Immunohistochemical localisation of intercellular adhesion molecule-1 in follicle associated epithelium of Peyer's patches

Y Fujimura, T Kihara

Abstract

The epithelium covering domes of lymphoid follicles in Peyer's patches includes membranous cells, which are sites of entry for various macromolecules, absorptive cells, a few goblet cells, and many migrating lymphoid cells. The mechanism of migration of lymphoid cells into the follicle associated epithelium of lymphoid follicles in Peyer's patches is still unknown. This study investigated the relation between localisation of intercellular adhesion molecule-1 (ICAM-1) and the follicle associated epithelium of Peyer's patches immunohistochemically. It was found that subepithelial fibroblasts expressed ICAM beneath the follicle associated epithelium on lymphoid follicles, but not on surrounding villi. The results show that the massive lymphocytic traffic between follicle associated epithelium and lymphoid follicles may be related to ICAM-1 expression.

(Gut 1994; 35: 46–50)

Peyer's patches are important for the generation of secretory IgA by B cells, which eventually 'home' back to the lamina propria at mucosal sites. Immature B and T cells from Peyer's patches migrate by efferent lymphatics to mesenteric lymph nodes where further maturation occurs, and then by the thoracic duct into the systemic circulation. Lymphocytes home to lymphoid tissue by adhering to and traversing postcapillary high endothelial venules. There are anti-genetically and functionally distinct lymphocyte high endothelial venules recognition systems that control the homing of lymphocytes to peripheral lymph nodes and mucosal lymphoid organs such as Peyer's patches and the appendix.^{1,2} In 1964, Gowans and Knight³ first recognised that lymphocytes migrate from blood to the lymph nodes by a morphologically distinct high endothelium of high endothelial venules. Stamper and Woodruff⁴ showed that rat lymphocytes bind selectively to the endothelium of high endothelial venules exposed in frozen sections of lymphoid tissues. Jalkanen *et al.*,² who adapted this high endothelial venules binding assay to mice, provided strong evidence for an important role of lymphocyte high endothelial venules interaction in determining the tissue specificity of lymphocyte migration. They proposed that this organ specificity was conveyed by interactions between specific 'homing' receptors on the lymphocyte cell surface and the tissue specific ligand of high endothelial venules. The comparative intensity with which lymphocytes bind to high endothelial venules in different lymphoid

organs varies with the lymphocyte subset and stage of differentiation and activation of the lymphocytes. Although a claim has been made that MEL-14⁵ and Hermes antigen (CD44)⁶ are lymphocyte 'homing receptors', they are not lymphocyte specific in their expression. Several adhesion molecules have been recognised.⁷

Owen *et al.*⁸ first found distinctive epithelial cells, which enclosed lymphocytes in attenuated cytoplasm among the follicle associated epithelial cells of Peyer's patches and solitary lymphoid follicles of the small intestine. These cells have been called 'microfold cells'⁸ or 'membranous cells'⁹ and are known as 'M cells'. The function of M cells has been investigated in morphological studies using various macromolecules^{10–13} and organisms.^{14–21} Shimazui²² investigated the morphological relation between M cells and lymphocytes, with particular regard to whether they make contact in intercellular or intracellular space. McClugage *et al.*²³ reported a porosity of the basal lamina covering lymphoid follicles by scanning electron microscopy after removing epithelia, and suggested these pores are an important pathway for migrating cells in the mucosal lymphoid tissue. The mechanism, however, by which immunocytes migrate to the covering epithelial layer of lymphoid follicles is still unknown. We investigated the cell to cell relation in follicle associated epithelium (FAE) of Peyer's patches using the monoclonal antibody to intercellular adhesion molecule-1 (ICAM-1).

Materials and methods

Tissue specimens of Peyer's patch were obtained from the jejunum and ileum of five week old female Wistar rats weighing about 250 g. The rats were fasted for 48 hours but had free access to water. They were then killed by inhalation anesthesia with ether. The specimens were excised immediately and processed for electron microscopic and immunohistochemical study.

