

Acta Medica Okayama

Volume 49, Issue 3

1995

Article 5

JUNE 1995

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Abstract

To clarify the immunological function of 'M' (microfold or membranous) cells in the large intestine, we examined the expression of intercellular adhesion molecule-1 (ICAM-1) and HLA-class II antigens immunohistochemically in M cells and follicle-associated epithelia (FAE) covering isolated lymphoid follicles of the human colon in comparison with their expression in Peyer's patches of the small intestine. In Peyer's patches of the small intestine, ICAM-1 was not expressed on the epithelial cells covering the lymphoid follicles, but their cell surfaces were stained positively for HLA-DR. In contrast, colonic M cells expressed ICAM-1 on their cell surfaces but were negative for HLA class II antigens. By immunoelectron microscopy, ICAM-1 was seen to be distributed on the surface of microfolds, on the membranes of apical vesicles and on part of the basolateral plasma membranes of M cells, but was not expressed on adjacent FAE. These findings imply that the M cells in the colon and in Peyer's patches have different immunological roles. In addition, identification of ICAM-1 expression on the colonic M cells should help elucidate the pathogenesis of some inflammatory colonic diseases which appear to start in the lymphoid follicles of the colonic mucosa.

KEYWORDS: ICAM-I, M cell, follicle-associated epithelial cells, HLA antigen, immunoelectron microscopy

*PMID: 7676845 [PubMed - indexed for MEDLINE]

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Expression of ICAM-1 on M Cells Covering Isolated Lymphoid Follicles of the Human Colon

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To clarify the immunological function of 'M' (microfold or membranous) cells in the large intestine, we examined the expression of intercellular adhesion molecule-1 (ICAM-1) and HLA-class II antigens immunohistochemically in M cells and follicle-associated epithelia (FAE) covering isolated lymphoid follicles of the human colon in comparison with their expression in Peyer's patches of the small intestine. In Peyer's patches of the small intestine, ICAM-1 was not expressed on the epithelial cells covering the lymphoid follicles, but their cell surfaces were stained positively for HLA-DR. In contrast, colonic M cells expressed ICAM-1 on their cell surfaces but were negative for HLA class II antigens. By immunoelectron microscopy, ICAM-1 was seen to be distributed on the surface of microfolds, on the membranes of apical vesicles and on part of the basolateral plasma membranes of M cells, but was not expressed on adjacent FAE. These findings imply that the M cells in the colon and in Peyer's patches have different immunological roles. In addition, identification of ICAM-1 expression on the colonic M cells should help elucidate the pathogenesis of some inflammatory colonic diseases which appear to start in the lymphoid follicles of the colonic mucosa.

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gut epithelia are exposed to various foreign antigens such as foods, drugs, viruses, and bacteria. Follicle-associated epithelia (FAE) covering Peyer's patches of the human small intestine and 'M' (microfold or membranous) cells within FAE are considered to have specialized functions in mucosal immune responses to these antigenic materials (1); M cells transport antigens

from the intestinal lumen to engulfing lymphocytes and macrophages (2). M cells are also present on the isolated lymphoid follicles of the human colon (3-5). Despite the accumulation of information regarding the functions of M cells in Peyer's patches in the small intestine (1, 6, 7), the immunological function of M cells in the large intestine remains unexplored.

Recent studies have shown that various cell adhesion molecules play important roles in various steps of cellular immune responses (8). Among these, intercellular adhesion molecule-1 (ICAM-1), a ligand for lymphocyte function-associated antigen-1 (9), is involved in the initial step of adhesion of cytotoxic T cells to target cells (10) or of helper T cells to antigen-presenting cells (11). Cotransfection of human ICAM-1 and HLA-DR genes into mouse L cells reconstituted antigen-presenting capabilities to human T cells (12).

To clarify the immunological function of M cells in the large intestine, we examined the expression of ICAM-1 and HLA-class II antigens immunohistochemically in M cells and FAE covering isolated lymphoid follicles of the human colon in comparison with the expression of these molecules in Peyer's patches of the small intestine.

Materials and Methods

Tissues. Tissue specimens of normal colon were obtained from grossly and histologically normal colonic mucosa by surgical resection from 8 patients (5 men and 3 women; mean age, 65 years) with colorectal cancer. Tissues of the human small intestine were obtained from the terminal ileum by endoscopic biopsy from 7 patients (4 men and 3 women; mean age, 40 years) with colon polyps or without apparent colorectal diseases. Informed consent was obtained from each patient.

