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Lack of relation between expression of HLA-DR and secretory component (SC) in follicle-associated epithelium of human Peyer's patches

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SUMMARY

Follicle-associated epithelium (FAE) of normal human Peyer's patches (PP) was studied with regard to expression of HLA-DR determinants and secretory component (SC); the latter acts as a receptor for polymeric immunoglobulins (pIg). Putative M cells were identified in FAE by lack of a brush border with alkaline phosphatase. These cells were virtually negative for HLA-DR whereas the remaining FAE was strongly positive like villous epithelium. Conversely, the complete FAE showed no SC expression and was negative for IgA. These findings suggested that the FAE (including the M cells) does not participate in SC-mediated transport of pIgA, which in the gut mainly takes place through columnar crypt cells. The FAE (excepting the M cells) may be involved in an MHC class II-restricted antigen-presenting function as recently suggested for villous epithelium. The role of M cells may hence be limited to uptake and transport of luminal antigens.

Keywords Peyer's patches M cells intestinal immunity secretory component HLA-DR

INTRODUCTION

Major histocompatibility complex (MHC) class II-determinants, which act as restriction elements in immune responses (Benaceraf, 1981), are known to be expressed by villous epithelial cells of the gut (Wiman *et al.*, 1978; Scott *et al.*, 1980; Natalie *et al.*, 1981; Selby *et al.*, 1981). This fact indicates that these cells may function as antigen-presenting cells—a possibility recently supported by experiments with rat enterocytes (Bland & Warren, 1986). The follicle-associated epithelium (FAE) covering the dome region of lymphoid follicles in the gut, including Peyer's patches (PP), is assumed to play an important role in the transport of luminal antigens to the intestinal lymphoid cells (Landsverk, 1981; Elson & Ealding, 1984; Bockman, Boydston & Beezhold, 1983). Within this epithelium there are specialized cells, the so-called 'membrane' or M cells (Bockman & Cooper, 1973; Owen & Jones, 1974). They differ morphologically and enzymatically from the adjacent absorptive cells, show preferential affinity for certain micro-organisms (Inman & Cantey, 1983; Wolf *et al.*, 1983; Owen, 1983), and take up and transport antigens inwards (Owen, 1977; Rosen *et al.*, 1981). M cells apparently can not process antigens by lysosomal degradation (Owen, Apple & Bhalla, 1981), and it is uncertain if they can present antigens to T cells (Carlson & Owen, 1987).

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Owen & Jones (1974), who supported the theory of antigen sampling by demonstrating numerous vesicles in the apical portion of the M cells, did not exclude the possibility that they may have a secretory function. Later it was suggested that this may include immunoglobulin (Ig) transport (Bockman, Boydston & Beezhold, 1983; Bockman, 1983). FAE of rat bronchus has in fact been reported to contain IgA (Komatsu *et al.*, 1980; van der Brugge-Gamelkoorn *et al.*, 1986a,b). Epithelial uptake of IgA in FAE of human PP was not observed in a preliminary study from our laboratory (Bjerke & Brandtzaeg, 1983).

The mature M cells are distinguished by a central hollow enclosing one or more mononuclear cells, probably T lymphocytes (Owen & Jones, 1974; Bye, Allan & Trier, 1984). This intimate spatial relationship suggests an antigen-presenting role for the M cells. In preliminary studies we found that the FAE covering the human Peyer's patches expressed MHC class II (HLA-DR) determinants (Bjerke & Brandtzaeg, 1983; Brandtzaeg, 1985), and this has subsequently been confirmed (Spencer, Finn & Isaacson, 1986). It was therefore of interest to examine whether the M cells were DR-positive or not.

