Qualitative and Quantitative Morphologic Study of Peyer's Patches of the Mouse after Neonatal Thymectomy and Hydrocortisone Injection [1]

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ABSTRACT Peyer's patches in normal adult mice, neonatally thymectomized mice and mice injected with hydrocortisone were studied qualitatively and quantitatively by light microscopy. The patch was divided into germinal center, follicular area, parafollicular area and dome area. In normal mice, the volumetric ratio of the germinal center to the entire patch was 30.9%; that of the follicular area, 33.3%; that of the parafollicular area, 27.7%; and that of the dome area, 8.2%. Thymus-dependent small lymphocytes were 40% of small lymphocytes in the patch. Out of the total thymus-dependent small lymphocytes in the patch, 13% were included in the germinal center; 19%, in the follicular area; 62%, in the parafollicular area; and 6%, in the dome area. Hydrocortisone-sensitive small lymphocytes were 65% of the total small lymphocytes in the patch, the germinal center contained 9%; the follicular area, 84%; the parafollicular area, 2%; and the dome area, 5%. The epithelium over the dome area was invaded by numerous small lymphocytes. Forty-eight percent of lymphocytes within the epithelium over the dome were thymus-dependent and 67% were hydrocortisone-sensitive.

It is concluded that Peyer's patch may be considered as a peripheral lymphatic tissue, functionally as well as morphologically.

At present lymphocytes are generally divided into two major lines, thymus dependent (T) lymphocytes and bursa-derived (B) lymphocytes. The thymus and the avian bursa of Fabricius have been considered as central lymphatic tissues providing an epithelial microenvironment for uncommitted stem cells to differentiate into T- or B-lymphocytes that are seeded into peripheral lymphatic tissues (Elves, '72; Greaves et al., '73). In mammals, although the bursa does not exist, the existence of the bursa-equivalent has been suggested and some of the gut-associated lymphatic tissues have been proposed as bursa-equivalent (Cooper et al., '66; Fichtelius, '66; Craig and Cebra, '75).

In this relation, Peyer's patches of the mouse have recently attracted much attention and have been morphologically and functionally studied in detail (Faulk et al., '71; Sobhon, '71; Levin et al., '73; Waksman et al., '73), because they represent a major gut-associated lymphatic tissue (Abe and Ito, '77). However, it remains uncertain whether the Peyer's patch is bursa-equivalent or peripheral lymphatic tissue (Evans et al., '67; Levin et al., '73; Friedberg and Weissman, '74). In a previous paper, we reported that the Peyer's patch is histologically divisible into four areas (Abe and Ito, '77). In peripheral lymphatic tissues, T- and B-lymphocytes are generally known to populate different areas, thymus-dependent and thymus-independent (Parrott and De Sousa, '71). Following neonatal thymectomy, the thymus-dependent areas are depleted of lymphocytes and the thymus-independent areas remain almost unchanged (Parrott et al., '66). The two types of lymphocytes are different in susceptibility to corticosteroids; T-lymphocytes are relatively resistant and B-lymphocytes are sensitive to hydrocortisone (Esteban, '68; Greaves et al., '73). Therefore, it would be possible to remove either T-lymphocytes or B-lymphocytes from lymphatic tissues following neonatal thymec-

Accepted September 8, '77.
[1] This study was supported by grants from the Japanese Ministry of Education, 1976 (Nos. 112114, 167001).

tomy or administration of hydrocortisone, respectively. By means of stereological methods (Weibel, '69; Ito and Abe, '76), the number of lymphocytes contained in an area in lymphatic tissue can be obtained from the volume of the area and the density of lymphocytes in the area. It has been suggested that the Peyer's patch contains both T- and B-lymphocytes (Levin et al., '73; Friedberg and Weissman, '74; Müller-Schoop and Good, '75). However, no information is available about the quantity of T- and B-lymphocytes contained in each area of the patch. Therefore, this study was undertaken to estimate the numbers of lymphocytes included in each area of the patch and the numbers of T- and B-lymphocytes in each area from the numbers of small lymphocytes lost after neonatal thymectomy or hydrocortisone injection, respectively.

MATERIALS AND METHODS

Forty-five dd-mice of both sexes were used in this study. Twenty-two mice were thymectomized within 16 hours after birth and killed at 50 to 60 days of age. At autopsy completeness of thymectomy was confirmed. Ten mice received two successive daily subcutaneous injections of 0.5 mg hydrocortisone at 50 to 60 days of age and were killed 24 hours after the second injection. Thirteen mice, 50 to 60 days of age, were used as controls. At autopsy Peyer's patches were removed from three different parts, the upper, middle and lower thirds of the small intestine, after the luminal contents of the intestine had been washed away with the fixing fluid. They were fixed in a mixture of Zenker-formol-acetic acid (18:2:1) for six hours, embedded in paraffin, and cut serially at 5 μm. The sections were stained with periodic acid Schiff (PAS) reagent-hematoxylin-fast green. For quantitative study the following procedures were used.