Immunohistochemistry. The tissues were

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fixed in periodate-lysine-paraformaldehyde (13), and cryostat sections were stained using an indirect peroxidase-labeled antibody method (14). The following monoclonal antibodies were used as primary antibodies: 84H10 antibody to the 105-kDa epitope of ICAM-1 (Immunotech, Marseille, France) (9) and anti-HLA-DP, -DQ, -DR antibodies (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) (15). As negative controls, normal mouse IgG1 (DAKOPATTS, Glostrup, Denmark) and phosphate-buffered saline (PBS) were used instead of the primary antibodies. After incubation with the primary antibodies, the sections were reacted with horseradish peroxidase-labeled Fab' fragments of rabbit anti-mouse immunoglobulins, prepared as described (14), then with diaminobenzidine (DAB) containing hydrogen peroxide. The stained sections were counterstained with methyl green, dehydrated, and mounted.

For immunoelectron microscopy, sections were reacted with the primary antibodies and the peroxidase-labeled secondary antibody, post-fixed in 2% glutaraldehyde, incubated sequentially with DAB and DAB containing hydrogen peroxide. The stained sections were osmicated, dehydrated, and embedded in Epon-Araldite as described (16). Ultrathin sections were observed under an electron microscope (Hitachi-H-700H) without additional staining.

Results

M cells and FAE in Peyer's patches of the small intestine. In Peyer's patches of the small intestine, 8 lymphoid follicles in 7 specimens were examined. Although ICAM-1 was present on mononuclear cells beneath the epithelial layer and on cells in the central area of the lymphoid follicles which were likely to be follicular dendritic cells in the germinal center, it was not

