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MESENTERIC LYMPH NODE TRANSPLANTATION IN SYNGENEIC RATS: CHANGES IN CELLULARITY AND ARCHITECTURE

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ABSTRACT

The cellular architectural and functional changes of transplanted mesenteric lymph nodes in rats were studied. After lymph nodes were transplanted with interruption of both the afferent and efferent lymphatics, the nodal cellular content gradually became depleted. One month after operation, the recirculating lymphocyte count in the transplanted node was only 3.5% of that in control nodes, whereas the number of mononuclear cells per mg tissue of transplanted node was only 28% of normal. In the transplanted nodal paracortex, the cells of high endothelial venules (HEV) became less prominent and gradually flattened altogether. In the transplanted nodal cortex, germinal centers and follicles also sharply decreased and later disappeared. Three months after transplantation, the normal compartmentalization of the transplanted nodes were no longer distinguishable. Regeneration of afferent lymphatics was not detected in transplanted nodes and the lack of circulating antigen and stimulated lymphocytes was probably responsible for the histological and functional involution of grafted mesenteric lymph nodes.

Excision of lymph nodes may alter lymph flow patterns, change local immunological defense and promote lymph stasis. Lymph node transplantation may restore lymphatic system function. During the past 60 years, many attempts have been made to transplant lymph nodes (1-8). Development of microsurgery in recent years, however, made transplantation of lymph nodes more technically feasible. Nonetheless, histological and functional changes of transplanted nodes are poorly documented. The first vascularized lymph node transplantation was reported by Schesol et al (3). After extirpation of hindlimb popliteal nodes in rats, epigastric nodes were transplanted into the now empty popliteal fossa. In another report (6) rat epigastric nodes were transplanted into the axilla after previous axillary nodal excision. In still another study (5), autologous inguinal nodes were transplanted into the popliteal space in dogs with experimental-induced lymphedema. In these studies, lymphatic functional restoration was documented radiographically. In contrast, Futrell and Rust (4) after transplanting rat popliteal nodes autogenously into variously sites failed to show functional restoration although the nodal grafts were histologically "viable."

The purpose of this study was to investigate the cellular, histological and functional changes of transplanted mesenteric lymph nodes in which both the afferent and efferent lymphatics were severed.

MATERIALS AND METHODS

Thirty-two syngeneic Wistar male rats weighing 150-200g were used. The transplantation procedure was as described before (7). In brief, mesenteric lymph nodes were isolated from the donor rat. The graft included an inferior portion of the pancreas with aortic segment, portal vein, adjacent mesenteric lymph nodes and surrounding fat. The whole "lymph node graft" was transferred to the recipient rat through a longitudinal incision near the right lumbar muscle. End-to-side anastomoses were performed between the grafted aortic segment and host aorta, and between the grafted portal vein and host inferior vena cava. The graft was placed subcutaneously through an orifice in the lateral abdominal musculature.

The grafts were examined at successive intervals at 1-6 months after transplantation. Regeneration of lymphatics was tested by 1) phagocytosis after injection of 1:10 India ink in the anticipated lymph drainage region 12 hours before sacrifice, and 2) the dye test of patent blue injection into the anticipated lymph drainage region and into the graft just before sacrifice. Thereafter, the transplanted nodes were prepared for light microscopy.

In addition, cytospin preparations were made from cell suspensions of 3 operated rats one month after transplantation and 3 non-operated rat lymph nodes (control). The number of mononuclear cells per mg tissue in transplanted node and host node were compared. Recirculating lymphocytes in the transplanted node were measured by ^{51}Cr trapping in 5 rats (9). In brief, lymphocytes of mesenteric lymph nodes were isolated from syngeneic rats, labeled with ^{51}Cr (50Mci for $1x10^6$ cells) and injected intravenously into the transplanted rats. Eighteen hrs later, spleen, liver, peripheral lymph nodes and grafted nodes were removed and radioactivity tested using a gamma counter (Workman, USA) according to the formula:

$$\frac{\text{organ radioactivity/radioactivity injected}}{\text{weight of the organ (gm)}} \times 100$$

Just before sacrifice when the transplanted grafts were prepared for cellular and histological examination, the vascular anastomoses were demonstrated to be patent with vigorous blood perfusion.

