Cell Tissue Res (1991) 266:29–36

Cell and Tissue Research

Morphological alteration of gut-associated lymphoid tissue after long-term total parenteral nutrition in rats

S. Tanaka, S. Miura, H. Tashiro, H. Serizawa, Y. Hamada, M. Yoshioka, and M. Tsuchiya

Division of Gastroenterology, Department of Internal Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160 Japan

Accepted June 5, 1991

Summary. The morphological alteration of gut-associated lymphoid tissue (GALT) induced by long-term absence of dietary stimulation was investigated. Male Wistar rats weighing ~230 g were maintained with total parenteral nutrition (TPN). Control rats were allowed to have the same amount of the solution orally. After two weeks, the morphological alteration of GALT was examined. Although no significant difference in weight gain was noted between the two groups, the area comprised by Peyer's patches was decreased in TPN rats. The number of transported lymphocytes and the ratio of helper T (T_h) cells to suppressor/cytotoxic T ($T_{s/c}$) cells in intestinal lymph were lowered after TPN treatment. In an immunohistochemical study of the rat ileum, the number of T cells and the $T_h/T_{s/c}$ ratio were decreased both in the intraepithelial spaces and in the lamina propria of TPN rats. The percentage of interleukin-2 receptor-positive cells and the number of IgA-containing cells in the lamina propria were significantly reduced in TPN rats. These results suggest that dietary stimulation might play a role in the maintenance of GALT function and morphology.

Key words: Parenteral feeding – Gut-associated lymphoid tissue – Peyer's patches – Immunoglobulin A – Immunohistochemistry – Rat (Wistar)

Total parenteral nutrition (TPN) is an effective alternative to gastrointestinal feeding in patients with gastrointestinal disorders. Parenteral feeding, however, bypasses the complex processes which normally occur in the gastrointestinal tract. Several studies have shown the morphological and functional changes of the small intestinal mucosa after TPN (Castro et al. 1975; Johnson et al. 1975; Schmitz et al. 1986). These reports suggest that the oral ingestion of food is necessary to maintain the structural integrity of the intestinal mucosa. The abundance of lymphoid tissue in the gastrointestinal tract, which is called gut-associated lymphoid tissue (GALT), plays an important defensive role against a variety of luminal antigens. Our previous studies revealed that intraluminal administration of carbohydrate or lipid increased intestinal lymph flow and the number of lymphocytes transported through mesenteric lymphatic ducts (Kobayashi et al. 1985; Miura et al. 1987). The possibility exists that nutritional absorption through the small intestine may contribute to the regulation of morphology and function of GALT. In the present study, we have investigated the morphological alteration of GALT induced by long-term absence of oral food intake in rats maintained by TPN.

Offprint requests to: M. Tsuchiya

Materials and methods

Methods of TPN

Male Wistar rats weighing ~230 g were maintained by TPN using a modified technique of Steiger et al. (1972). Briefly, a silastic catheter was inserted from the jugular vein into the vena cava or right atrium. The free end of the catheter was passed subcutaneously to the midscapular area and brought out through a specially constructed harness connected to a swivel apparatus to allow freedom of movement in a metabolic cage. The basic solution consisted of 20.6% dextrose and 3.3% amino acid with required amounts of electrolytes, vitamins, minerals and essential fatty acids (National Research Council 1978). The solution was continuously infused with an infusion pump at a speed of 3.1 ml/h for two weeks, providing 319 kcal/kg/day. Control rats were sham operated and allowed to have nearly equal amounts of the solution orally. The number of animals used in this study included 22 TPN rats and 23 control rats. In order to eliminate the possibility that the results were due to a difference in the nutritional state rather than the routes of administration, body weight gain, serum total protein levels, plasma glucose levels and the number of lymphocytes in peripheral blood were determined in both groups after two weeks.

Morphological study of Peyer's patches

Six TPN rats and 6 control rats were used for the morphological study of Peyer's patches. The major and minor axis of each Peyer's

patch that was regarded as ellipse were measured from serosal side along the small intestine to determine the area of Peyer's patches. Intestinal mucosal wet weight was also determined at this time. To determine lymphocyte subsets of total lymphocytes in Peyer's patches, all Peyer's patches were surgically removed from serosal side and cell suspensions were prepared by mechanically pressing the Peyer's patches between two slide glasses in RPMI medium 1640 (Life Technologies Inc., Gaithersburg, Md., USA) supplemented with 10% fetal calf serum. Lymphocyte subsets were assessed by means of fluorescence-activated cell sorter (FACS-440, Becton Dickinson, San Jose, Calif., USA). Lymphocytes that are positive to monoclonal antibodies W3/13, W3/25, MRC OX-8 and MRC OX-6 were regarded as $CD3^+$ T cells, $CD4^+$ helper T cells (T_h), $CD8^+$ suppressor/cytotoxic T cells ($T_{s/c}$) and Ia-positive cells, respectively. An immunoperoxidase technique was used to study the distribution of lymphocyte subsets in Peyer's patches.

Intestinal lymph collection

Five TPN rats and 6 control rats were used for the experiment with intestinal lymph. The mesenteric lymphatic duct was cannulated according to the method of Bollman et al. (1948). Rats were infused with saline via the jugular vein at a speed of 2.4 ml/h to prevent dehydration. After 2 h when the lymph flow rate had become constant, intestinal lymph flow and the lymphocyte output in intestinal lymph were measured for 3 h. Lymphocyte subsets in intestinal lymph were assessed using FACS-440.