Volume of the Peyer's patch

The volumetry was performed by means of a point-counting method (Weibel, '69; Ito and Abe, '76). Under light microscopy with an ocular grid which had small squares of unit edge length (125 μm) formed by lines in each of two directions, the numbers *(Pi)* of cross points of the square grid lying on objects to be measured were counted. The point-counting was made for each section at every interval of 100 μm in serial sections through an entire patch. Total number of points counted for one Peyer's patch was 500 to 2,000.

The volume *(V)* of the Peyer's patch was obtained from the total number (ΣPi) of points included in the patch and the volume ($125^2 \times 100\ \mu m^3$) which was represented by one point, as follows:

$$V\ (\mu m^3) = \Sigma Pi \times 125^2 \times 100.$$

For each patch, the volume ratio (V_V) of each area was obtained from the number *(Pi)* of points included in the area and the total number (ΣPi) of points contained in the patch as

$$V_V\ (\%) = \frac{Pi}{\Sigma Pi} \times 100.$$

Population density of small lymphocytes (Ni)

The density in each area of the patch was determined by the number of nuclei of small lymphocytes per unit area (4,500 μm^2).

Number of small lymphocytes

Stereologically if the average size of the nuclei of small lymphocytes is the same in each area, the number of nuclei per unit sectional area is proportional to that per unit volume. Our previous cytometric studies of small lymphocytes indicated that small lymphocytes in each area are the same in nuclear size (Abe et al., '73). Therefore, the relative number of small lymphocytes in each area can be estimated from the volume of each area of the patch and the number of small lymphocytes per unit area. The relative number *(ni)* of small lymphocytes in each area was represented as

$$ni = Ni \times V.$$

The value *(ni)* is proportional to the absolute number of small lymphocytes included in each area. The proportion (N_{ni}) of small lymphocytes included in each area to the total small lymphocytes contained in the patch was also obtained as

$$N_{ni}\ (\%) = \frac{ni}{\Sigma ni} \times 100.$$

Number of intraepithelial small lymphocytes

The number of lymphocytes encountered within the intestinal epithelium over the Peyer's patch was counted for the villi and the domes of the patch. The counting was carried out in the epithelium of a length of 250 μm on the lateral aspect of the domes and villi for each section. For each case, the counts were made in ten sections, so that the value of the total counts represents the number of lymphocytes contained within the epithelium of a length of 2.5 mm.

The statistical differences among the re-

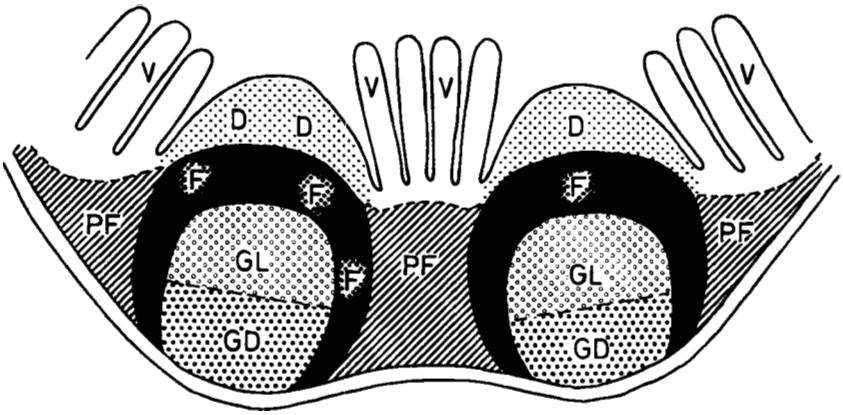

Fig. 1 Schematic diagram of the Peyer's patch. The intestinal lumen is above. The lower part of the patch is bounded by the intestinal muscular coat. GL, light zone of germinal center; GD, dark zone of germinal center; F, follicular area; PF, parafollicular area; D, dome area; V, villi.

sults obtained were evaluated by Student's *t* test.

RESULTS

(1) *Histological observations*

Peyer's patches in the normal mouse

As described in a previous paper (Abe and Ito, '77), the Peyer's patch of the mouse is an aggregation of lymphatic nodules in the intestinal mucosal layer and may be divided histologically into four portions; germinal center, follicular area, parafollicular area and dome area (figs. 1, 2).

The germinal center in the patch is large and consists of light and dark zones as seen in peripheral lymphatic tissues (Abe and Ito, '72, '73). The light zone has an apical pole directed toward the mucosal surface, and its light appearance is due to a relative abundance of reticular cells among which larger lymphocytes are scattered. The dark zone has a basal pole facing toward the muscular coat, and its dark appearance is caused by a dense population of larger lymphocytes. Small lymphocytes are scarcely distributed throughout the germinal center.

The follicular area surrounds the apical pole of the germinal center. This area is packed densely with small lymphocytes.