RESULTS

Normal Lymph Nodes

In these nodes three topographical areas were distinguished by shape and vascular pattern: an outer cortex, an inner layer or paracortex, and medulla (10). The parenchyma was traversed by an intermediate sinus and was surrounded by the marginal and medullary sinus. Typically, there were many follicles and germinal centers in the paracortex of the rat mesenteric lymph node. The characteristic postcapillary high endothelial venules (HEV) in the paracortex displayed many migrating lymphocytes between these endothelial cells. The lymph node consisted of both lymphocytes and non-lymphoid elements. The latter were comprised of macrophages, reticulum cells and cells of the blood microvascular wall (10).

Transplanted Nodes

No regenerated afferent lymphatics were identified using the patent blue dye tests and no India ink was taken up by the transplanted nodes. An efferent lymphatic was identified after injection of patent blue into the graft itself in two instances and it was connected to adjacent recipient lymphatics. In 30 of 32, the grafts were integrated without obvious absorption of surrounding fat and connective tissue. However, the grafted lymph nodes were smaller than normal counterparts at one month. With time the size of the transplanted nodes continued to decrease. Six months after transplantation, the nodes grossly were tiny, yellow, fragile nodules.

Light microscopy showed striking changes of both architecture and cellular content in the transplanted nodes. The plump cells characteristic of HEV became