LIGHT MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY

All tissue specimens were fixed in 2.5% glutaraldehyde at 4°C for two hours. Transmission electron microscopic samples were fixed for two hours in 1% osmium tetroxide, dehydrated through an ethanol series, transferred to propylene oxide, and embedded in epoxy resin. Ultrathin sections were cut with glass knives and a Porter-Blum MT2-B ultramicrotome. For light microscopy, 1 µm sections were stained by toluidine blue. For transmission electron micro-

Division of
Gastroenterology,
Department of Medicine,
Kawasaki Medical
School, Kurashiki,
Okayama, Japan
Y Fujimura
T Kihara

Correspondence to:
Dr Y Fujimura, Division of
Gastroenterology,
Department of Medicine,
Kawasaki Medical School,
577 Matsushima, Kurashiki,
Okayama, 701-01, Japan.

Accepted for publication
8 June 1993

scopy, these sections were stained with uranyl acetate and lead citrate and viewed with a Hitachi H-500 electron microscope.

IMMUNOHISTOCHEMICAL EXAMINATION

The specimens were immediately fixed in periodate-lysine-2% paraformaldehyde solution for six hours at 4°C, rinsed in a 0.01 M phosphate buffered saline series (pH 7.6) containing graded concentrations of sucrose, and embedded in OCT compound (Miles Scientific, Elkhart, USA). They were then sectioned at 10 µm by a cryostat, mounted on poly-L-lysine coated glass slides, and air dried at room temperature for three hours.

Sections were processed for examination of ICAM-1 antigen using an avidin-biotin-peroxidase complex method.²⁴ After the specimens were rinsed with 0.01 M phosphate buffered saline, they were treated with 0.3% hydrogen peroxide in methanol for 10 minutes to inactivate endogenous peroxidase. The samples were then immersed in 10% normal horse serum for 30 minutes to block any non-specific reaction. Each of the above incubations specimens were then rinsed in 0.01 M phosphate buffered saline and incubated with mouse monoclonal anti-rat ICAM-1²⁵ (Life Science, Seikagaku Co, Tokyo, Japan) diluted 1:50 in 0.01 M phosphate buffered saline containing 10% horse serum for 12 hours in moist chambers at 4°C, and biotinylated with horse anti-mouse IgG (diluted 1:50 in 0.01 M phosphate buffered saline containing 2% horse serum, rat absorbed, Vector Laboratories, Burlingame, CA) for 60 minutes. The specimens were then rinsed for 30 minutes, in 10% sucrose at 4°C and they were incubated with the avidin-biotin complex or ABC reagent (a mixture of avidin DH solution and biotinylated enzyme, each diluted 1:50 with 0.01 M phosphate buffered saline). The horse serum, biotinylated anti-mouse IgG, and ABC complex are reagents in the Vectastain Elite ABC kit (Vectorstain; Vector Laboratories, Burlingame, CA). The incubated specimens were rinsed in 0.01% phosphate buffered saline for 10 minutes, and then fixed in 2% glutaraldehyde in 0.01 M phosphate buffered saline. The specimens were then rinsed carefully six times in 0.01 M phosphate buffered saline for five minutes.

For light microscopy, the samples were reacted with 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) diluted in 0.05 M TRIS buffer, pH 7.6, for 30 minutes at room temperature and subsequently in 0.02% DAB solution containing 10 mM hydrogen peroxide and 10 mM sodium azide for five minutes.

For immunoelectron microscopy, specimens were treated as described above and then post-fixed in 2% osmium tetroxide in phosphate buffered saline for one hour. They were then dehydrated in graded ethanol solutions, embedded in epoxy resin, and allowed to stand three days for polymerisation. Ultrathin sections were cut with an MT2-B Porter-Blum ultramicrotome and examined in a Hitachi H-500 transmission electron microscope. For negative controls, non-immune mouse serum, mouse IgG1, and phosphate buffered saline were used in place of the first antibody.