Immunohistochemistry

Segments of ileum of approximately 1 cm in length were obtained from 6 TPN rats and 6 control rats. Each segment was incised along the mesenteric attachment and sliced into longitudinal strips. The strips were fixed in the periodate-lysine-paraformaldehyde solutions (McLean and Nakane 1974). After fixation the tissue was embedded by O.C.T. compound (Miles Inc., Elkhart, Ind., USA), quick-frozen in liquid nitrogen, and stored at $-80°$ C until cryostat sections were cut at 6 µm perpendicular to the mucosal surface. Endogenous peroxidase activity was inhibited according to the method of Streefkerk (1972). Sections were stained for immunoglobulin (IgA, IgM and IgG)-containing cells, T-cell subsets and interleukin-2 (IL-2) receptors. Goat antisera against rat IgA (Fc) (Binding Site Ltd., Birmingham, England) and horseradish peroxidase (HRP)-labelled rabbit anti-goat IgG (H&L) (Organon Teknika N.V., West Chester, N.C., USA) were used in the indirect immunoperoxidase technique for IgA staining. IgM and IgG were stained by a direct immunoperoxidase technique using HRP-labelled goat anti-rat IgM and HRP-labelled goat anti-rat IgG (Organon Teknika N.V.). T-cell subsets and IL-2 receptors were stained using an indirect immunoperoxidase technique. Monoclonal antibodies W3/13, W3/25, MRC OX-8 and MRC OX-39 were purchased from Serotec Ltd. (Kidlington, England); the reactive elements were regarded as $CD3^+$ T cells, $CD4^+$ helper T cells (T_h), $CD8^+$ suppressor/cytotoxic T cells ($T_{s/c}$) and IL-2 receptor-positive cells, respectively. HRP-labelled goat anti-mouse IgG(H) (Pharmacia Fine Chemicals, Uppsala, Sweden) was used as secondary antibody.

The peroxidase-positive cells were assessed by morphometry. Counts of T-cell subsets and immunoglobulin-containing cells were made with $\times 40$ objective and $\times 8$ eyepieces by scanning in the lamina propria and in the intraepithelial spaces. The width of each field was 0.35 mm, and thus a mean of 1 peroxidase-positive cell per field was equivalent to 28.5 cells/cm of intestine. The populations of each T-cell subset and immunoglobulin-containing cells were expressed as positive cells per 1 cm small intestine. In case of assessing the population of IL-2 receptor-positive cells, data were expressed as positive cells per 1000 mononuclear cells in the lamina propria. The $T_h/T_{s/c}$ ratio was expressed as the mean of the ratios of individual counts for each specimen.

Bacterial cultures of ileal luminal contents

Five TPN rats and 5 control rats were used for intestinal bacteriological studies. Segments of the ileum 10 cm proximal to the ileocecal orifice were removed and flushed with 10 ml sterile phosphate-buffered saline. The fluid was collected, homogenized, diluted serially in brain-heart infusion (BHI) media and 100 µl of each dilution plated (1) on Trypticase Soy (TS) agar for quantitative aerobic bacterial counts and (2) on Eggerth Gagnon (EG) agar, with the addition of 5% carbon dioxide, for anaerobic colony counts. The bacterial counts were expressed as $\log_{10}$ colony-forming units per ml of initial luminal washings.

Statistical analysis

Results were expressed as means $\pm$ SD. Statistical analysis was performed according to Student's *t*-test and differences were regarded as significant when $P < 0.05$.

Results

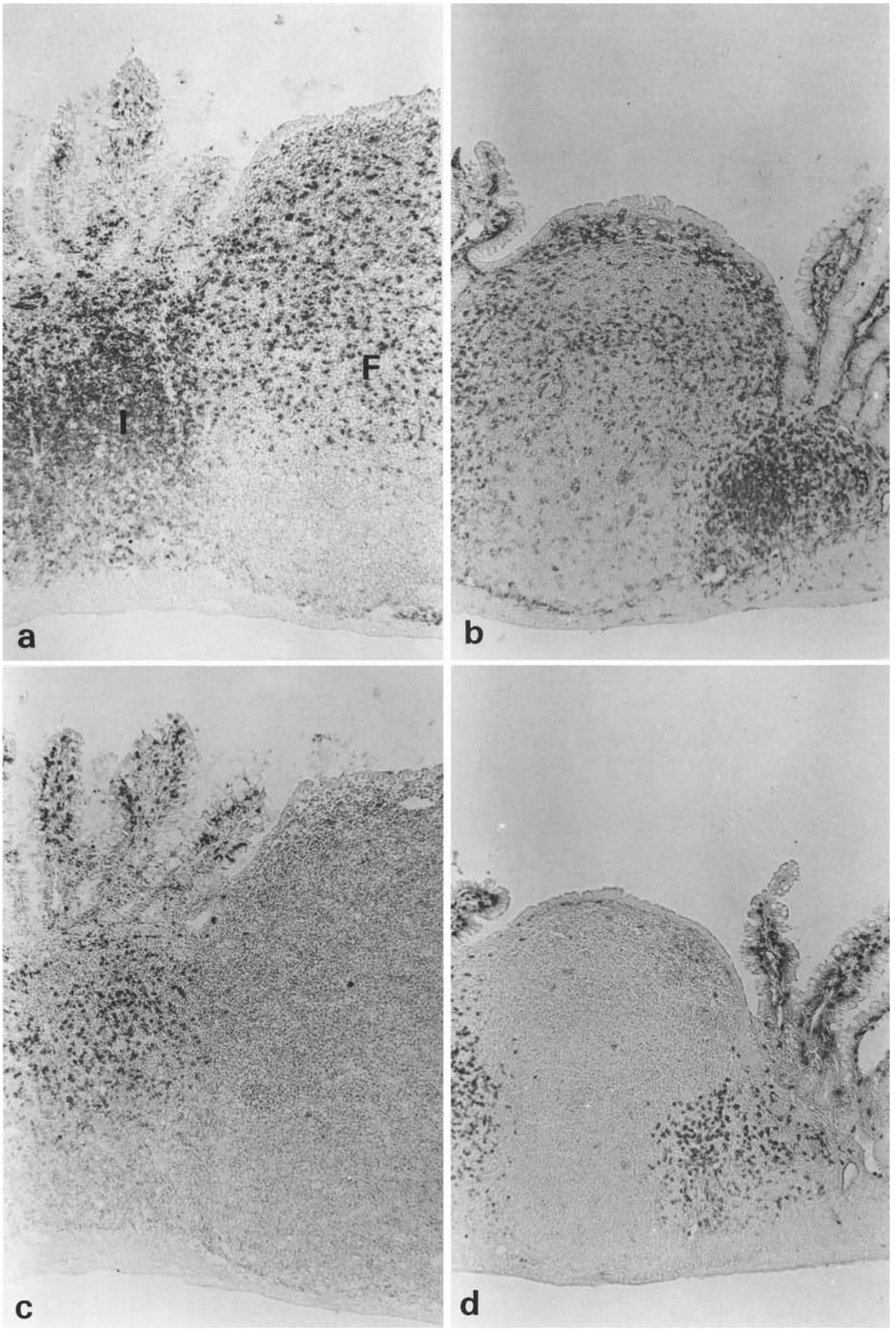
The average daily weight gains were 7.8 ± 2.2 g/kg/day ($n=6$) for TPN rats and 8.7 ± 2.0 g/kg/day ($n=6$) for