MATERIALS AND METHODS

Tissue specimens

Ileal mucosa was obtained from six kidney donors with maintained peripheral circulation. Small tissue blocks from

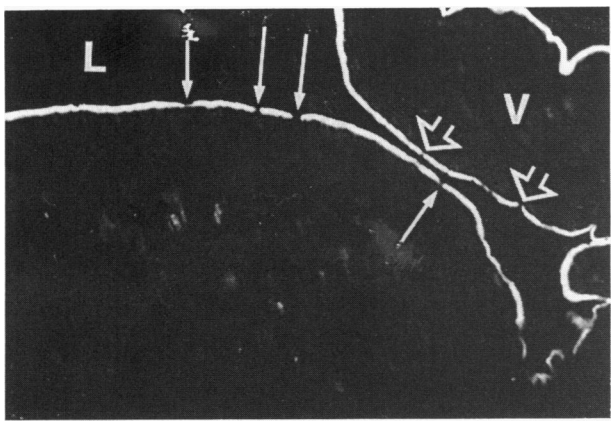

Fig. 1. Staining for alkaline phosphatase in section of ethanol-fixed normal human Peyer's patch (red fluorescence emission). The positive brush border of the follicle-associated epithelium (FAE) is intermittently broken by gaps indicating M cells (small arrows). Some gaps are also seen on the adjacent villus (V), representing opening of goblet cells (open arrows). L, lumen. × 100.

Peyer's patches were excised, fixed in cold ethanol and processed for paraffin embedding (Sainte-Marie, 1962).

Serial sections from appropriately oriented samples were cut at 1–2 μm or at 6 μm perpendicular to the surface of the mucosa.

Immunofluorescence and histochemical staining

Attempts to demonstrate M cells were performed by the method of Owen & Bhalla (1983) based on the absence of a brush border with alkaline phosphatase on these cells (Fig. (1). Co-staining for HLA-DR determinants and alkaline phosphatase was carried out as follows. Dewaxed sections of 1–2 μm thickness were first incubated for 20 h at room temperature with a biotinylated murine monoclonal antibody (1:24) to human HLA-DR (Becton-Dickinson, Mountain View CA) followed by fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories, Burlingame, CA) applied at 0·025 g/l for 30 min; thereafter naphtol phosphoric acid with fast red violet was applied for 3 min (1 mg naphtol AS-TR phosphate (N-6125; Sigma Chemical Co., St Louis, MO) dissolved in 100 μl dimethyl formamide mixed with 5 ml 0·1 M Tris HCl buffer, pH 9·0, containing 4 mg fast red violet LB salt (F-1625; Sigma)).