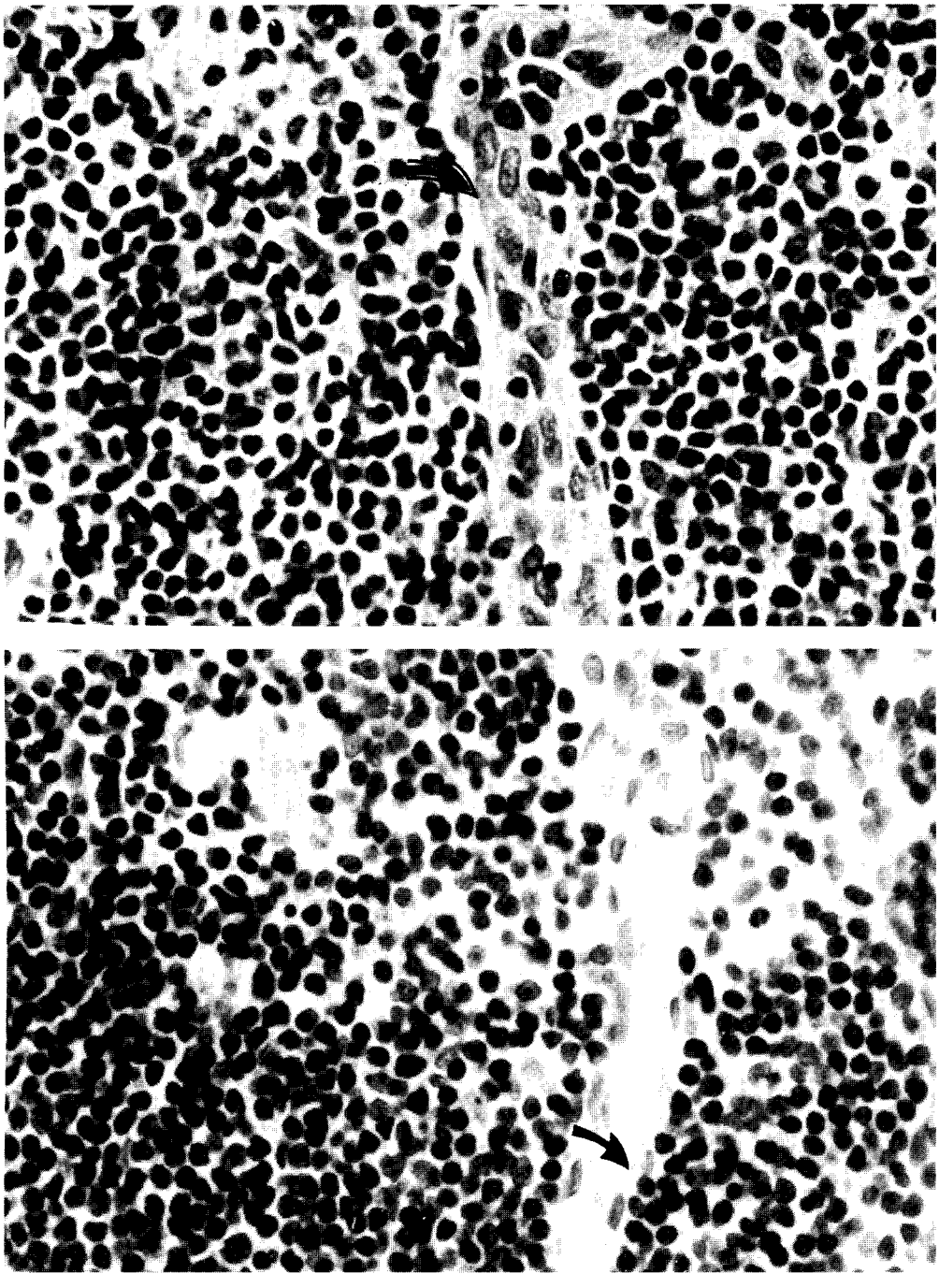

Fig. 1. A: Normal (non-transplanted) mesenteric lymph node showing abundant lymphocytes between venular high endothelial cells (arrow). B: Lymph node one month after transplantation. Few lymphocytes are seen migrating into now flattened venular endothelial cells (arrow). H.E. x400.

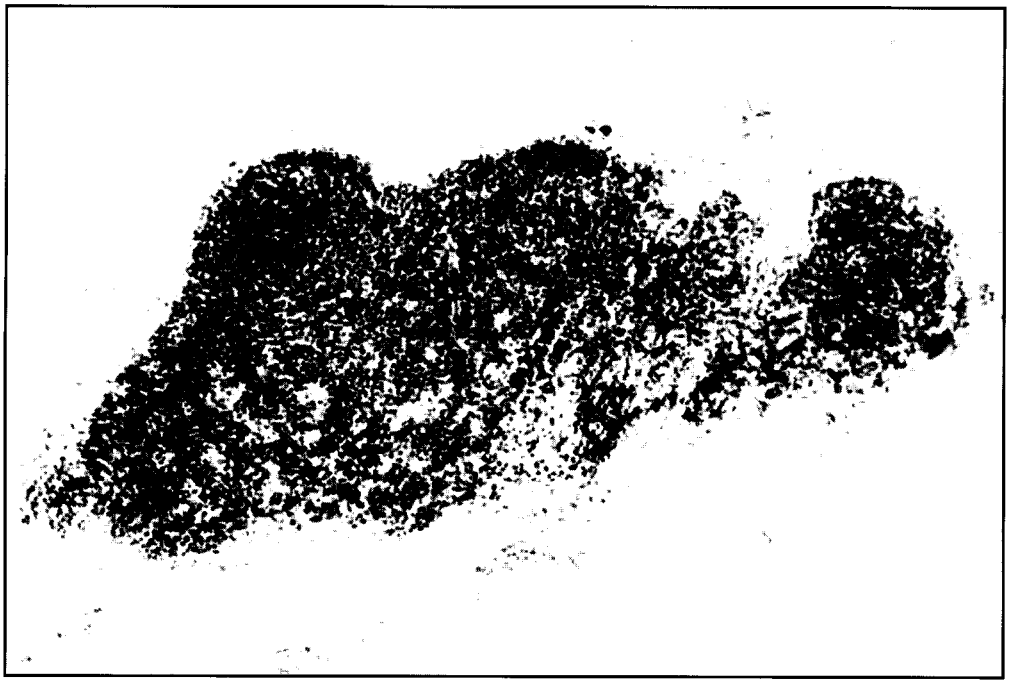

Fig. 2. Lymph nodes six months after transplantation. The volume of the lymph node is reduced and the distinction between cortex and medulla are no longer apparent. H.E. x100.

less prominent and by one month, fewer migrating lymphocytes passed through its wall. At three months, the HEV was hardly detectable *(Fig. 1)*.