Fig. 1. Helper T cells (**a, b**) and suppressor/cytotoxic T cells (**c, d**) of Peyer's patches from a control rat (**a, c**) and a TPN rat (**b, d**). Both helper T cells and suppressor/cytotoxic T cells are concentrated in the interfollicular areas (*I*) and scattered in the follicles (*F*). Although the size of lymphoid follicles is markedly diminished in the TPN rat, the distribution of both subsets is similar in TPN rat and control rat. $\times 100$

Table 1. Lymphocyte output and T-cell subsets in intestinal lymph. Values are means $\pm$ SD. TPN, Total parenteral nutrition; Th, helper T cells; Ts/c, suppressor/cytotoxic T cells

	Lymph flow (ml/h)	Lymphocyte output (10^7 counts/h)	T (%)	Th (%)	Ts/c (%)	Th/Ts/c (ratio)
Control ($n=6$)	2.32 ± 0.37	3.56 ± 0.76	95.3 ± 2.5	78.6 ± 3.6	21.1 ± 2.6	3.78 ± 0.66
TPN ($n=5$)	1.11 ± 0.24***	2.10 ± 0.42**	93.4 ± 3.3	72.0 ± 4.5*	26.4 ± 5.4	2.85 ± 0.72*

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$


I
F
a
b
c
d

control rats. This difference was not statistically significant. Serum total protein levels were 6.4 ± 1.9 g/dl ($n=6$) for TPN rats and 6.7 ± 2.3 g/dl ($n=6$) for control rats; there was no statistical difference between the two groups. In TPN rats, there was a marked reduction in mean mucosal wet weight of the small intestine, which fell from 2.56 ± 0.67 g/small intestine ($n=6$) in control rats to 1.26 ± 0.34 g/small intestine. This reduction amounted to 50% and was statistically significant ($P<0.01$). The reduction in the area of Peyer's patches in TPN rats was also statistically significant ($P<0.01$). The average area of TPN rats was 120.4 ± 24.4 mm^2/10 cm intestine; this amounted to 51% of the control values (234.3 ± 35.9 mm^2/10 cm intestine).

Table 2. T-cell subsets of intraepithelial lymphocytes of rat ileum

	Positive cells per 1 cm intestine			Th/Ts/c (ratio)
	T	Th	Ts/c	
Control ($n=6$)	2755 ± 506	521 ± 162	2365 ± 629	0.22 ± 0.06
TPN ($n=6$)	1196 ± 377***	102 ± 35***	1138 ± 411**	0.09 ± 0.03**

Transported lymphocytes in intestinal lymph

Intestinal lymph flow, lymphocyte output and lymphocyte subsets in intestinal lymph of both groups are shown in Table 1. Intestinal lymph flow was markedly reduced in TPN rats ($P<0.001$), and the average flow was 48% of the controls. The average number of lymphocytes transported through mesenteric lymphatic ducts was 2.10×10^7/h in TPN rats, which was 60% of the control rats ($P<0.01$). Although the percentage of T cells in intestinal lymph was not statistically different between the two groups, the percentage of T_h cells was significantly decreased in TPN rats. The $T_h/T_{s/c}$ ratio showed a statistically significant decrease after TPN treatment. On the other hand, the number of lymphocytes in peripheral blood was 1538 ± 308/mm^3 for the control rats and 1342 ± 350/mm^3 for the TPN rats, which was not statistically different between the two groups.