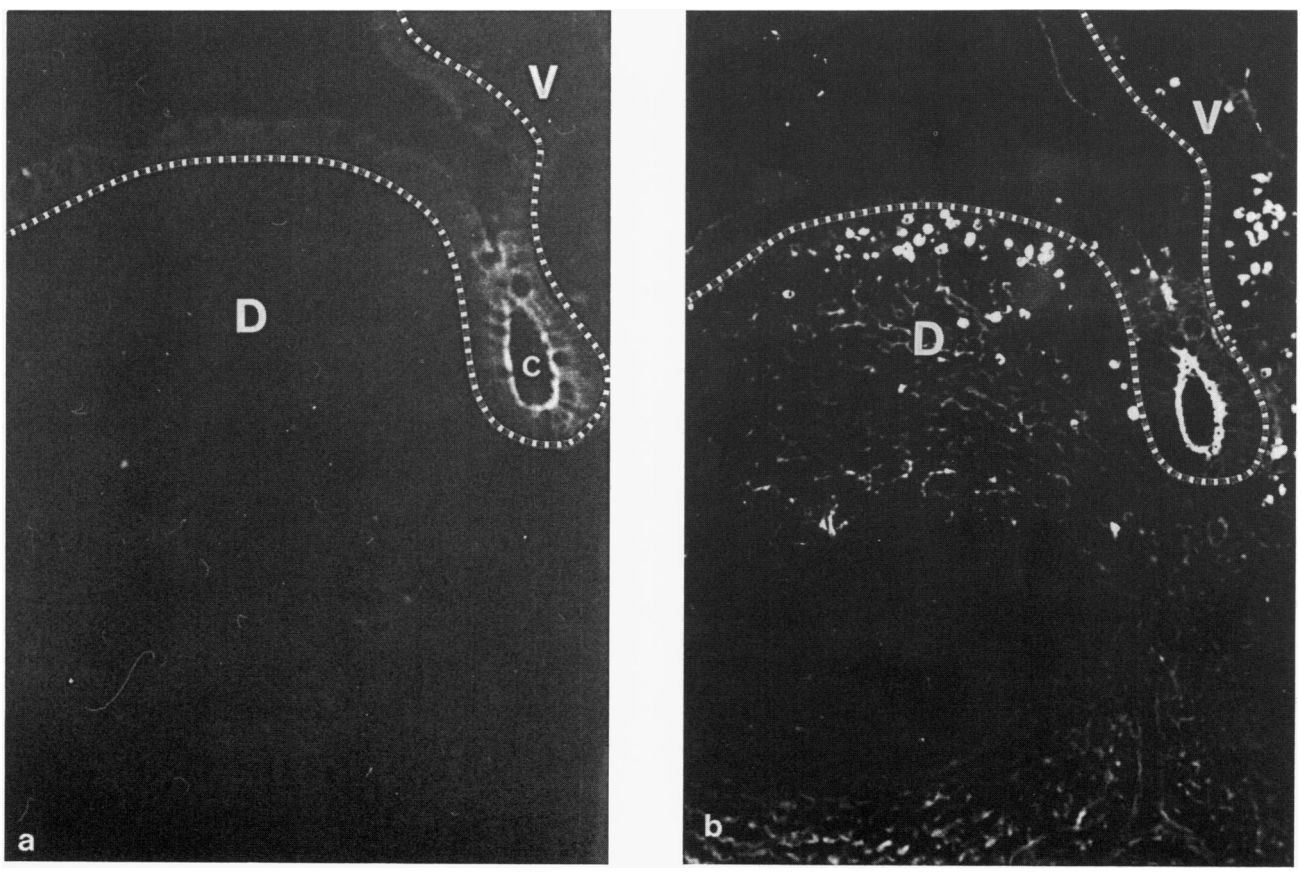

Fig. 2. Paired immunofluorescence staining for (a) SC (FITC) and (b) IgA (TRITC) in the same field from a section of ethanol-fixed normal Peyer's patch. SC is restricted to the epithelium adjacent crypt (C), whereas the dome epithelium (left) and the villus epithelium (right) are negative. Epithelial uptake of IgA is indicated only in crypt epithelium despite the presence of several IgA-producing plasma cells in the dome area. Broken line indicates basement membrane. D, dome; V, villus.

Goblet cells, which also cause interruptions in the brush border staining for alkaline phosphatase, could be excluded because of their empty cytoplasm. For a control the sections were subsequently stained with alcian blue and Periodic Acid Schiff (PAS) that should detect most or all mucin in this part of the gut (Mowry, 1956; Bancroft & Cook, 1984).

Dewaxed sections of 6 μm thickness were subjected to paired immunofluorescence staining with an FITC-labelled rabbit IgG anti-human IgA conjugate combined with tetramethylrhodamine isothiocyanate (TRITC)-labelled sheep IgG anti-human SC for 30 min at room temperature. Preparation and characterization of these fluorochrome conjugates have been detailed elsewhere (Brandtzaeg, 1981).

Microscopy

The fluorescence microscope was a Leitz Orthoplan equipped with an Osram HBO 200W lamp for red fluorescence emitted by TRITC or by the fast red colour product, and with an XBO 150 W lamp for green (FITC) fluorescence. A Ploem-type epi-illuminator was used for narrow-band excitation and selective filtration of the fluorescence colours. Single- and double-exposed colour slides were obtained with Agfa 1000 RS daylight film.