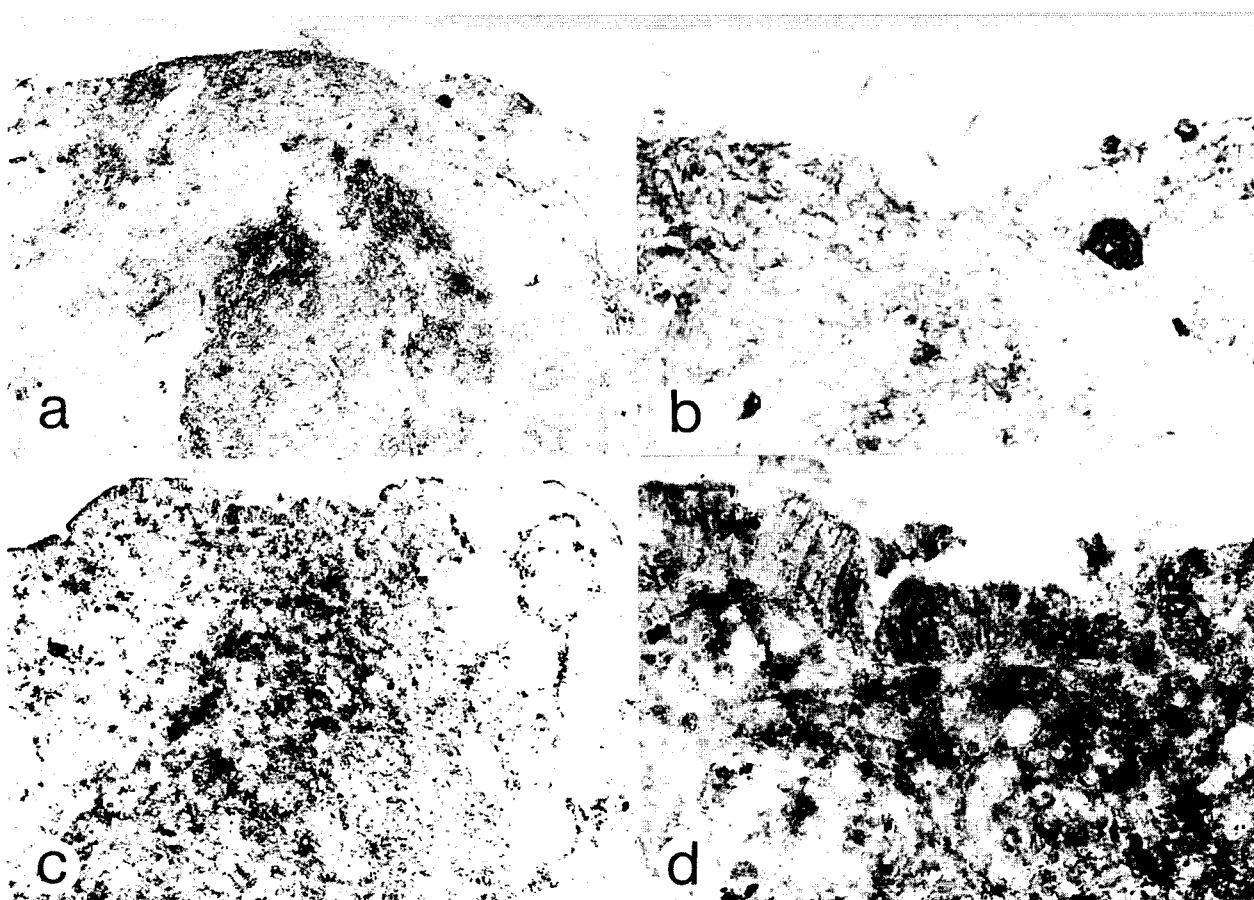


Fig. 1

expressed on the epithelial cells covering the lymphoid follicles in any of the specimens examined (Fig. 1 a, b). In contrast, HLA-DR was expressed on cells covering the lymphoid follicles in all the specimens which probably included both M cells and FAE; it was present along the basolateral membranes of these cells (Fig. 1 c, d).

HLA-DR was also stained on cells beneath the epithelial layer and in the peripheral area of the lymphoid follicles (Fig. 1 c). Neither HLA-DP nor HLA-DQ was expressed on the epithelial cells covering the lymphoid follicles. No staining was observed in the colonic mucosa reacted with control normal mouse IgG or PBS.