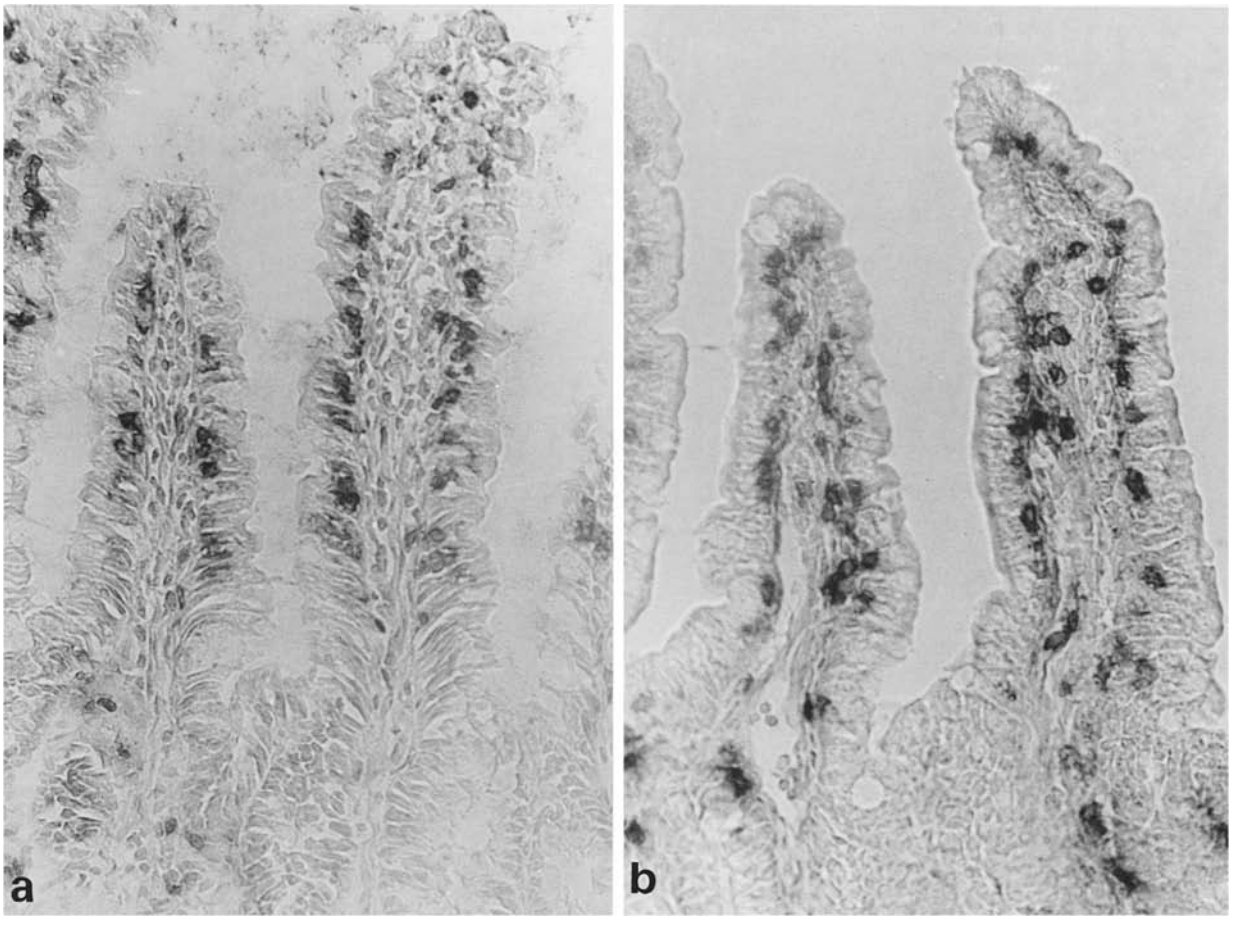


Fig. 2a, b. Suppressor/cytotoxic T ($T_{s/c}$) cells in the ileal mucosa from a control rat (**a**) and a TPN rat (**b**). The number of $T_{s/c}$ cells is decreased in the intraepithelial spaces and is increased in the lamina propria of the TPN rat. Almost all $T_{s/c}$ cells in the intraepithelial spaces are situated in the basal third of the villous epithelium in the TPN rat, whereas some $T_{s/c}$ cells could be found in the distal two-thirds of the villous epithelium in the control rat. ×400

Table 3. T-cell subsets and IL-2 receptor-positive cells in the lamina propria. *IL-2R*, Interleukin-2 receptor-positive cells

	Positive cells per 1 cm intestine			Th/Ts/c (ratio)	IL-2R (per 1000 mononuclear cells)
	T	Th	Ts/c		
Control ($n=6$)	3282±422	3065±421	621±183	4.94±1.33	110.8±19.0
TPN ($n=6$)	2537±339**	2002±487**	919±321	2.18±0.91**	70.6±16.3**