Results

LIGHT MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY

Light microscopically, subepithelial fibroblasts were recognised by toluidine blue staining as spindle shaped cells beneath epithelial cells in villi and lymphoid follicles. In lymphoid follicles, many lymphoid cells lay close to them. By transmission electron microscopy, lymphocytes were sometimes recognised in lymphoid follicles in disrupted spaces in the basal lamina. There was a well developed endoplasmic reticulum in the subepithelial fibroblasts from which cytoplasmic processes extended with sheet like and branched configuration to the reticular lamina (Fig 1).

IMMUNOHISTOCHEMICAL EXAMINATION OF ICAM-1 EXPRESSION IN PEYER'S PATCHES

ICAM-1 was expressed on the vascular endothelium of high endothelial venules (Fig 2) in interfollicular areas, in dendritic cells or macrophage like cells and in the subepithelial areas of lymphoid follicles (Fig 3A) in Peyer's patches.

Figure 1: Transmission electron micrograph shows a subepithelial fibroblast (Fb) beneath an absorptive cell (A) in a Peyer's patch. It has a well developed endoplasmic reticulum (open arrow) and cytoplasmic processes (arrows), which have a finger like or tentacle configuration in collagenous fibrils of the basal lamina. A lymphocyte (L) is closely apposed to subepithelial fibroblasts.

Figure 2: Immunohistochemical examination shows a high endothelial venule (HEV) of a Peyer's patch, which reacts (arrows) strongly with anti-ICAM-1 antibody (original magnification $\times 300$).

Figure 3: (A) Immunohistochemical localisation of ICAM-1 in a dome epithelium of a lymphoid follicle (LF) in a Peyer's patch. Although ICAM-1 is positive in the basal lamina on dome epithelium, it is negative on the surrounding villi (V) (original magnification $\times 160$). (B) Enlargement of Fig 3(A) shows a positive reaction of fibroblast like cells (arrow) (original magnification $\times 320$). (C) Control staining with non-immune mouse serum (original magnification $\times 160$). There is no reaction in the basal lamina on either the dome epithelium or the surrounding villi.

Figure 4: Immunoelectron micrograph of a section treated with anti-ICAM-1 antibody. Electron dense areas (arrows) of positive reaction are prominent on subepithelial fibroblasts (Fb), but not on an M cell (M). AN=nucleus of an absorptive cell, MN=nucleus of an M cell.

There was no expression of ICAM-1, however, in the subepithelial areas of surrounding villi (Fig 3A). Figure 3B is an enlarged photomicrograph of Fig 3A and shows that spindle shaped subepithelial cells with ICAM-1 expression are fibroblast like cells. There was no labelling of the subepithelial cells of Peyer's patches or surrounding villi with control stainings (Fig 3C).

IMMUNOELECTRON MICROSCOPIC EXAMINATIONS OF ICAM-1 EXPRESSION OF PEYER'S PATCHES
On ultrathin sections of Peyer's patches stained with anti-ICAM-1 antibody, ICAM-1 expression was recognised on the surface of subepithelial fibroblasts in lymphoid follicles, but there was no expression of ICAM-1 in M cells (Fig 4). Careful study, however, showed that subepithelial fibroblasts with long and sheet like cytoplasmic processes had a strong positive reaction to ICAM-1 (Fig 5A). The cytoplasmic processes of the fibroblasts extended among collagen fibrils of the basal laminae into the epithelial cells above the lymphoid follicles (Fig 5B, C). The subepithelial fibroblasts of surrounding villi showed no expression of ICAM-1.