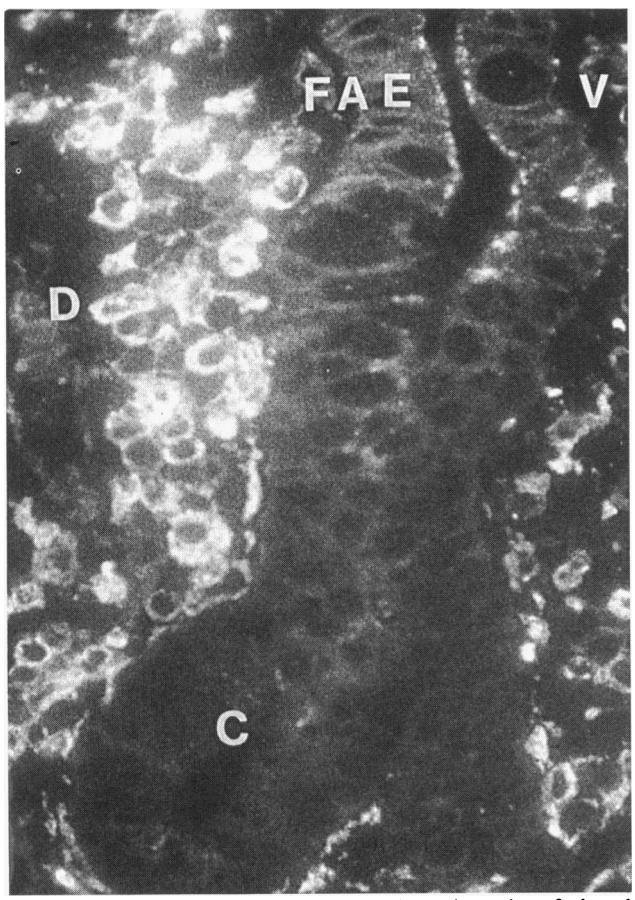

Fig. 3. Immunofluorescence staining for HLA-DR in section of ethanol-fixed normal Peyer's patch with adjacent crypt region. There is only faint DR expression in the epithelium of the crypt mouth region in contrast to strong plasma membrane-related and apical expression seen in follicle-associated epithelium (FAE) and villous epithelium (V). Numerous subepithelial DR-positive histiocytic cells are present in the dome area. C, crypt; D, dome. ×600.

RESULTS

Secretory component and IgA were mainly present in the epithelium of the crypts. There were no signs of IgA transport through FAE despite a considerable number of IgA-producing cells in the dome (Fig. 2)

Epithelial HLA-DR expression was distinctly localized to the apical portion of the FAE with patchy luminal intensification in a pattern similar to that observed for the villous epithelium. In addition, there was faint staining of the cytoplasm with intensification along the basolateral borders (Fig. 3). Numerous DR-positive sub-epithelial histiocytic cells of various morphology were observed, particularly in the dome area (Fig. 3).

There was no expression of HLA-DR (or at least less) in those regions of the FAE where the brush border showed a lack of alkaline phosphatase, regardless of absence or presence of staining for mucin (Fig. 4). We evaluated 48 putative M cells (range per subject: 5–14); none of them showed convincingly the patchy apical intensification of HLA-DR staining. However, for seven of the 48 cells faint staining was observed. A possible basolateral DR expression on the M cells could not be excluded because of the strong staining of the adjacent FAE cells.

DISCUSSION

In this study we tried to identify human M cells indirectly by the method of Owen & Bhalla (1983), using absence of the brush border alkaline phosphatase as a characteristic of these cells. We found that the putative M cells showed no HLA-DR expression (or largely reduced expression) compared with the remaining FAE. They were likewise negative for SC, which is the polymeric Ig (pIg) receptor mediating epithelial transport of J-chain-containing pIgA and pIgM (Brandtzaeg & Prydz, 1984).

The M cell has been the subject of much speculation concerning its possible function. Electron-microscopic studies demonstrating cytoplasmic vesicles (Bockman & Cooper, 1973; Owen & Jones, 1974) indicate that it performs transport. M cells of the mouse ileum were shown to endocytose and translocate macromolecules (Bockman & Cooper, 1973; Owen, 1977) and bacteria (Owen *et al.*, 1982) from the gut lumen. These observations suggested that the FAE is a preferred site for antigen uptake and transport. The importance of surface receptors on M cells in this process is still poorly defined. Wolf *et al.* (1981) described the effects of host variables (age and strain) and of reovirus serotype on the transport through M cells; in neonatal mice, type 1 reovirus attached only to M cells, whereas type 3 adhered to both M cells and other intestinal absorptive cells. It has been speculated that MHC molecules may mediate cellular transport (Unanue & Allen, 1986); but the absence of apical HLA-DR expression on putative human M cells in the present study renders this possibility unlikely for these cells.