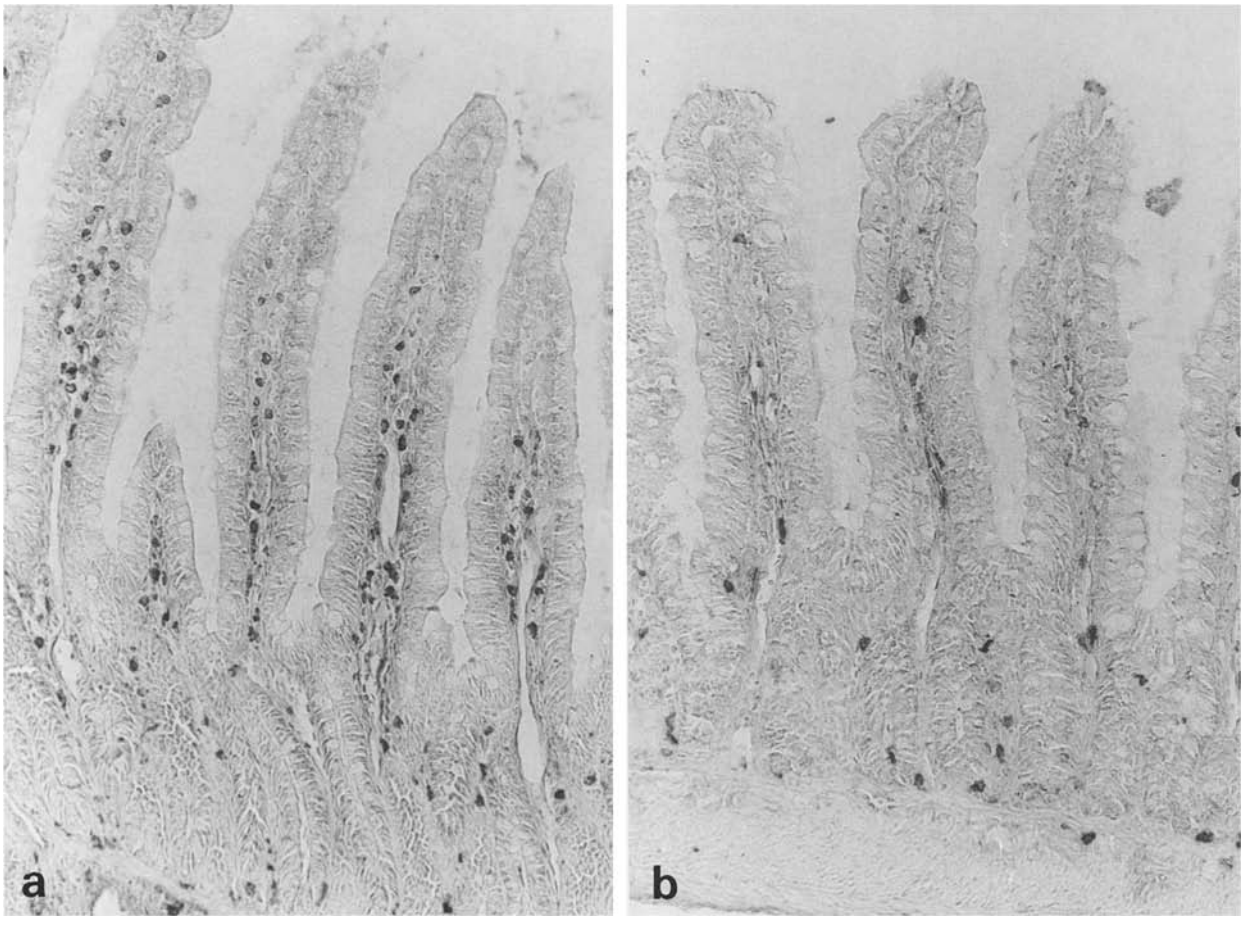


Fig. 3a, b. IgA-containing cells in the ileal mucosa from a control rat (**a**) and a TPN rat (**b**). The number of IgA-containing cells in the lamina propria is markedly decreased in the TPN rat. ×200

Lymphocyte subsets in Peyer's patches

The percentages of T cells, T_h cells, $T_{s/c}$ cells and Ia-positive cells in Peyer's patches of control rats were 29.5±8.2%, 29.0±3.3%, 13.9±6.1% and 68.5±8.8% ($n=5$), and those in TPN rats 35.4±3.7%, 33.0±6.6%, 17.6±10.2% and 64.1±4.5% ($n=5$), respectively. The $T_h/T_{s/c}$ ratio was 2.35±0.86 for control rats and 2.42±1.29 for TPN rats. Although the size of Peyer's patches was markedly reduced after TPN treatment, the percentage of each subset and the $T_h/T_{s/c}$ ratio were not significantly different in both groups. Fig. 1 shows the distribution of T_h cells and $T_{s/c}$ cells in Peyer's patches from control and TPN rats. The distribution of both T-cell subsets showed a similar pattern in the two groups.

T-cell subsets in ileal mucosa

The populations of T-cell subsets of intraepithelial lymphocytes (IEL) are summarized in Table 2. A marked decrease in the number of each T-cell subset of IEL was found in TPN rats. A greater decrease in the number of T_h cells of IEL was seen in TPN rats, resulting in a decrease in the $T_h/T_{s/c}$ ratio in these animals. The population of T-cell subsets and the percentage of IL-2 receptor-positive cells in the lamina propria are shown in Table 3. The number of $T_{s/c}$ cells in the lamina propria showed an increasing tendency after TPN treatment, but it was not statistically significant. The population of T cells and T_h cells in the lamina propria decreased significantly in TPN rats, resulting in a decrease in the $T_h/T_{s/c}$

Table 4. Immunoglobulin-containing cells in the lamina propria

	Positive cells per 1 cm intestine		
	IgA	IgG	IgM
Control ($n=6$)	1860 ± 452	319 ± 133	26 ± 15
TPN ($n=6$)	931 ± 311**	216 ± 96	32 ± 18

ratio in these animals. Although ~11% of the mononuclear cells in the lamina propria were found to have IL-2 receptors in control rats, the percentage fell to 7% for TPN rats, which was statistically significant.

There was a noticeable difference in the distributional pattern of $T_{s/c}$ cells between the two groups (Fig. 2) In TPN rats, the number of $T_{s/c}$ cells of IEL was decreased as compared with control rats. An increasing tendency of the number of $T_{s/c}$ cells in the lamina propria was seen in TPN rats. Moreover, almost all IEL were situated in the basal third of the villous epithelium of TPN rats, whereas some IEL could be found in the distal two-thirds of the villous epithelium in control rats.

Immunoglobulin-containing cells in the lamina propria

The number of immunoglobulin-containing cells in the lamina propria of the ileum was compared in both groups. As shown in Table 4 and Fig. 3, the number of IgA-containing cells in the lamina propria was markedly decreased in TPN rats. However, there was no statistical difference in the number of IgG-containing cells and IgM-containing cells in the lamina propria between the two groups.