Discussion
ICAM-1 is a cell surface glycoprotein, the ligand for LFA-1, and its expression can be induced on a variety of cell types. These include vascular endothelium,²⁶ dermal fibroblasts²⁶ and keratinocytes,²⁷ dendritic cells,²⁶ thymic epithelial cells,²⁶ mucosal epithelial cells in the tonsils,²⁸ renal tubular cells,²⁹ and rheumatoid synovium.³⁰ In vitro, the normally low basal expression of ICAM-1 on dermal fibroblasts, keratinocytes, and endothelial cells can be dramatically increased by treatment with the inflammatory mediators interferon γ , IL-1, tumour necrosis factor, or lymphotoxin. The increased ICAM-1 expression directly correlates with increased LFA-1 dependent adhesion of lymphocytes to the induced cells.^{26 31 32} These findings suggest that ICAM-1 may play a part in localising the immune response to sites of inflammation. Recently, it was reported that expression of ICAM-1 positively correlated with enterocytic maturation of colon adenocarcinoma.³³ The distribution of ICAM-1 in tissue specimens is similar to that of HLA-DR.³⁴⁻³⁶ Although M cells have been shown to express HLA-DR³⁷ or Ia antigen³⁸ on their surfaces, thus indicating that M cells are antigen presenting cells to their associated lymphocytes, our investigation showed no expression of ICAM-1 in the M cells of Peyer's patches.

The epithelium covering lymphoid follicles is specialised and differentiated epithelium, which includes M cells, a number of intraepithelial lymphocytes, and a few goblet cells. Although the origin of M cells is still unknown, there are two hypotheses: that M cells develop directly from undifferentiated crypt cells^{39 40} or indirectly from mature absorptive cells.⁴¹⁻⁴³ It may be suggested that the subepithelial fibroblasts of lymphoid follicles affect the development of follicle associated epithelium including M cells. It has been shown that a cytotoxic/suppressor

Figure 5 (A): Immunoelectron micrograph of a section treated with anti-ICAM-1 antibody. An electron dense area of strong positive reaction in a fibroblast (Fb) and its cytoplasmic processes. L=lymphocyte, M=M cell. (B) Enlargement of the fibroblast in Fig 5 (A) (*) shows a strongly positive reaction of a cytoplasmic process (open arrow). (C) Enlargement of the fibroblast in Fig 5 (A) (**) shows a strongly positive reaction of a cytoplasmic process. Fine processes (arrows) from the fibroblast extend among collagen fibrils of the basal lamina to the epithelial cells. L=lymphocyte, M=M cell.

phenotype of intraepithelial T cells predominated over a helper phenotype in follicle associated epithelium,³⁸ and this phenotype was similar to those in villus epithelium.⁴⁴ Our study is the first to show ICAM-1 expression of subepithelial fibroblasts in the Peyer's patches of rats, but not in the surrounding villi. It may be possible that both the Ia expression of M cells and the ICAM-1 expression of subepithelial fibroblasts are induced by an interferon γ like factor emitted by T cytotoxic/suppressor lymphocytes in close contact on passage through the disrupted basal lamina.

Additionally, we found that sheet like and branched cytoplasmic processes of subepithelial fibroblasts covering lymphoid follicles closely extended to epithelial cells in the reticular lamina and the lamina densa. These findings were similar to subepithelial fibroblasts of villi.⁴⁵ Although the function of subepithelial fibroblasts is not fully known, they may give signals to epithelial cells and lymphocytes and may play a part in the differentiation and development of a variety of epithelial cell populations in Peyer's patches.

The porosity of the basal lamina of the dome epithelia of Peyer's patches has been shown.²³

Ohtani *et al*⁴⁶ found that the sheet of collagen fibrils underlying the basal lamina also possesses morphologically heterogeneous pores corresponding to those within the basal lamina. They speculated that the pores were primarily there to provide migrating cells with passageways between the spaces formed by M cells and the reticular spaces of the dome. Our findings suggest that the ICAM-1 expression of subepithelial fibroblasts may facilitate the migration of lymphocytes into follicle associated epithelium.

Recently, Barker *et al*⁴⁷ showed that interferon γ injected into normal skin intensified the ICAM-1 expression of dermal endothelial cells and induced keratinocyte expression of ICAM-1. In addition, LFA-1 positive lymphocytes were found along the basement membrane zone close to basal keratinocytes, which have been found to be ICAM-1 positive *in vivo* in humans.