The parafollicular area surrounds the follicular area. The area is packed less densely with small lymphocytes than the follicular area. Larger lymphocytes and plasma cells are also intermingled among small lymphocytes. In this area there are postcapillary venules with a characteristically high endothelium (fig. 3). The venules join veins just under the muscularis mucosae or just above the muscularis exterior. Lymphatic vessels are seen close to the veins. The mucosal layer above the parafollicular area has villi and crypts.

The dome area is situated just above the follicular area and projects toward the intestinal lumen. The area is populated with lymphocytes, plasma cells and macrophages. Lymphocytes are mainly of small type. The epithelium over the dome area contains many small lymphocytes.

Peyer's patches after neonatal thymectomy

In neonatally thymectomized mice, Peyer's patches were reduced in size, and the parafollicular area showed marked depletion of small lymphocytes (figs. 2, 3). In the parafollicular area plasma cells were often grouped, especially around the veins and lymphatics, and larger lymphocytes were more frequent than those in normal controls. The germinal center was almost unchanged in structure, but it occasionally was smaller in size or lacking. The follicular area was as well developed as seen in normal controls. In some patches, however, the follicular area was depleted of small lymphocytes and the germinal center appeared naked. The dome area remained almost unchanged, but it had occasional accumulations of mature plasma cells. The epithelium over the dome area generally contained fewer lymphocytes than that in normal controls. Particularly in patches where small lymphocytes were significantly depleted from the follicular and parafollicular areas, few or almost no intraepithelial lymphocytes were observed.

