The number of migrating lymphocytes according to days after antigenic stimulation. *: p<0.001.*

Twenty Wistar rats of both sexes weighing 200-250 g were used. Twelve were selected as "stimulated" group and received paratyphoid vaccine (0.1ml) (Refik Saydam Protection Institute, Ankara-Turkey) subcutaneously through the cervical region. The rest were used as control (0 day) and given saline (0.1ml). In both control and stimulated rats, lymph nodes of the superficial cervical region were removed and weighed on 1st, 2nd, 3rd, 4th and 5th day. The lymph nodes from both groups were processed for routine paraffin and electron microscopy. Paraffin sections (4, 6, or 8 μm) were stained using hematoxylin and eosin (H&E). Semi-thin (1 μm) sections were stained with Toluidine Blue-Azur II (8).

Evaluation was performed on HEV cross sections near the corticomedullary junction. 300 different HEV sections from a total of 36 superficial cervical lymph nodes were examined by histometrical methods. Measurements were performed with an ocular micrometer (Leitz Wetzlar, periplan 6.3 xm). Diameters of venules seen in cross-section were determined by measuring from the endothelial basal membrane (BM) (9). In evaluating lymphocyte number in HEV sections, only the class 2 (endothelial) lymphocytes were taken into consideration (10). Statistical analyses were performed by means of calculation program SPSS/PC. The data were subjected to analysis of Student's *t* test. Histological sections were photographed by a Carl Zeiss photomicroscope.

## *RESULTS*

The mean weight of lymph nodes were as follows: 10.9, 13.8, 14.3, 17.2, 18.3, and 21.1 mg on 0, 1st, 2nd, 3rd, 4th, and 5th days, respectively.

The changes in diameter and endothelial height of HEVs, and migrating lymphocyte number obtained by examination of 300 HEV cross sections in the total of 36 superficial cervical lymph nodes are presented in *Figs. 1-3*, respectively.

HEVs were easily identified in the paracortex with their unique stuffed endothelial cells *(Figs. 4-6)*. The nuclei of endothelial cells were open, ovoid and large *(Figs. 4a,6a)*. The cells lay on a thick, densely eosinophilic BM *(Fig. 4b,4c)*. However, after stimulation, BM was discontinuous because of migrating lymphocytes *(Fig. 4d)*. After the antigenic stimulation, HEVs were larger and there were more lymphocytes between the HEV endothelial cells when compared with the controls *(Figs. 4d,6b)*.

On the first day of the antigenic stimulation, measurements of the HEV diameters and the height of the endothelial cells were similar to the control group ($p>0.05$) *(Fig. 6a)*. On the second day of antigenic stimulation, the paracortex was enlarged and the boundary between cortex and paracortex disappeared. When compared with the control group, the increases of the parameters on the third and fourth days were evident ($p<0.001$) *(Figs. 1-3)*. On the fourth day, the largest HEV diameters were measured, and most lymphocytes were between the endothelial cells on transverse sections *(Fig. 4d)*. On the fifth day of antigenic stimulation, an increase in the number of the HEVs was identified; however, there were fewer lymphocytes between the HECs. The highest length of the HECs also occurred on the fifth day after stimulation *(Figs. 5,6c)*.

After the second day of antigenic stimulation, the number of erythrocytes also increased in the HEV lumens *(Figs. 4c,4d,6b)*. Moreover, capillaries and other blood vessels were hyperemic. In the cortex of stimulated lymph nodes, some blood vessels had HECs but considerably smaller diameter than HEV *(Fig. 6d)*.

## *DISCUSSION*

It was first demonstrated by Gowans et al (11) in a rat model that lymphocytes selectively emigrate from the blood circulation into lymph nodes through specialized postcapillary venules in the paracortex. These specialized vessels termed HEVs were