Interestingly, Nakamura *et al*⁴⁸ suggested that ontogenically Peyer's patches develop from aggregates of spindle cells with a morphology resembling that of endothelial cells or fibroblasts in the lamina propria under villus epithelial cells in newborn rats. Although the mechanism of generation of lymphoid follicles is still unknown, the expression of ICAM-1 by subepithelial fibroblasts, as shown in this paper, may be related to ontogenic development of the dome epithelium of lymphoid follicles.

One interesting finding in Crohn's disease has been that early aphthoid ulcers are typically seen over lymphoid follicles.^{49, 50} This may be related to the presence of an active immune response against a given antigen that has a predisposition for the M cell as its route of entry. Although no specific organism has been identified in a large number of previous studies, it seems that the location of aphthoid ulcers may be evidence for an infectious cause and the effect of ICAM-1 in targeting lymphoid cells to lymphoid follicles may play a part in producing aphthoid ulcers in Crohn's disease. The administration of anti-ICAM-1 may provide a new treatment for chronic inflammatory bowel disease.

This study was supported by Project Research Grant No 4-603 from Kawasaki Medical School. The authors are grateful to Mr K Uehira for his excellent technical assistance and Dr Y Higashi for his technical advice. We also wish to thank to Ms N Moriya for preparing the manuscript.

- Gallatin M, John TP St, Siegelman M, Reichert R, Butcher EC, Weissman IL. Lymphocyte homing receptors. *Cell* 1986; **44**: 673-80.
- Jalkanen S, Reichert RA, Gallatin WM, Bargatze RF, Weissman IL, Butcher EC. Homing receptors and the control of lymphocyte migration. *Immunol Rev* 1986a; **91**: 39-60.
- Gowans JL, Knight EJ. The route of recirculation of lymphocytes in the rat. *Proc R Soc Lond (Biol)* 1964; **159**: 257-82.
- Stamper HB Jr, Woodruff JJ. Lymphocyte homing into lymph nodes: *in vitro* demonstration of the selective affinity of recirculating lymphocytes for high endothelial venules. *J Exp Med* 1976; **144**: 828-33.
- Gallatin WM, Weissman IL, Butcher EC. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* 1983; **304**: 30-4.
- Jalkanen S, Bargatze RF, de los Toyos J, Butcher EC. Lymphocyte recognition for high endothelium: antibodies to distinct epitopes of an 85-95 kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. *J Cell Biol* 1987; **105**: 983-90.
- Salmi M, Jalkanen S. Regulation of lymphocyte traffic to mucosa-associated lymphatic tissues. *Gastroenterol Clin North Am* 1991; **20**: 495-510.
- Owen RL, Jones AL. Epithelial cell specialization within human Peyer's patches. *Gastroenterology* 1974; **66**: 189-2034.