Bacterial counts of ileal luminal contents

Control rats had 6.82 ± 0.48 $\log_{10}$ colony forming units (CFU)/ml aerobes and 7.02 ± 0.27 $\log_{10}$ CFU/ml anaerobes ($n=5$), while TPN rats had 6.44 ± 0.62 $\log_{10}$ CFU/ml aerobes and 6.59 ± 0.48 $\log_{10}$ CFU/ml anaerobes ($n=5$) in the ileal luminal washings. Both the aerobic and anaerobic counts in the ileal luminal washings did not differ significantly between the two groups.

Discussion

According to our observations, TPN appears to compromise the mucosal immunity of the gastrointestinal tract. Several authors have emphasized the immunological superiority of oral feeding over parenteral feeding. Kudsk et al. (1983) demonstrated increased survival in rats with *Escherichia coli* hemoglobin peritonitis when they were enterally fed. Alverdy et al. (1985) showed that enterally fed rats maintained higher secretory IgA levels in bile than parenterally fed rats. Clinical significance of the changes of GALT induced by TPN is great and one should be aware of the importance of oral feeding from an immunological standpoint. Marsh et al. (1983) reported that TPN predisposes surgical patients to bacteremia of *Candida* species which are constituents of the normal intestinal flora. From this study, it is conceivable that TPN compromises mucosal immunity, thus weakening the mucosal defense against bacteria or fungi.

Luminal antigens are taken up by specialized M cells overlying Peyer's patches (Owen and Jones 1974) and reach the follicular area of Peyer's patches for processing. The lymphocytes that have responded to luminal antigens or microenvironmental stimuli emigrate from Peyer's patches and enter the thoracic duct via the intestinal lymphatics. They are, then, released into the systemic circulation and home to the distant mucosal sites (Gowans and Knight 1964; Guy-Grand et al. 1974). This process of lymphocyte circulation is thought to be important for the intestinal mucosal defense system. However, the regulatory mechanisms of the lymphatic circulation are not fully understood. Our previous experiments revealed that the number of lymphocytes transported in the mesenteric lymphatic ducts analyzed by the use of a high-speed microscopic video system, increased during lipid absorption (Miura et al. 1987), and that T_h cells were the major population of cells increased in intestinal lymph after lipid administration (Kobayashi et al. 1985). The possibility exists that dietary stimulation is an important factor influencing the lymphocyte transport, especially T_h cell transport in the intestinal lymphatic system.

There was a significant reduction in lymphocyte output and the percentage of T_h cells in intestinal lymph of TPN rats that were free of dietary stimulation for 2 weeks. Several investigators have examined the origin of lymphocytes in intestinal lymph. This lymph contains cells that might have left mesenteric lymph nodes (MLN); therefore, the data obtained from rats with lymphatic fistula may receive some modification by MLN. For example, macrophages account for several percent of the cells in the peripheral intestinal lymph, but few are found in lymph draining from MLN (Hall et al. 1977). However, it has been observed that the peripheral intestinal lymph from mesenteric lymphadenectomized lamb had approximately the same cell output as the efferent mesenteric lymph from control lamb (Reynolds 1988). Gowans and Knight (1964) demonstrated the recirculation of lymphocytes which passed from the blood into the lymph at the post-capillary venules of lymphoid tissue including Peyer's patches of the intestine. The importance of Peyer's patches in contributing to the cellular content in intestinal lymph was supported by the 10-fold increase in the cell content of lacteals draining an area of gut containing a Peyer's patch (Baker 1933; Steer 1980). Reynolds et al. (1982, 1984) showed that recirculating lymphocytes were the major population of cells in sheep ileal lymph and most T cells in the ileal Peyer's patches represented a portion of the recirculating pool, which spend less than 24 h in the tissue. The reduction of the lymphocyte output in intestinal lymph of TPN rats could be due to the reduction of the recirculating lymphocyte pool in GALT, especially in Peyer's

patches. Marked decrease in the area of Peyer's patches and lymphocyte content in the intestinal mucosa observed in TPN rats correspond well with the decrease in the number of recirculating lymphocytes. On the other hand, no significant change in lymphocyte number in peripheral blood was observed between the two groups. Other mucosal defense systems including BALT (bronchial associated lymphoid tissue) may contribute to the maintenance of the number of recirculating lymphocytes in the peripheral blood of TPN rats.

Antigenic stimulation is thought to be important to maintain the appearance of GALT. Nagura et al. (1982) showed that the size of Peyer's patches and the number of IgA-containing cells in the intestinal mucosa were reduced in germfree rats. Carlson et al. (1986) demonstrated that the number of Peyer's-patch leukocytes more than doubled during the course of *Giardia muris* infection; however, the percentages of T-cell subsets in Peyer's patches remained constant during the hypertrophy of Peyer's patches. It has been reported that TPN influenced intestinal flora (Hughes et al. 1980) and secretions of bile and pancreatic juice (Towne et al. 1973). However, in the present study, both the aerobic and anaerobic bacterial counts in the ileum were not significantly altered after TPN treatment. This result suggests that changes in GALT after TPN treatment are not secondary due to an alteration in intestinal bacterial population but probably are rather due to the absence of dietary absorption itself.