In normal mesenteric lymph nodes, prominent germinal follicles indicative of antigenic stimulation and immunological activity were apparent. Early after nodal transplantation, remnants of germinal centers and follicles were still present. By $1^1/_2$ months only an occasional follicle was seen and at 3 months, none were detected. At one month, the transplanted lymph node contained only 28% of the normal number of mononuclear cells per mg tissue *(Table 1)*, and "circulating" lymphocytes in the grafted node were only 3.5% of normal *(Table 2)*.

After three months, it was difficult to distinguish lymph nodal compartmentalization, and by six months, there were evenly distributed small lymphocytes with rare macrophages and histocytes *(Fig. 2)*.

DISCUSSION

Lymph nodes have a dual vascular system, namely, blood and lymph vessels. Normally, circulating antigens, macrophages and Langerhans cells enter the lymph node via afferent lymphatics. Recirculating lymphocytes and monocytes pass from the bloodstream into lymph nodes through high endothelial venules (10-12). The immunogenic content of the afferent lymph stimulates compartmentalization in the lymph node (13).

In this study, syngeneic transplantation of mesenteric lymph nodes with interruption of both the afferent and efferent lymphatics brought about progressive involution of the nodes. The number of lymphoid and non-lymphoid cells progressively decreased. Cells of HEV became less prominent and

TABLE 1
Total Nucleated Cell Number/mg Tissue of Mesenteric Lymph Node ($x10^6$)

Rat	Transplanted Node	Host Node
8	144	484
2	119	453
2	64	205
mean	109	380
SD	40.9	152.9

TABLE 2
^{51}Cr Radioactivity in Mesenteric Lymph Node (%)

Rat	Transplanted Node	Host Node
1	0.29	10.2
2	0.08	12.2
3	0.1	16.6
4	1.8	23.8
5	0.6	18.8
mean	0.57	16.4
SD	0.7	5.3
$p<0.01$		

eventually flattened altogether. The number of lymphocytes in transplanted nodes became notably less than that in control nodes. Germinal centers and follicles disappeared indicating that the transplanted nodes lost immunologic function.

The histologic changes of these transplanted lymph nodes are similar to the alterations that take place in a "deafferented" lymph node (10,11). Thus, ligating the afferent lymphatic results in a reduced number of macrophages and interdigitating cells, a progressive decrease in lymphoid cells and germinal centers and degeneration and eventual disappearance of high endothelial venules.

Neither phagocytosis nor the patent blue dye test revealed regeneration of afferent lymphatics in the transplanted nodes. Failure of afferent lymphatic reconstitution was likely responsible for the structural and functional deterioration in the grafted nodes. Under physiological conditions, macrophages and antigens supply lymph nodes via afferent lymphatics and the bulk of recirculating lymphocytes enter lymph nodes through the HEV (10). The latter phenomenon probably depends on the presence of antigen and chemotactic factors produced by stimulated lymphocytes (10-12). Failure of afferent lymphatic regeneration blocks the influx of macrophages, lymphocytes and antigen into the lymph node. Deprivation of antigen and perhaps lack of other factors including non-lymphoid cells that normally arrive through afferent lymphatics, in turn, extinguishes immunologic activity (9). Moreover, exclusion of antigen and chemotactic factors (10) result in changes and disappearance of HEV, thereby decreasing the migration of lymphocytes and monocytes into transplanted nodes. Progressively depleted of its cellular content, the normal architecture of the transplanted node disappears. Accordingly, reestablishment of afferent lymphatic inflow is critical both for restoration of nodal architecture and function of transplanted lymph nodes.

Thus far, only one report (8) has shown histologic regeneration of afferent lymphatic, i.e., reappearance of germinal centers after antigenic stimulation of an implanted lymph node slice. In other studies (3,5,6), restoration of transplanted nodal function was displayed by lymphoscintigraphy, isotope scanning and conventional lymphography. In these studies, however, lymph nodes were transplanted as a free flap or island flap with fat, connective tissue, subcutis and skin attached to the nodes. In these examples, the afferent lymphatics were probably included in the nodal graft without interruption.

The reason for failure of regeneration of afferent lymphatics after syngeneic lymph nodal transplantation is unknown, but before this technique is clinically applicable,

this deficiency will need to be remedied.

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