- 9 Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine. *Gastroenterology* 1977; 72: 440-51.
- 10 Bockman DE, Cooper MD. Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study. *Am J Anat* 1973; 136: 455-78.
- 11 LeFevre ME, Olivo R, Vanderhoff JW, Joel DD. Accumulation of latex in Peyer's patches and its subsequent appearance in villi and mesenteric lymph nodes. *Proc Soc Exp Biol Med* 1978; 159: 298-302.
- 12 Von Rosen L, Podjaski D, Bettmann I, Otto HF. Observations on the ultrastructure and function of the so-called 'microfold' or 'membranous' cells (M cells) by means of peroxidase as a tracer. *Virchow Arch [A]* 1981; 390: 289-312.
- 13 Shakhlamov VA, Gaidar YA, Baronov VN. Electron-cytochemical investigation of cholera toxin absorption by epithelium of Peyer's patches in guinea pigs. *Bulletin of Experimental Biology and Medicine* 1981; 90: 1159-61.
- 14 Wolf JL, Rubin DH, Finberg R, Kauffman RS, Sharpe AH, Trier JS, et al. Intestinal M cells. A pathway for entry of reovirus into the host. *Science* 1981; 212: 471-2.
- 15 Fujimura Y. Functional morphology of Microfold cells (M cells) in Peyer's patches. Phagocytosis and transport of BCG by M cells into rabbit Peyer's patches. *Gastroenterol Jpn* 1986; 21: 325-35.
- 16 Owen RL, Pierce NF, Apple RT, Cray WC Jr. M cell transport of *Vibrio cholerae* from the intestinal lumen into Peyer's patches. A mechanism for antigen sampling and for microbial trans epithelial migration. *J Infect Dis* 1986; 153: 1108-18.
- 17 Marcial MA, Madara JL. Cryptosporidium; cellular localization, structural analysis of absorptive cell-parasite membrane-membrane interactions in guinea pigs, and suggestion of protozoan transport by M cells. *Gastroenterology* 1986; 90: 583-94.
- 18 Kohbata S, Yokoyama H, Yabuuchi E. Cytopathogenic effect of *Salmonella typhi* GIFU 10007 on M cells of murine ileal Peyer's patches in ligated ileal loops; an ultrastructural study. *Microbiol Immunol* 1986; 30: 1225-37.
- 19 Uchida J. An ultrastructural study on active uptake and transport of bacteria by microfold cells (M cells) to the lymphoid follicles in the rabbit appendix. *Journal of Clinical Electron Microscopy* 1987; 20: 379-94.
- 20 Grutzkau A, Hanski C, Hahn H, Riecken EO. Involvement of M cells in the bacterial invasion of Peyer's patches; a common mechanism shared by *Yersinia enterocolitica* and other enteroinvasive bacteria. *Gut* 1990; 31: 1011-5.
- 21 Fujimura Y, Kihara T, Mine H. Membranous cells as a portal of *Yersinia pseudotuberculosis* entry into rabbit ileum. *J Clin Electron Microscopy* 1992; 25: 35-46.
- 22 Shimazui T. An ultrastructural study of the pathway and the localization of migrating lymphocytes through the intestinal microfold cells (M cells). *J Clin Electron Microscopy* 1985; 18: 127-40.
- 23 McClugage SG, Low FN, Zimny ML. Porosity of the basement membrane overlying Peyer's patches in rats and monkeys. *Gastroenterology* 1986; 91: 1128-33.
- 24 Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem, Cytochem* 1981; 29: 577-80.
- 25 Tamatani T, Kotani M, Miyasaka M. Characterization of the rat leukocyte integrin, CD11/CD18, by the use of LFA-1 subunit-specific monoclonal antibodies. *Eur J Immunol* 1991; 21: 627-33.
- 26 Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer T. Induction by IL 1 and interferon- γ : tissue distribution, biochemistry, and function of A natural adherence molecule (ICAM-1). *J Immunol* 1986; 137: 245-54.
- 27 Dustin ML, Singer KH, Tuck DT, Springer TA. Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon- γ and is mediated by intercellular adhesion molecule-1 (ICAM-1). *J Exp Med* 1988; 167: 1323-40.
- 28 Reibel J, Sørensen CH. Association between keratin staining patterns and the structural and functional aspects of palatine tonsil epithelium. *APMIS* 1991; 99: 905-15.