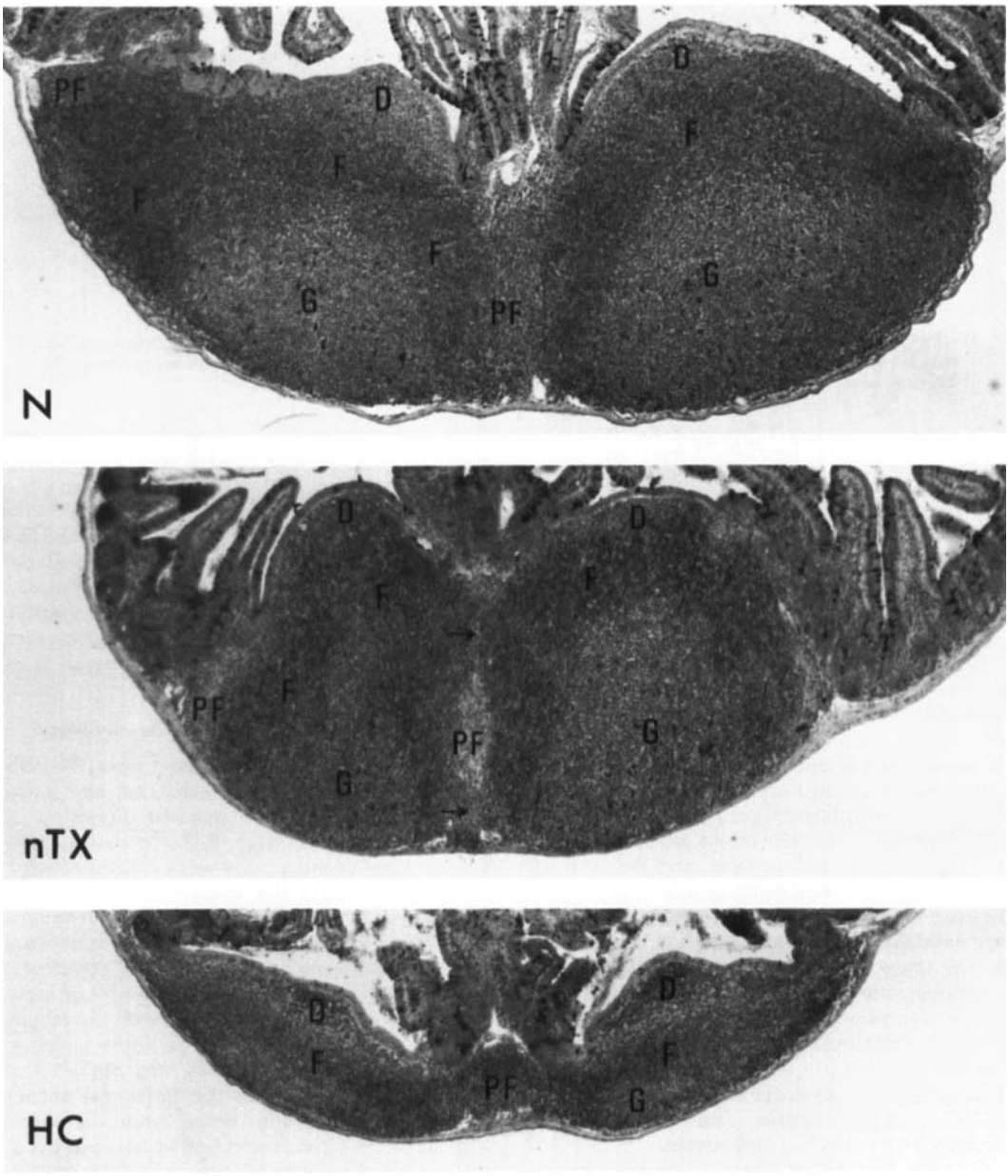

Fig. 2 Cross section of Peyer's patches from normal mouse (N), neonatally thymectomized mouse (nTX) and mouse injected with hydrocortisone (HC). In nTX, the parafollicular area appears to be lighter due to depletion of small lymphocytes. There are accumulations of plasma cells in the parafollicular area (arrows). In HC, the patch is smaller in size and the density of lymphocytes in the parafollicular area seems to be similar to that in the follicular area. G, germinal center; F, follicular area; PF, parafollicular area; D, dome area. × 60.

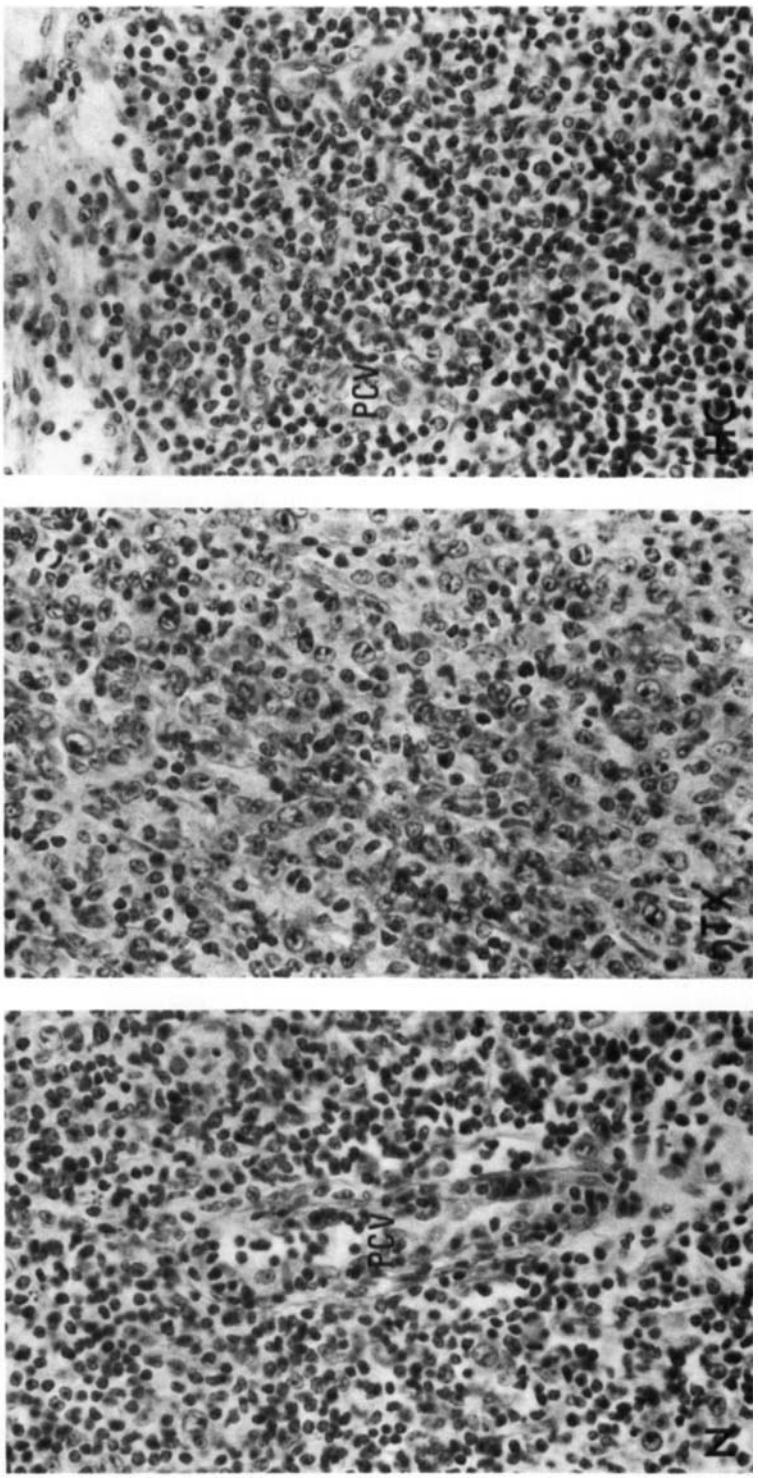

Fig. 3 Parafollicular area of the patch. N, normal mouse; nTX, neonatally thymectomized mouse; HC, mouse injected with hydrocortisone. Note differences in density of small lymphocytes between N, nTX and HC. In nTX, small lymphocytes are markedly depleted and large lymphocytes and plasma cells are prominent. In HC, small lymphocytes populate more densely. PCV, postcapillary venule. × 400.