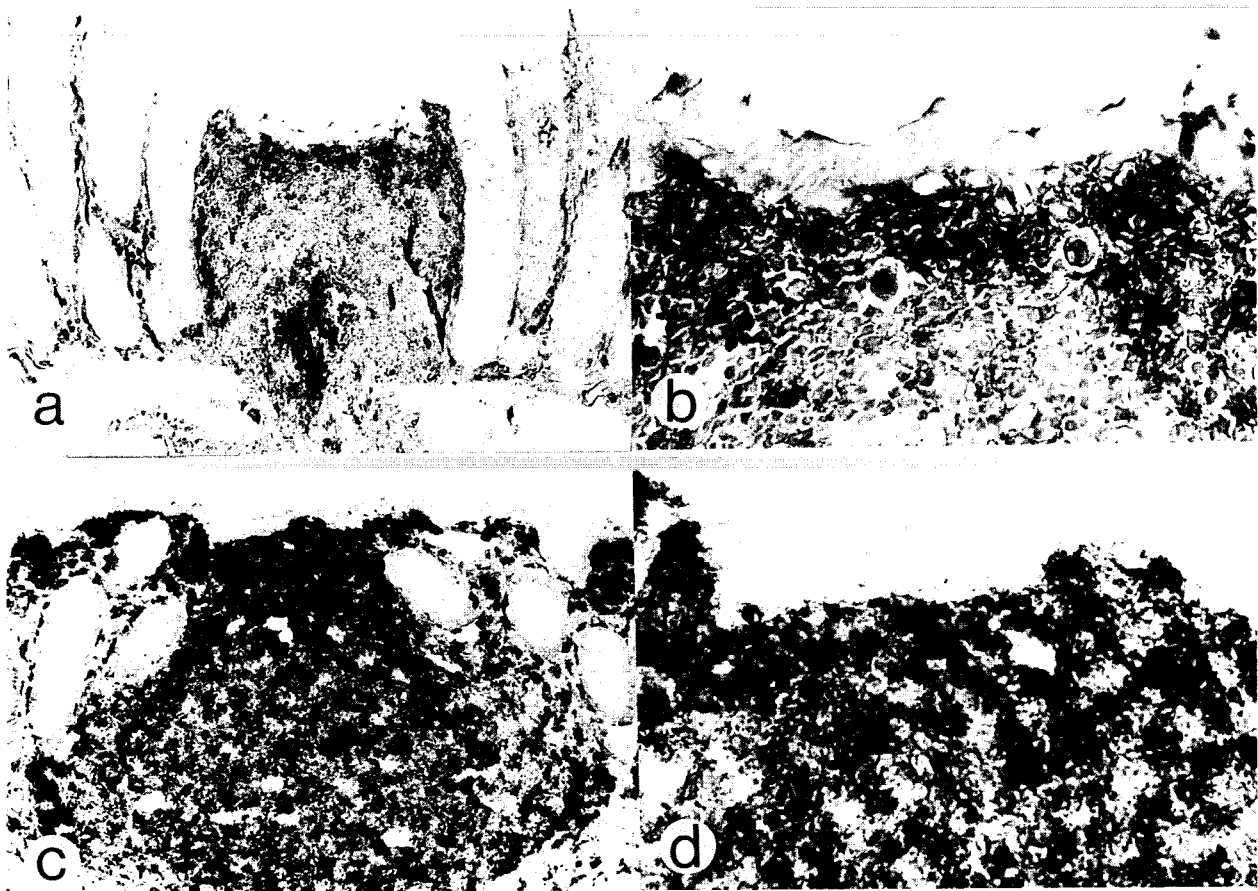


Fig. 2 Immunohistochemical localization of ICAM-1 and HLA-DR in isolated lymphoid follicles of the colon. (a) ICAM-1 is seen to be present on mononuclear cells beneath the epithelial layer and cells likely to be follicular dendritic cells in the germinal center of the lymphoid follicles, and also on the epithelial cells covering the lymphoid follicles (x100). (b) At higher magnification, ICAM-1 expression is seen on the apical surface of the epithelial cells covering the lymphoid follicles in a studded pattern (x400). (c) HLA-DR immunoreactivity is intense on the cells beneath the epithelial layer and in the lymphoid follicles (x100). (d) At higher magnification, the epithelial cells covering the lymphoid follicle is seen to be entirely negative for HLA-DR (x400).

Fig. 1 Immunohistochemical localization of ICAM-1 and HLA-DR in Peyer's patches of the small intestine. (a) ICAM-1 immunoreactivity is observed on mononuclear cells beneath the epithelial layer and cells in the central area of the lymphoid follicles likely to be follicular dendritic cells in the germinal center of the lymphoid follicles (x100). (b) At higher magnification, ICAM-1 is seen not to be expressed on the epithelial cells covering the lymphoid follicles (x400). (c) HLA-DR is expressed ubiquitously on cells covering the lymphoid follicles. Thus, it is likely that the positive cells include both M cells and FAE. Positive staining for HLA-DR is also seen on the cells beneath the epithelial layer and in the peripheral area of the lymphoid follicles (x100). (d) At higher magnification, HLA-DR immunoreactivity is observed along the basolateral membranes of M cells and FAE (x400).