- 29 Faull RJ, Russ GR. Tubular expression of intercellular adhesion molecule 1 (ICAM-1) during renal allograft rejection. *Transplantation* 1989; 48: 226-30.
- 30 Hale L, Martin ME, McCollum DE, Nunley JA, Springer TA, Singer KH, et al. Immunohistologic analysis of the distribution of cell adhesion molecules within the inflammatory synovial microenvironment. *Arthritis Rheum* 1989; 32: 22-30.
- 31 Pober JS, Gimbrone MA Jr, Lapierre LA, Mendrick DL, Fiers W, Rothlein R, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor and immune interferon. *J Immunol* 1986; 137: 1893-6.
- 32 Pober JS, Lapierre LA, Stolpen AH, Brock TA, Springer TA, Fiers W, et al. Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin 1 species. *J Immunol* 1987; 138: 3319-24.
- 33 Kaiserlian D, Rigal D, Abello J, Revillard JP. Expression, function and regulation of the intercellular adhesion molecule-1 (ICAM-1) on human intestinal epithelial cell lines. *Eur J Immunol* 1991; 21: 2415-21.
- 34 Inaba K, Steinman RM. Resting and sensitized T lymphocytes exhibit distinct stimulatory (antigen-presenting cell) requirement for growth and lymphokine release. *J Exp Med* 1984; 160: 1717-35.
- 35 Hirschberg H, Bergh OJ, Thorsby E. Antigen presenting properties of human vascular endothelial cells. *J Exp Med* 1980; 152: 249-555.
- 36 Hirschberg H. Presentation of viral antigens by human vascular endothelial cells in vitro. *Hum Immunol* 1981; 2: 235-46.
- 37 Hirata I, Austin LL, Blackwell WH, Weber JR, Dobbins WO. Immunoelectron microscopic localization of HLA-DR antigen in control small intestine and colon and in inflammatory bowel disease. *Dig Dis Sci* 1986; 31: 1317-30.
- 38 Jarry A, Robaszekiewicz M, Brousse N, Potet F. Immune cells associated with M cells in the follicle-associated epithelium of Peyer's patches in the rat. An electron- and immunoelectron-microscopic study. *Cell Tissue Res* 1989; 255: 293-8.
- 39 Bye WA, Allan CH, Trier JS. Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. *Gastroenterology* 1984; 86: 789-801.
- 40 Madara JL, Bye WA, Trier JS. Structural features and cholesterol distribution in M cell membranes in guinea pig, rat, and mouse Peyer's patches. *Gastroenterology* 1984; 87: 1091-103.
- 41 Smith MW, Peacock MA. M cell distribution in follicle-associated epithelium of mouse Peyer's patch. *Am J Anat* 1980; 159: 167-76.
- 42 Bhalla DK, Owen RL. Cell renewal and migration in lymphoid follicles of Peyer's patches and cecum. An autoradiographic study in mice. *Gastroenterology* 1982; 82: 232-42.
- 43 Smith MW, Peacock MA. Lymphocyte induced formation of antigen transporting "M" cells from fully differentiated mouse enterocytes. In: Robinson JW, Dowling RH, Riecken ED, eds. *Intestinal adaptation and its mechanisms*. Lancaster: MTP Press Ltd, 1982: 573-83.
- 44 Cerf-Bensussan N, Guy-Grand D, Lisowska-Grospierre B, Griscelli C, Bhan AK. A monoclonal antibody specific for rat intestinal lymphocytes. *J Immunol* 1986; 136: 76-82.
- 45 Marsh MN, Trier JS. Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. *Gastroenterology* 1974; 67: 622-35.
- 46 Ohtani O, Kikuta A, Ohtsuka A, Murakami T. Organization of the reticular network of rabbit Peyer's patches. *Anat Rec* 1991; 229: 251-8.
- 47 Barker JNWN, Allen MH, MacDonald DM. The effect of in vivo interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. *J Invest Dermatol* 1989; 93: 439-42.
- 48 Nakamura S, Sumi Y, Nagura H. Ontogenic development of gut-associated lymphoid tissue in the rat. An immunohistochemical study. *Acta Pathol Jpn* 1988; 38: 1267-83.
- 49 Rickert RR, Carter HW. The 'early' ulcerative lesion of Crohn's disease: correlative light- and scanning electron-microscopic studies. *J Clin Gastroenterol* 1980; 2: 11-9.
- 50 Fujimura Y, Hosobe M, Kihara T. Ultrastructural study of M cells from colonic lymphoid nodules obtained by colonoscopic biopsy. *Dig Dis Sci* 1992; 37: 1089-98.