Peyer's patches after hydrocortisone injection

The Peyer's patch was flattened and thinned in mice injected with hydrocortisone (figs. 2, 3). The germinal center was markedly reduced in size and consisted of only a few immature lymphocytes. The follicular area around the germinal center was less prominent, due to depletion of small lymphocytes. The parafollicular area showed little or almost no changes in size, and the area appeared to be populated more densely with small lymphocytes. The dome area was flattened and did not protrude into the intestinal lumen.

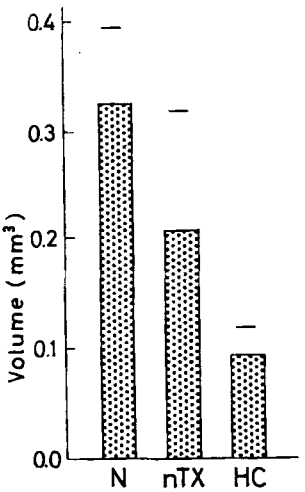

Fig. 4 Volume of a single follicle of the Peyer's patch. N, normal mouse; nTX, neonatally thymectomized mouse; HC, mouse injected with hydrocortisone. Bars represent standard deviations.

(2) *Quantitative observations*

Volume of the Peyer's patch

The volume of the Peyer's patch varies depending on the number of follicles contained. In general the volume of a single follicle in the patch varies in proportion to the average patch volume. The volume of a single follicle of the patch can be obtained from the volume of the patch and the number of the follicles contained. The volume of the patch thus obtained in normal and experimental mice is presented in figure 4. As seen in this figure, the volume was significantly smaller in neonatally thymecitomized mice than in normal ($P < 0.001$) and it was significantly smaller in mice injected with hydrocortisone than in neonatally thymectomized mice ($P < 0.001$).

In normal mice, the volumetric ratio (mean ± S.D.) of the germinal center to the Peyer's patch was 30.9 ± 4.9%; that of the follicular area, 33.3 ± 3.6%; that of the parafollicular area, 27.7 ± 4.4%; and that of the dome area, 8.2 ± 1.6%, respectively (fig. 5). Thus the germinal center, follicular area and parafollicular area were almost similar in volume, and the dome area occupied only a small portion of the patch.

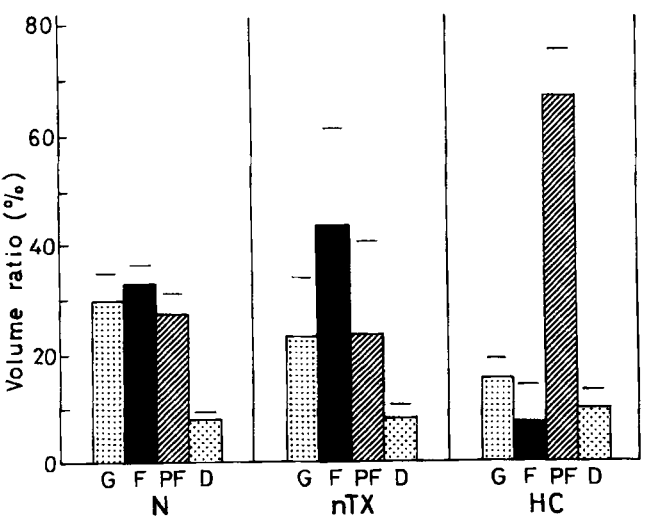

Fig. 5 Volumetric ratio of each area of the patch. N, normal mouse; nTX, neonatally thymectomized mouse; HC, mouse injected with hydrocortisone; G, germinal center; F, follicular area; PF, parafollicular area; D, dome area. Bars represent standard deviations.

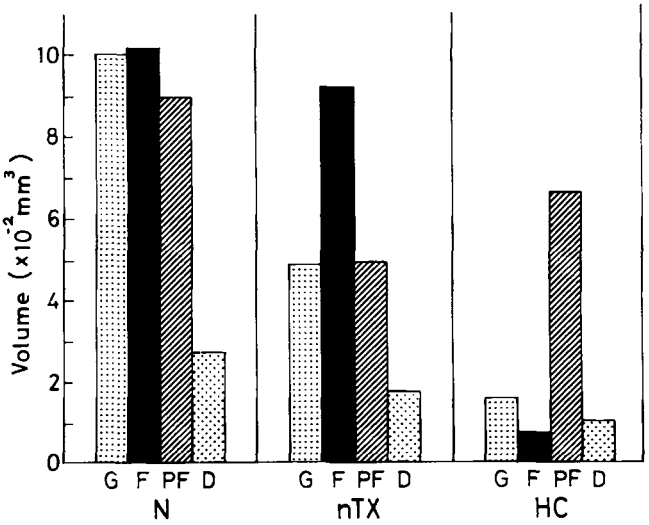

Fig. 6 Volume of each area in a single follicle of the patch. Abbreviations are the same as in figure 5.