In the present immunohistochemical study of control rats, monoclonal antibody for T_h cells (W3/25) stained more lamina propria lymphocytes than did that for $T_{s/c}$ cells (OX-8) and it stained only rare IEL, whereas $T_{s/c}$ cells were found in large number in the intraepithelial spaces of ileal villi. In Peyer's patches, both T_h and $T_{s/c}$ cells were concentrated in the interfollicular areas and scattered in the follicles. T_h cells considerably outnumbered $T_{s/c}$ cells in these areas, which was supported by the data of each subset analyzed by FACS. These findings in control rats are consistent with the previous reports using monoclonal antibodies for rat lymphocytes (Lyscom and Brueton 1982; Mayrhofer et al. 1983; Westermann et al. 1989).

A marked reduction in the number of T cells and IgA-containing cells was found in the intestinal mucosa of TPN rats. There is now a great deal of evidence to show that precursors of gut T cells and IgA plasma cells arise in Peyer's patches (Craig and Cebra 1971; Guy-Grand et al. 1978), and the migration of these precursors into the gut is antigen independent (Husband and Gowans 1978). Atrophy of Peyer's patches and a decreased lymphocyte migration from Peyer's patches in the absence of dietary stimulation are possibly responsible for a reduction of the lymphocyte content in the intestinal mucosa of TPN rats. A decreased ratio of $T_h/T_{s/c}$ could suppress the proliferation of immunocytes in the intestinal mucosa of TPN rats. An altered intraluminal environment induced by TPN, such as the decreased secretion of bile and pancreatic juice and loss of direct nutritional stimuli, may also influence the lymphoid cell response in the intestinal mucosa.

Alterations in the distributional pattern of $T_{s/c}$ cells, which composed the major population of IEL, were found after TPN treatment. Although the origin and fate of IEL are not fully understood, the possibility that these lymphocytes could recycle through the lamina propria was suggested by Darlington et al. (1966) and Marsh et al. (1975). The decreased number and alteration in the distributional pattern of IEL could implicate the change in lymphocyte migration, such as activated migration of lymphocytes from the epithelium to the lamina propria or to the intestinal lumen.

The mechanisms of the morphological alterations in GALT induced by TPN are not known. Recently, several types of cytokines were found to be important for immunological processes. IL-2 is known to interact with IL-2 receptors of activated T and B cells, inducing cell growth, cell division and cytotoxic activity (Pike et al. 1984; Fiocchi et al. 1988). Recent studies demonstrated that IL-5 and IL-4 produced by T cells from Peyer's patches enhanced IgA secretion from activated B cells (Murray et al. 1987; Lebman and Coffman 1988). An altered cytokine network induced by absence of dietary stimulation could influence lymphocyte proliferation and IgA production and could also play a role in the morphological alteration of GALT in TPN rats. On the other hand, several hormones and neurotransmitters have been focussed as immunomodulators. Specific receptors for VIP (vasoactive intestinal peptide) (Ottaway and Greenberg 1984), substance P (Stanisz et al. 1987) and somatostatin (Scicchitano et al. 1987) have been demonstrated on Peyer's patch T and B cells. Ottaway et al. (1987) showed innervation of Peyer's patches by VIPergic nerves. Moore et al. (1988) demonstrated that VIP depressed lymphocyte output from sheep popliteal lymph nodes with a selective effect on $CD4^+$ lymphocytes. Dietary stimulation could potentially influence the neurohumoral system in the gut, resulting in the maintenance of GALT morphology and function. Further investigations are necessary to elucidate the potential role of humoral and neural factors on GALT during nutrient absorption.

Acknowledgement. This work was supported by grants from the Ministry of Education of Japan and Keio University.

References

Alverdy J, Chi HS, Sheldon GF (1985) The effect of parenteral nutrition on gastrointestinal immunity. Ann Surg 202:681–684

Baker RD (1933) The cellular content of chyle in relation to lymphoid tissue and fat transportation. Anat Rec 55:207–221

Bollman JL, Cain JC, Grindlay JH (1948) Techniques for collection of lymph from liver, small intestine or thoracic duct of the rat. J Lab Clin Med 33:1349–1352

Carlson JR, Heyworth MF, Owen RL (1986) Response of Peyer's patch lymphocyte subsets of *Giardia muris* infection in BALB/c mice. Cell Immunol 97:44–50

Castro GA, Copeland EM, Dudrick SJ, Johnson LR (1975) Intestinal disaccharidase and peroxidase activities in parenterally nourished rats. J Nutr 105:776–781

Craig SW, Cebra JJ (1971) Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. J Exp Med 134:188–200

Darlington D, Rogers AW (1966) Epithelial lymphocytes in the small intestine of the mouse. J Anat 100:813–830

Fiocchi C, Youngman KR, Yen-Lieberman B, Tubbs RR (1988) Modulation of intestinal immune reactivity by interleukin 2. Dig Dis Sci 33:1305–1315

Gowans JL, Knight EJ (1964) The route of recirculation of lymphocytes in the rat. Proc R Soc Lond [Biol] 159:257–282

Guy-Grand D, Griscelli C, Vassalli P (1974) The gut-associated lymphoid system: nature and properties of the large dividing cells. Eur J Immunol 4:435–443

Guy-Grand D, Griscelli C, Vassalli P (1978) The mouse gut T lymphocyte, a novel type of T cell. Nature, origin and traffic in mice in normal and graft-versus-host conditions. J Exp Med 148:1661–1677

Hall JG, Hopkins J, Orlans E (1977) Studies on the lymphocytes of sheep. III. Destination of lymph-borne immunoblasts in relation to their tissue of origin. Eur J Immunol 7:30–37

Hughes CA, Prince A, Dowling RH (1980) Speed of change in pancreatic mass and in intestinal bacteriology of parenterally fed rats. Clin Science 59:329–336

Husband AJ, Gowans JL (1978) The origin and antigen-dependent distribution of IgA-containing cells in the intestine. J Exp Med 148:1146–1160