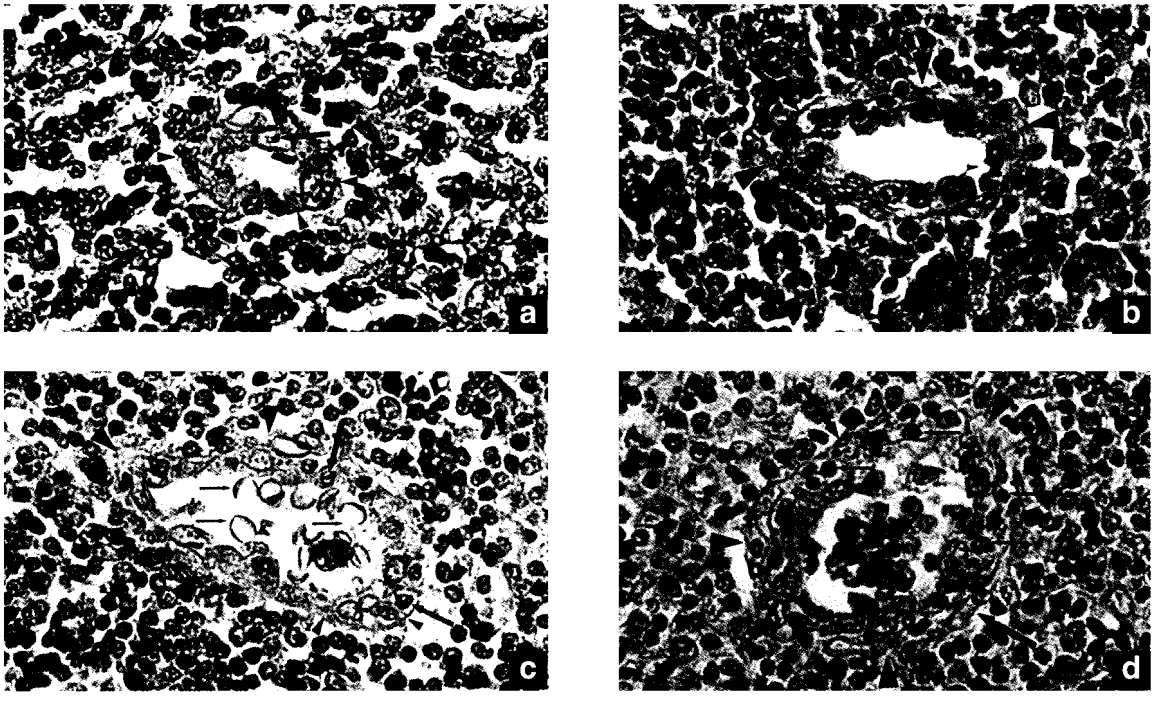


*Fig. 4. Several HEV cross sections near the corticomedullary junction in the paracortex of cervical lymph nodes. a) HEV (arrows) cross section of control lymph node. Nuclei of HEC (arrowhead). b) 2 days after stimulation, HEV (arrowheads) cross section, BM (arrow) is distinct. c) 3 days after stimulation, HEV (arrowheads) cross section. The erythrocytes (small arrow) in the HEV lumen. BM (small arrowhead) and migrating lymphocytes (arrows). d) 4 days after stimulation, HEV (arrowheads) cross section. BM (large arrow). Several lymphocytes (small arrows) can be seen in various stages of progress through the wall between the HECs. H&E x240*

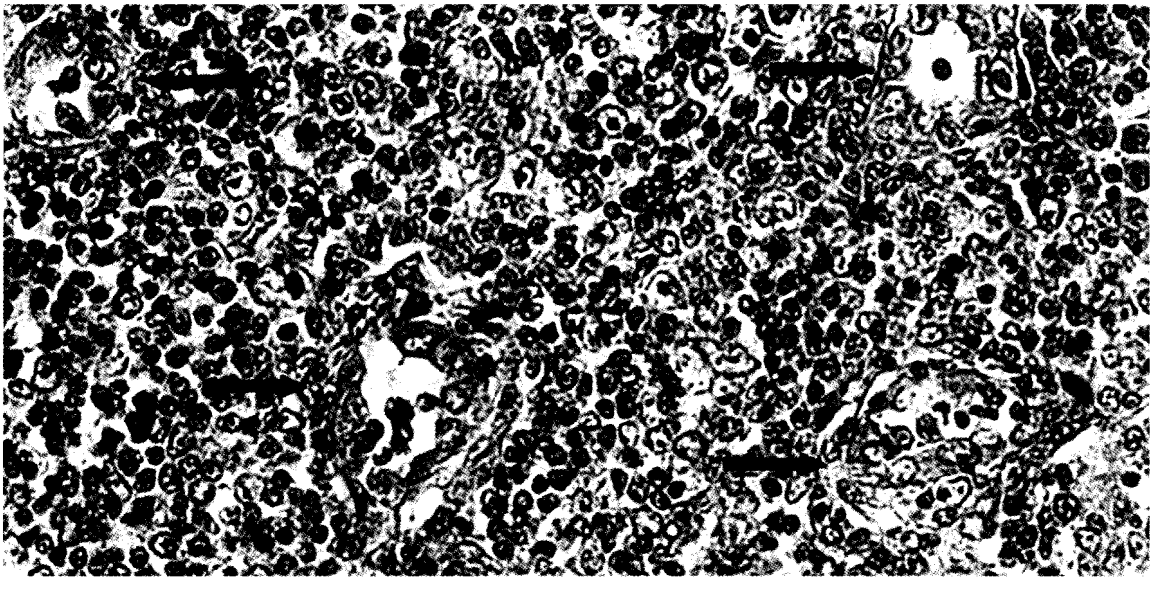

*Fig. 5. Five days after stimulation, HEV cross sections near the corticomedullary junction in the paracortex of cervical lymph nodes (arrows). H&E x240.*