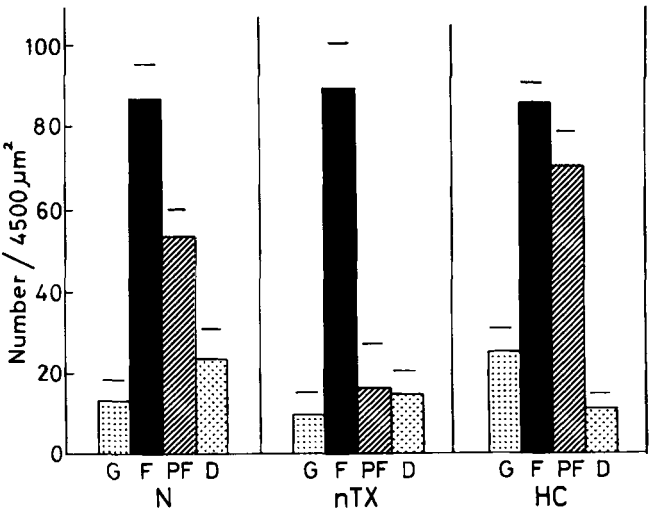

Fig. 7 Population density of small lymphocytes in each area. Abbreviations are the same as in figure 5. Bars represent standard deviations.

As seen in figure 5, either thymectomy or hydrocortisone injection caused changes in the volume ratio of each area. However, changes in the volume ratio do not represent changes in the volume of each area. The volume of each area (fig. 6) could be obtained from the volume of the Peyer's patch (fig. 4) and the volume ratio of each area (fig. 5). In neonatally thymectomized mice, as seen in figure 6, the parafollicular area was reduced in volume to half of that in normal mice. After hydrocortisone injection, the follicular area underwent a significant decrease in volume. The germinal center and dome area were smaller in neonatally thymectomized mice than in normal. They were much smaller in volume in mice injected with hydrocortisone.

Population density of small lymphocytes

In normal mice the population density of small lymphocytes was less in the parafollicular area than in the follicular area (fig. 7). In neonatally thymectomized mice, the density in the parafollicular area was significantly de-

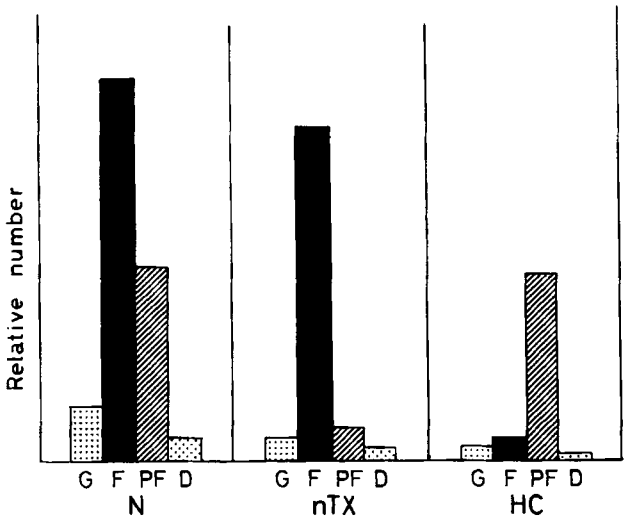

Fig. 8 Relative number of small lymphocytes in each area. Abbreviations are the same as in figure 5.

creased. In particular, the density was very low when the area contained accumulations of plasma cells. The density in the dome area was slightly decreased. In mice treated with hydrocortisone, the density of small lymphocytes in the parafollicular area was increased, but that in the dome area was decreased. Changes in the population density of small lymphocytes after thymectomy and hydrocortisone injection are thought to have resulted from changes in the number of small lymphocytes that will be described below.

Number of small lymphocytes

Relative number of small lymphocytes in the Peyer's patch is presented in figure 8. The number was estimated from the population density of small lymphocytes (fig. 7) and the volume of each area (fig. 6). From the relative number of small lymphocytes in each area (fig. 8) the proportion of small lymphocytes in each area to the total small lymphocytes in the patch could be estimated. In normal mice, of the total small lymphocytes in the patch, 8.4% were contained in the germinal center; 58.2%, in the follicular area; 29.7%, in the parafollicular area; and 3.8%, in the dome area, respectively. Thus the follicular area contained twice as many small lymphocytes as the parafollicular area, and the dome area contained relatively few small lymphocytes.

Figure 8 shows also changes in the number of small lymphocytes in each area after thymectomy and hydrocortisone injection. In neonatally thymectomized mice, the number of small lymphocytes in the parafollicular area was significantly decreased and the numbers in the germinal center and dome area were decreased to about a half of that in normal mice. Small lymphocytes in the follicular area, however, was almost unchanged in number. Out of small lymphocytes remaining in the patch after thymectomy, 84% were contained in the follicular area. In hydrocortisone-injected mice, the numbers of small lymphocytes in the germinal center and dome were less than those in thymectomized mice. Small lymphocytes in the parafollicular area were almost unchanged in number. Out of small lymphocytes remaining in the patch after hydrocortisone injection, 81% were contained in the parafollicular area.