A secretory function for M cells was proposed by Bockman & Stevens (1977) when they noted that intravenously injected peroxidase appeared in the cytoplasm of these cells. As pointed out by others (Lupetti & Dolfi, 1980), however, this observation might reflect luminal uptake after removal of peroxidase substrate by the liver with subsequent secretion into the

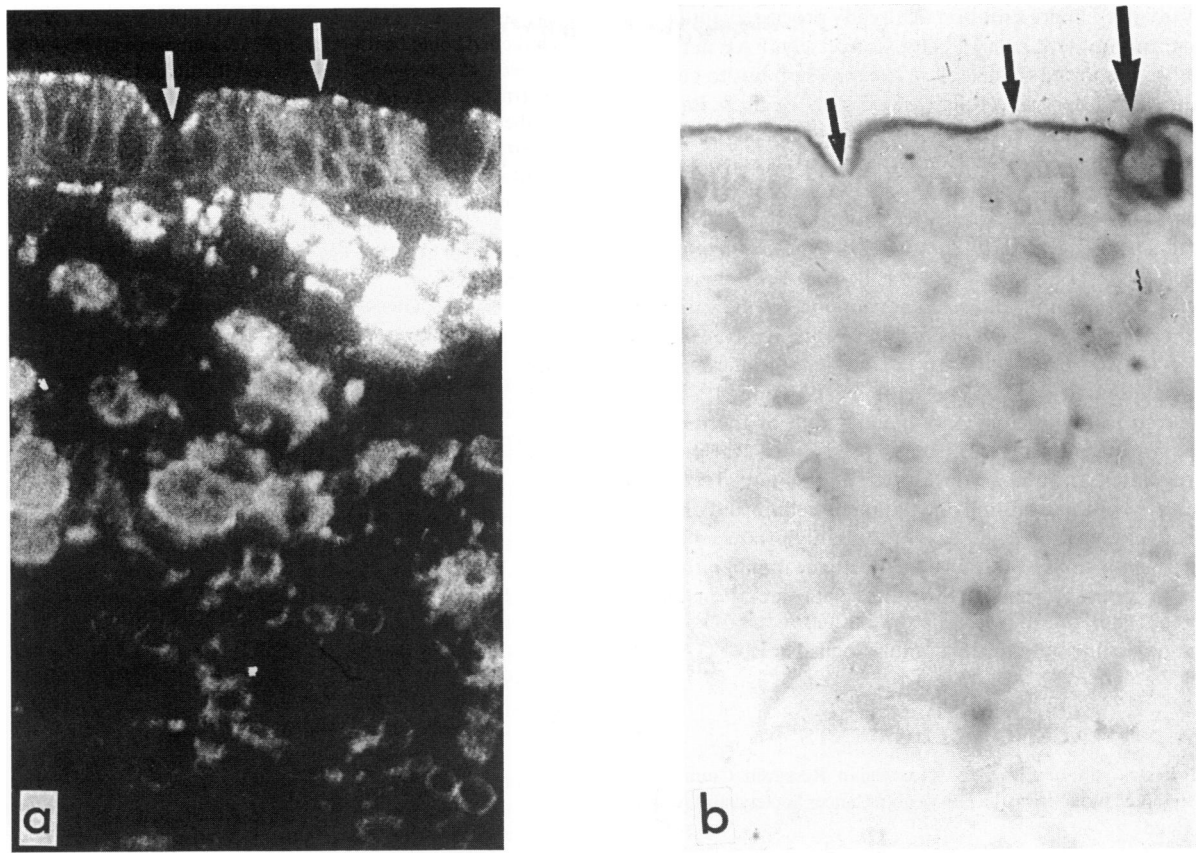

Fig. 4. Co-staining for (a) HLA-DR(immunofluorescence with FITC) and (b) alkaline phosphatase and mucine (alcian blue/PAS) in same field from section of ethanol-fixed normal Peyer's patch. Interruptions in the brush border staining for alkaline phosphatase indicate M cells (thin black arrows). The goblet cell is positive for mucin (bold black arrow). Note that there is little or no epithelial expression of HLA-DR corresponding to putative M cells (white arrows). There are numerous HLA-DR-positive histiocytic cells in the subepithelial dome area. ×600.