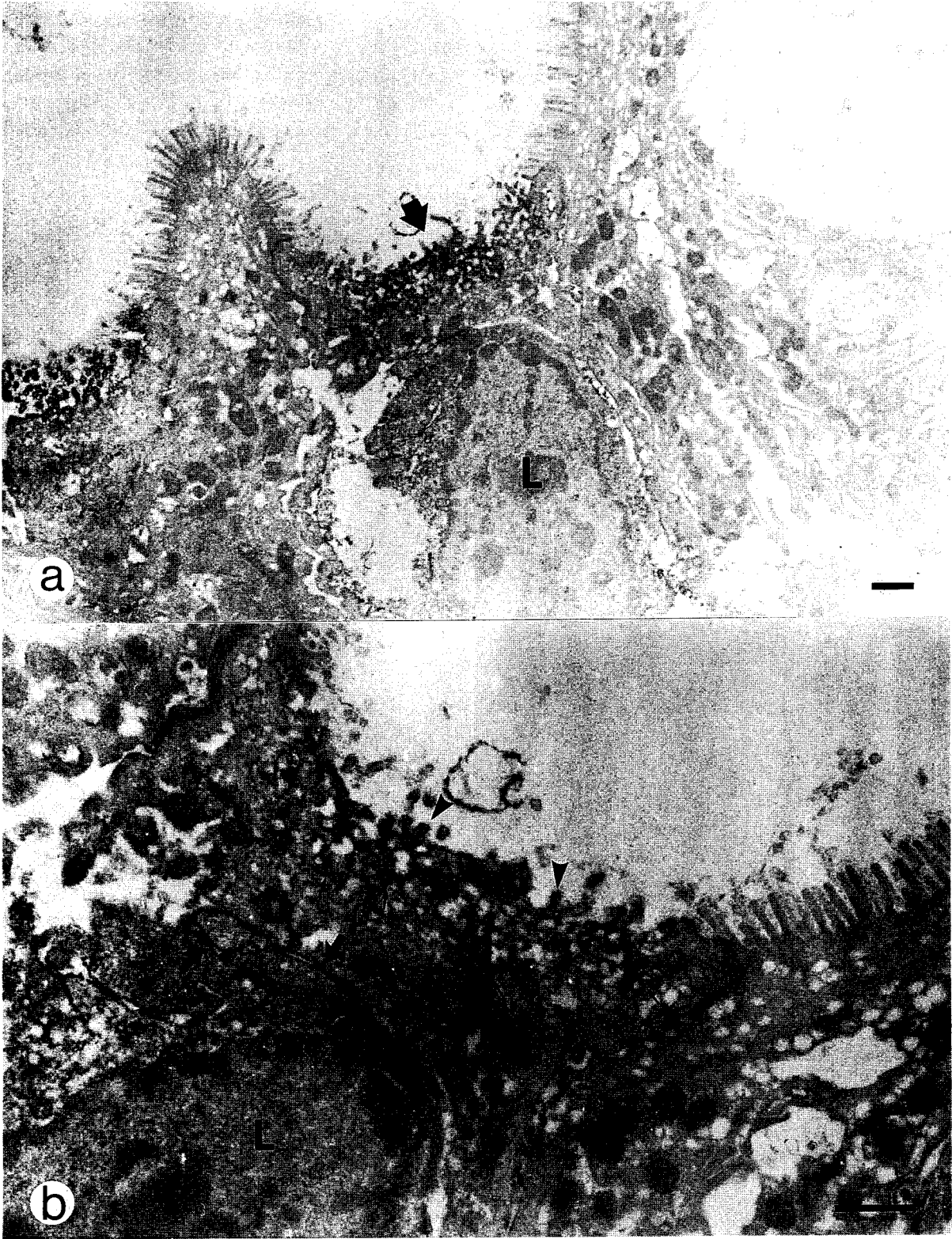


Fig. 3

M cells and FAE covering isolated lymphoid follicles of the colon. In 8 specimens from the colon, 8 of 11 lymphoid follicles were associated with covering epithelial cells. Expression of ICAM-1 was observed on mononuclear cells beneath the epithelial layer and on cells likely to be follicular dendritic cells in the germinal center of the lymphoid follicles (Fig. 2 a), as seen in Peyer's patches in the small intestine. In contrast to Peyer's patches, ICAM-1 was also expressed on the apical surface of the epithelial cells covering the lymphoid follicles in a studded pattern (Fig. 2 a, b). ICAM-1 positive-epithelial cells were identified in all 8 lymphoid follicles associated with covering epithelial cells.

By immunoelectron microscopy, ICAM-1 was seen on the cells which had morphological characteristics of M cells; *i.e.*, short sparse irregular microfolds, abundant vesicles beneath the apical membrane and engulfed lymphocytes (Fig. 3). ICAM-1 was positive on all 10 M cells in the 3 specimens examined. In the M cells, ICAM-1 was distributed on the surface of microfolds, the membranes of the apical vesicles and on part of the basolateral plasma membrane surrounding lymphocytes (Fig. 3). However, it was not expressed on adjacent FAE with numerous uniform microvilli (Fig. 3).

HLA-DR staining was intense on the cells beneath the epithelial layer and in the lymphoid follicle, but the epithelial cells covering the lymphoid follicle were negative for the antigen in all the specimens examined (Fig. 2 c, d). Neither HLA-DP nor HLA-DQ was expressed on these cells.

Discussion

In this study, we demonstrated immunohistochemically that M cells covering isolated lymphoid follicles of the human colon expressed ICAM-1, but not HLA class II antigens. By immunoelectron microscopy, we localized ICAM-1 to the apical microfolds, the membrane of apical vesicles and the basolateral plasma membrane of M cells. Identification of cells in the human colon which closely

resemble M cells on Peyer's patches in the small intestine was first reported by Jacob *et al.* in 1987 (3). The colonic 'M' cells were shown to have sparse, short and distorted microvilli, a well-developed tubulovesicular network and to surround lymphocytes (3, 5); the cells overlying the colonic lymphoid follicles which were positive for ICAM-1 showed these distinctive features of M cells. Thus, the expression of ICAM-1 on the colonic M cells was confirmed by immunoelectron microscopy.