The relative number of small lymphocytes from each area after thymectomy could be obtained from the relative number of small lymphocytes in each area in normal and thymectomized mice (fig. 8). The relative number of small lymphocytes lost from each area after thymectomy represents the relative number of thymus-dependent small lymphocytes included in each area. Thus the proportion of thymus-dependent small lymphocytes in the patch was estimated. In thymectomized mice, 40% of small lymphocytes were lost from the Peyer's patch (fig. 9). Out of the total thymus-dependent lymphocytes in the patch, 13% were included in the germinal center; 19%, in the follicular area; 62%, in the parafollicular area; and 6%, in the dome area; respectively. Thus it is likely that the parafollicular area is

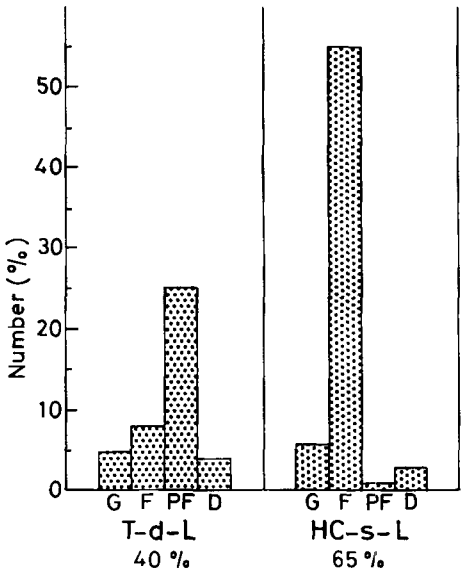

Fig. 9 Estimated proportion of thymus-dependent small lymphocytes (T-d-L) and hydrocortisone-sensitive small lymphocytes (HC-s-L) in each area of the patch. Abbreviations are the same as in figure 5.

populated most densely with thymus-dependent lymphocytes.

The relative number of hydrocortisone-sensitive small lymphocytes in the patch was obtained from the relative number of small lymphocytes in each area in normal and hydrocortisone-injected mice (fig. 8). Hydrocortisone injection caused a marked decrease of small lymphocytes in the patch. The decrease was estimated to be 65% of the total small lymphocytes in the patch (fig. 9). Out of the total hydrocortisone-sensitive lymphocytes in the patch, the germinal center contained 9%; the follicular area, 84%; the parafollicular area, 2%; and the dome area, 5%; respectively. Thus the follicular area is thought to be populated largely with hydrocortisone-sensitive small lymphocytes.

In order to facilitate comparison between the numbers of thymus-dependent and hydrocortisone-sensitive small lymphocytes included in each area, the results are summarized in figure 9. As seen in this figure, the Peyer's patch contained more hydrocortisone-sensitive small lymphocytes than thymus-dependent small lymphocytes. The number of thymus-dependent small lymphocytes in the parafollicular area is about half that of hydrocortisone-sensitive small lymphocytes in the follicular area, and the germinal center and dome area contain almost equal amounts of thymus-dependent small lymphocytes and hydrocortisone-sensitive small lymphocytes.

Number of intraepithelial wandering cells

The epithelium of the patch, particularly that over the dome area, was invaded by numerous wandering cells, most of which were small lymphocytes. In normal mice, about half of the intraepithelial lymphocytes were located above the nuclear level of the epithelial cells (fig. 10). Intraepithelial lymphocytes

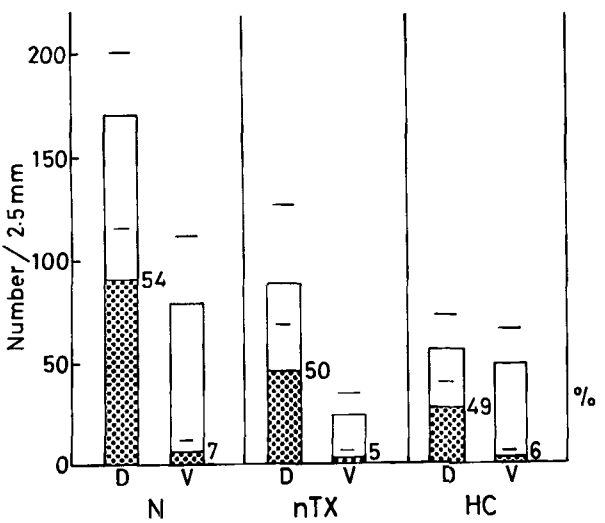

Fig. 10 Numbers of intraepithelial lymphocytes in the dome (D) and villi (V). Dotted bars and percentages represent intraepithelial lymphocytes above the nuclear level of the epithelial cells. N, normal mouse; nTX, neonatally thymectomized mouse; HC, mouse injected with hydrocortisone. Bars represent standard deviations.