intestine. Nevertheless, after ligation of the jejunum, peroxidase could still be found in the M cells (Bockman, Boydston & Beezhold, 1983). The lack of SC expression by the whole FAE, as shown in the present study, contradicted previous suggestions that M cells may participiate in secretion of IgA. Absence of both SC and IgA in the FAE was in striking contrast to their presence in the columnar cells of crypts juxtaposed to PP. Moreover, IgA producing cells in the dome area show reduced J-chain expression compared with those adjacent to crypts (Bjerke & Brandtzaeg, 1986).

The topical absence of external IgA transport and the scarcity of mucin-producing goblet cells in the FAE, along with the porosity of its basement membrane (McClugage, Low & Zimny, 1986), probably promote antigen uptake at these sites of organized lymphoepithelial structures. This is most likely to be advantageous to the immunological role of the gut-associated lymphoid tissue (GALT), such as PP, in which most gastrointestinal immune responses are thought to be elicited (reviewed by Brandtzaeg, 1985; Carlson & Owen, 1987). However, these characteristics of the FAE may be assumed to render the lymphoid nodules covered by it particularly vulnerable in terms of infection (Owen, 1983).

Both human and murine M cells have cytoplasmic extensions enfolding mononuclear cells (Owen & Jones, 1974; Bye *et al.*, 1984). In human PP we recently found indications of a spatial relationship between intraepithelial T cells and M cells (Bjerke, Brandtzaeg & Fausa, 1988). On the basis of such observations it is tempting to speculate that M cells may have an antigen-presenting function. MHC class II molecules act as genetic restriction elements when antigen is presented to $CD4^+$ T cells (Uanue, 1984), and Hirata *et al.* (1986) recently demonstrated by immunoelectron microscopy HLA-DR expression on two of three studied M cells. This observation favoured the notion about an antigen-presenting function of these cells, but their location in relation to solitary lymphoid nodules or PP was not defined.

Several studies have shown that the density of MHC class II-molecules varies considerably among cells expressing such determinants and this disparity may influence their ability to contribute to immune responsiveness (Uanue, 1984; Janeway *et al.*, 1984). Our study, therefore, did not support the possibility that M cells of PP have an important antigen-presenting role. Most of the M cells showed no apical DR expression in contrast to the remaining FAE which was strongly positive like villous epithelium. It is therefore possible that the function of M cells is restricted to transport of non-degraded luminal material to underlying macrophages or other DR-positive antigen-presenting cells (Brandtzaeg, 1985), whereas the remaining FAE may be

directly involved in presentation of already processed antigen to the numerous intraepithelial T cells. Conversely, FAE in the rat gut has been reported to lack class II expression but to contain class II-positive dendritic cells (Mayrhofer, Pugh & Barclay, 1983; Wilders, Sminia & Janse, 1983). This disparity may be a matter of differences in local immunological activity because FAE of rat bronchus-associated tissue was recently shown to become strongly class II positive upon antigen challenge (Simecka, Davis & Cassel, 1986; van der Brugge-Gamelkoorn *et al.*, 1986b).

In conclusion, our findings did not support the view that the FAE (including the M cells) of human PP is involved in SC-mediated transport of IgA. Both HLA-DR molecules and SC seem to belong to the Ig supergene family (Williams, 1984) and are involved in the afferent and efferent limb of the mucosal immune system, respectively. Their expression is clearly differently regulated in human intestinal epithelium. HLA-DR molecules are normally found on villous epithelium and on FAE but hardly in the crypts, whereas SC is mainly produced by the crypt epithelium. Since MHC class II-positive villous epithelium recently has been shown to perform antigen presentation (Bland & Warren, 1986), this may likewise be a function of FAE, which thereby may contribute to the immunological role of GALT.

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