Johnson LR, Copeland EM, Dudrick SJ, Lichtenberger LM, Castro GA (1975) Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. Gastroenterology 68:1177–1183

Kobayashi K, Miura S, Hamada Y, Asakura H, Tsuchiya M (1985) Possible dietary regulation of lymphocyte traffic through intestinal lymph. In: Casley-Smith JR, Piller NB (eds) Progress in lymphology X. University of Adelaide Press, Adelaide, Australia, pp 94–96

Kudsk KA, Stone JM, Carpenter G, Sheldon GF (1983) Enteral and parenteral feeding influences mortality after hemoglobin-*E. coli* peritonitis in normal rats. J Trauma 23:605–609

Lebman DA, Coffman RL (1988) The effects of IL-4 and IL-5 on the IgA response by murine Peyer's patch B cell subpopulations. J Immunol 141:2050–2056

Lyscom N, Brueton MJ (1982) Intraepithelial, lamina propria and Peyer's patch lymphocytes of rat small intestine: isolation and characterization in terms of immunoglobulin markers and receptors for monoclonal antibodies. Immunology 45:775–783

Marsh MN (1975) Aspects of proliferation and migration of epithelial lymphocytes in the small intestine of mice. Gut 16:674–682

Marsh PK, Tally FP, Kellum J, Callow A, Gorbach SL (1983) *Candida* infections in surgical patients. Ann Surg 198:42–47

Mayrhofer G, Pugh CW, Barclay AN (1983) The distribution, ontogeny and origin in the rat of Ia-positive cells with dendritic morphology and of Ia antigen in epithelia, with special reference to the intestine. Eur J Immunol 13:112–122

McLean IW, Nakane PK (1974) Periodate-lysine-paraformaldehyde fixative: a new fixative for immunoelectron microscopy. J Histochem Cytochem 22:1077–1083

Miura S, Sekizuka E, Nagata H, Oshio C, Minamitani H, Suematsu M, Suzuki M, Hamada Y, Kobayashi K, Asakura H, Tsuchiya M (1987) Increased lymphocyte transport by lipid absorption in rat mesenteric lymphatics. Am J Physiol 253:G596–G600

Moore TC, Spruck CH, Said SI (1988) Depression of lymphocyte traffic in sheep by vasoactive intestinal peptide (VIP). Immunology 64:475–478

Murray PD, McKenzie DT, Swain SL, Kagnoff MF (1987) Interleukin 5 and interleukin 4 produced by Peyer's patch T cells selectively enhance immunoglobulin A expression. J Immunol 139:2669–2674

Nagura H, Hasegawa H, Yoshimura S, Aihara K, Watanabe K, Sawamura S, Ozawa A (1982) Comparative immunohistochemical studies on conventional and germfree rat intestinal mucosa: with special reference to microbiol flora and secretory IgA. In: Sasaki S, Ozawa A, Hashimoto K (eds) Recent advances in germfree research. Tokai University Press, Tokyo, pp 511–517

National Research Council (1978) Nutrient requirements of domestic animals. Nutrient requirements of laboratory animals, no 10. National Academy of Science, Washington DC

Ottaway CA, Greenberg GR (1984) Interaction of vasoactive intestinal peptide with mouse lymphocytes: specific binding and the modulation of mitogen responses. J Immunol 132:417–423

Ottaway CA, Lewis DL, Asa SL (1987) Vasoactive intestinal peptide-containing nerves in Peyer's patches. Brain Behav Immunity 1:148–158

Owen RL, Jones AL (1974) Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology 66:189–203

Pike BL, Raubitschek A, Nossal GJV (1984) Human interleukin 2 can promote the growth and differentiation of single hapten-specific B cells in the presence of specific antigen. Proc Natl Acad Sci USA 81:7917–7921

Reynolds JD (1988) Lymphocyte traffic associated with the gut: a review of in vivo studies in sheep. In: Husband AJ (ed) Migration and homing of lymphoid cells, Vol II. CRC Press, Boca Raton, pp 113–136

Reynolds JD, Pabst R (1984) The emigration of lymphocytes from Peyer's patches in sheep. Eur J Immunol 14:7–13

Reynolds JD, Heron I, Dudler L, Trnka Z (1982) T-cell recirculation in the sheep: migratory properties of cells from lymph nodes. Immunology 47:415–421

Schmitz CG, Metayer EL, Audran E, Hemet J, Colin R (1986) Decreased brush border hydrolase activities without gross morphologic changes in human intestinal mucosa after prolonged total parenteral nutrition of adults. Gastroenterology 90:373–378

Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM (1987) Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immunity 1:173–184

Stanisz AM, Scicchitano R, Dazin P, Bienenstock J, Payan DG (1987) Distribution of substance P receptors on murine spleen and Peyer's patch T and B cells. J Immunol 139:749–754

Steer HW (1980) An analysis of the lymphocyte content of rat lacteals. J Immunol 125:1845–1848

Steiger E, Vars HM, Dudrick SJ (1972) A technique for long-term intravenous feeding in unrestrained rats. Arch Surg 104:330–332

Streefkerk JG (1972) Inhibition of erythrocyte pseudoperoxidase activity by treatment with hydrogen peroxide following methanol. J Histochem Cytochem 20:829–831

Towne JB, Hamilton RF, Stephenson DV (1973) Mechanism of hyperalimentation in the suprression of upper gastrointestinal secretions. Am J Surg 126:714–716

Westermann J, Ronneberg S, Fritz FJ, Pabst R (1989) Proliferation of lymphocyte subsets in the adult rat: a comparison of different lymphoid organs. Eur J Immunol 19:1087–1093