over the villi were almost half as numerous as those over the dome area, and they were generally located below the nuclear level of the epithelial cells. In thymectomized mice, intraepithelial lymphocytes over the dome area were almost half as many as those in normal controls. In mice injected with hydrocortisone, the epithelium contained one-third as many lymphocytes as that in normal mice. Intraepithelial lymphocytes over the villi were also decreased markedly in number in both thymectomized and hydrocortisone-injected mice. Thus intraepithelial lymphocytes showed a marked decrease in number following either thymectomy or hydrocortisone injection. In the dome or villi, however, the proportion of intraepithelial lymphocytes located above the nuclear level to those below the nuclear level of the epithelial cells was almost unchanged in thymectomized and hydrocortisone-injected mice. From these data it could be estimated that 48% of lymphocytes within the epithelium over the dome were thymus-dependent and 68% were hydrocortisone-sensitive and that 70% of lymphocytes within the villous epithelium were thymus-dependent and 40% were hydrocortisone-sensitive. Thus the proportion of thymus-dependent to hydrocortisone-sensitive lymphocytes within the epithelium was almost the same as in the whole patch.

DISCUSSION

In this paper, T- and B-lymphocyte compartments in the Peyer's patch were demonstrated quantitatively. The results showed that the parafollicular area in the patch can be regarded as representing the thymus-dependent area populated largely with T-lymphocytes, and that the follicular area is the thymus-independent area consisting largely of B-lymphocytes. Such a distribution pattern of T- and B-lymphocytes in the Peyer's patch has been demonstrated qualitatively by earlier investigators (Friedberg and Weissman, '74). The parafollicular area contains postcapillary venules with characteristically high endothelium such as seen in the thymus-dependent area in lymph nodes (Anderson et al., '76). T-lymphocytes are thought to enter the patch via the postcapillary venules and belong to a recirculating pool of lymphocytes (Gowans and Knight, '64; Perey and Milne, '75). The follicular area is generally well developed in the gut-associated lymphatic tissue. In the rabbit, the appendix, that is regarded as a major gut-associated lymphatic tissue, has been considered as bursa-equivalent in nature (Cooper et al., '66; Waksman et al., '73). The appendix consists of well developed follicles with narrow thymus-dependent area and contains a high proportion of B-lymphocytes (Calkins et al., '75). In the Peyer's patch of the mouse, however, the amount of B-lymphocytes is estimated to be approximately half of that of the total small lymphocytes contained. Thus the amount of B-lymphocytes is almost equal to that in peripheral lymphatic tissues (Calkins et al., '75). In the patch, as shown in these results, the thymus-dependent and thymus-independent areas contain about 90% of the total small lymphocytes included.

In peripheral lymphatic tissues, germinal centers appear as a results of antigenic stimulation, and lymphocytes in germinal centers are B-lymphocytes (Cottier et al., '67). As seen in these results, large lymphocytes in germinal centers of the patch were hydrocortisone-sensitive and thymus-independent in nature. However, about half of small lymphocytes contained within the germinal center of the patch were T-lymphocytes which may have emigrated from the surrounding areas. Germinal centers in the Peyer's patch are structurally similar to those in other peripheral lymphatic tissues as described in previous papers (Abe and Ito, '72, '73).

The close relation of lymphocytes in the dome to the overlying epithelium has suggested that the dome area may be similar in nature to the avian bursa and that the dome area, including the overlying epithelium, may serve as mammalian bursa-equivalent (Bockman and Cooper, '73; Waksman et al., '73; Owen and Jones, '74). As seen in the present results, however, the dome area constitutes only a small portion of the patch and contains very small numbers of lymphocytes. The proportions of T- and B-lymphocytes in the dome area and the overlying epithelium are similar to those in the entire patch. The numbers of lymphocytes decrease in proportion to those in the patch after either thymectomy or hydrocortisone injection. Thus lymphocytes in the dome area and the overlying epithelium seem to have migrated from both the thymus-dependent and thymus-independent areas in the patch. In addition, it seems likely that lymphocytes in the dome area, at least in part, pass through the epithelium into the lumen, because almost half of the intraepithelial lymphocytes over the dome area were located

above the nuclear level of the epithelial cells.

As previously reported (Abe and Ito, '72), peripheral lymphatic tissues such as the spleen and lymph nodes are essentially similar in structural organization. The marginal zone in the spleen and the marginal sinuses of lymph nodes are localized peripheral to the follicles, and they are considered as sites through which antigens appear to gain access to the lymphatic tissue (Nossal et al., '68). The dome area is likely to be a region where antigens invading from the intestinal lumen reach first (Joel et al., '70; Waksman et al., '73). Thus the dome area of the Peyer's patch seems to correspond to the marginal zone of the spleen and the marginal sinus of lymph nodes.

In conclusion, Peyer's patches can be considered as peripheral lymphatic tissue, functionally as well as morphologically. The patches appear to differ in architectural details from other peripheral lymphatic tissues, possibly due to the continual antigenic stimulation and route of antigen entry.

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