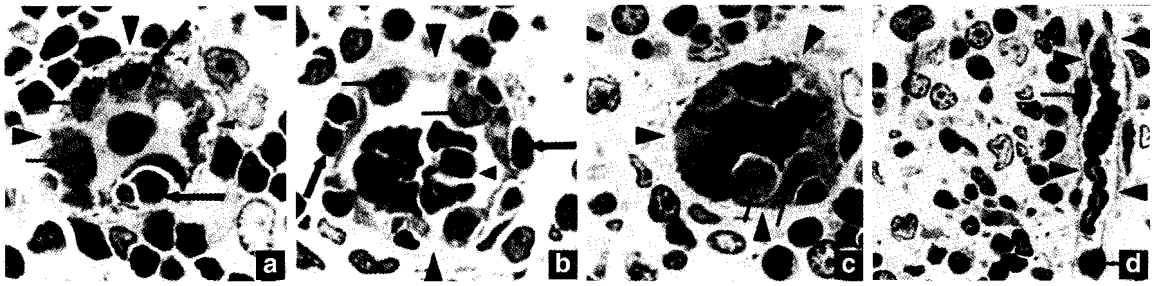


*Fig. 6. Semi-thin sections of HEVs. a) 1 day after stimulation, HEV (arrowheads) in paracortex. BM (small arrowhead) is distinct. HECs (small arrows) and migrating lymphocytes (arrows). Nuclei of HEC (n). b) 3 days after stimulation, HEV (arrowheads) in paracortex. Several erythrocytes (small arrowhead) in the HEV lumen. HECs (small arrows) and migrating lymphocytes (arrows). c) 5 days after stimulation, HEV (arrowheads) in paracortex. HECs (arrows). d) 5 days after stimulation, a blood vessel (arrowheads) which has HECs (arrows) is apparently smaller than HEVs in cortex. Toluidine Blue-Azure II x480.*

examined in various species such as human, rabbit, rat, mouse and calf (12-15). While human and rodent HEVs share most characteristics, in several aspects they differ (12-14). In the present study, the light microscopic appearances of the paracortical HEVs were similar to the earlier descriptions.

Twisk et al (2) reported that after bacterial lipopolysaccharide administration, neither size, localization nor receptor specificity for lymphocyte subsets of HEV differed from control HEV. On the contrary, some changes of HEV size and endothelial cell height occurring after antigenic stimulation were recognized by several researchers (6,16). Csanaky et al (16) concluded that dilatation of HEVs occurred on day 2 and the endothelial cell height of HEVs increased on days 4 and 5 after antigenic stimulation in rat lymph nodes. Herman et al (6) also reported that there was a significant increase in diameter and density of the subcapsular and medullary cord capillaries beginning on the second day and reaching its peak on the fifth day. In the present study, we also demonstrated that HEVs were dilated on days 2 to 4, and endothelial cell height of HEVs was increased on days 2 to 5 after antigenic stimulation. The changes of the endothelium size of postcapillary venules in lymphatic tissue are due to the migrating lymphocytes (15).

The HEV diameters near the medullary area were determined to be larger, which is in harmony with the findings of others (6,12,14,17). It was reported that the size of HEVs was 10-50 µm and the height of HECs was 5-7 µm in peripheral lymph nodes of the unstimulated mouse (9). In unstimulated rat lymph nodes, the diameter of HEVs found was 30-40 µm (12). These findings paralleled those of the HEV diameter and height of HEC in the control group determined as average 41.7 µm and 5.45 µm, respectively. Herman et al (6) reported that HEV size reached up to 150 µm near the cortico-medullary junction in the rabbit popliteal lymph node on the third day after antigenic stimulation. In the present study, the highest diameter of HEV was determined as 80 µm on the fourth day after antigenic stimulation.

The HEVs of the lymph nodes are sites for transvascular lymphocyte trafficking. Features typical of the HEC are stimulated to emerge by antigens and the proper lymphocytes and mediators (7). Following antigenic stimulation, the enlargements of the HEV could result in an increased entry of lymphocytes into the lymph node (6). In the present study, most migrating lymphocytes were determined on day four after antigenic stimulation at which time the largest HEV diameter was found. In addition, erythrocytes

in the HEV lumen were increased after the second day of antigenic stimulation. The migration of lymphocytes is directly influenced by increased blood flow to lymph nodes after antigenic stimulation (18,19). The subendothelial spaces of HECs are sites of interactions between drained lymphocytes, HECs and recruited blood lymphocytes (7).

It has been proposed that during the primary immune response, the early phase of increased blood flow was due to hyperemia, whereas a later phase had a significant angiogenesis component (18). Using micro-angiographic techniques, a redistribution and increase in diameter and density of lymph node capillaries have been described as well as the opening of direct arteriovenous shunts (6,12). During the capillary redistribution phase of the primary immune response, a redistribution of HEVs also occurs. The redistribution of the capillaries and HEVs throughout the cortex of the lymph node facilitates increased interchange between the lymphatic tissue and the circulating blood (6). Both the increased blood flow and the shunting shortly after antigenic stimulus is important in the evolution of the immune response (20). In this study, increased number of HEVs on day five after antigenic stimulation suggest that a redistribution phase occurred, which likely plays a pivotal regulative role in the immune response.

In conclusion, the changes of HEVs determined after antigenic stimulation favor that these specialized vessels play an important regulatory role in the primary immune response.

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