We observed ICAM-1 expression partly on the basolateral membrane of colonic M cells at the site of lymphocyte adhesion. Recently, M cells in the rat jejunum were shown to contain lysosomal elements and were thus suggested to play a role in both processing and presentation of endocytosed antigens to adjacent intra-epithelial lymphocytes (1). ICAM-1 mediates most of the lymphocyte/lymphocyte, lymphocyte/antigen-presenting cell, and leukocyte/endothelial cell interactions in the immune responses (17). Our findings imply that M cells and engulfed lymphocytes interact immunologically via ICAM-1-dependent cell adhesion, but it is unlikely that colonic M cells act as antigen-presenting cells since HLA-DR antigen was not detected on their cell surface.

We also showed that ICAM-1 was present on the apical surface of M cells. An important implication of this finding is that ICAM-1 expressed on the apical surface of M cells may act as a receptor for certain pathogens. There are several pathogens which are known to use intestinal M cells as a portal of entry to the body: Rabbit M cells have been shown to transport *Vibrio cholerae* into Peyer's patches (18); murine colorectal M cells were reported to take up and transport reovirus particles introduced via the anus (19); poliovirus enters from the intestinal lumen into the lymphoid follicles through M cells (20). Poliovirus is a member of Picornaviridae as are the rhinoviruses which use ICAM-1 as a receptor (21, 22), and the receptor for poliovirus belongs to the immunoglobulin superfamily as does ICAM-1 (23). It is shown that the binding domain for poliovirus-receptor interaction is common to the binding domain for rhinovirus-ICAM-1

Fig. 3 Immunoelectron microscopic analysis of ICAM-1 expression on the epithelial cells covering isolated lymphoid follicles of the colon. (a) ICAM-1 is present on cells with short sparse irregular microfolds, abundant vesicles beneath the apical membrane and engulfed lymphocytes, which are distinct features of intestinal M cells (an arrow). (b) At higher magnification, ICAM-1 immunoreactivity is seen to be distributed on M cells on the surface of microfolds (arrowheads), the membrane of the apical vesicles (small arrows) and part of the basolateral plasma membrane surrounding a lymphocyte (large arrows), but it is not observed on adjacent FAE with numerous uniform microvilli. L: lymphocyte. Bars = 1 μ m.

interaction (24). Therefore, poliovirus may enter and infect the human host via interaction with ICAM-1 on the apical surface of M cells. Our finding may also be of importance in understanding the pathogenesis of some inflammatory colonic diseases which appear to start in lymphoid follicles. Early lesion of Crohn's colitis, the etiology of which remains unknown, is localized to the colonic lymphoid follicles. The lesion could be induced by an unidentified agent which is taken up by M cells by way of the ICAM-1 pathway.

In the present immunohistochemical study, colonic M cells were shown to express ICAM-1 but were negative for HLA-DR. Whereas simultaneous expression of HLA-DR and ICAM-1 has been observed on various types of cells (25-27), the differential expression of these molecules was also reported in gastric epithelial cells of *Helicobacter pylori*-associated gastritis (28). Expression of ICAM-1 and HLA-DR is regulated by various cytokines, but the cytokines responsible for the expression of each molecule are variable (26, 27), which may explain the unrelated expression of ICAM-1 and HLA-DR on M cells. In addition, a contrasting pattern of expression of ICAM-1 and HLA-DR was observed between the colon and the small intestine. In contrast to colonic M cells, M cells in Peyer's patches of the small intestine were positive for HLA-DR, as previously reported (6), but were negative for ICAM-1, which is consistent with findings in the rat (29). The environmental milieu with regard to such factors as luminal contents and bacterial flora is different between the colon and the small intestine. Lipopolysaccharides of certain bacteria have been shown to induce ICAM-1 expression on human dermal microvascular endothelial cells (26) and human ureteral epithelial cells (30). In addition, bacterial lipopolysaccharides (31) and membrane lipoproteins of mycoplasma (32) have been shown to induce cytokine production in human monocytes. Thus, the differences in local milieu could account for the different expression patterns of ICAM-1 on M cells in the colon and those in the small intestine directly or via different profiles of cytokine induction.

In conclusion, our findings suggest that the colonic M cells and the M cells in Peyer's patches have different immunological roles. In addition, identification of ICAM-1 expression on the colonic M cells should help elucidate the pathogenesis of colonic diseases affecting lymphoid follicles.

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Received February 15, 1995; accepted March 13, 1995.