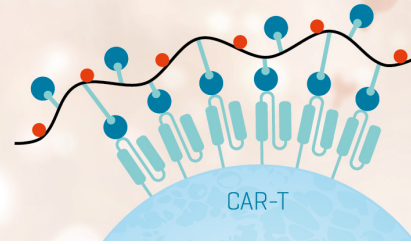


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Selective Adherence of IgA to Murine Peyer's Patch M Cells: Evidence for a Novel IgA Receptor¹

Nicholas J. Mantis,^{2*} Marsha C. Cheung,^{*} Koteswara R. Chintalacheruvu,[†] Jacques Rey,[‡] Blaise Corthésy,[‡] and Marian R. Neutra^{*}

M cells represent the primary route by which mucosal Ags are transported across the intestinal epithelium and delivered to underlying gut-associated lymphoid tissues. In rodents and rabbits, Peyer's patch M cells selectively bind and endocytose secretory IgA (SIgA) Abs. Neither the nature of the M cell IgR nor the domains of SIgA involved in this interaction are known. Using a mouse ligated ileal loop assay, we found that monoclonal IgA Abs with or without secretory component, but not IgG or IgM Abs, bound to the apical surfaces of Peyer's patch M cells, indicating that the receptor is specific for the IgA isotype. Human serum IgA and colostral SIgA also bound to mouse M cells. The asialoglycoprotein receptor or other lectin-like receptors were not detected on the apical surfaces of M cells. We used recombinant human IgA1 and human IgA2 Abs and domain swapped IgA/IgG chimeras to determine that both domains C α 1 and C α 2 are required for IgA adherence to mouse Peyer's patch M cells. This distinguishes the M cell IgA receptor from CD89 (Fc α I), which binds domains C α 2-C α 3. Finally, we observed by immunofluorescence microscopy that some M cells in the human ileum are coated with IgA. Together these data suggest that mouse, and possibly human, M cells express an IgA-specific receptor on their apical surfaces that mediates the transepithelial transport of SIgA from the intestinal lumen to underlying gut-associated organized lymphoid tissues. *The Journal of Immunology*, 2002, 169: 1844–1851.

The intestinal mucosa represents a vast surface area covered by a single layer of epithelial cells. Secretory Abs, especially secretory IgA (SIgA),³ provide an important immunological barrier that prevents commensal and pathogenic bacteria from gaining access to epithelial cell surfaces (1, 2). For mucosal Ags to elicit a SIgA response, they must first be transported across the epithelium and delivered to the organized mucosa-associated lymphoid tissues (O-MALT). Selective Ag transport is accomplished by M cells, a specialized epithelial cell located exclusively within the follicle-associated epithelium (FAE) overlying O-MALT (3). The apical and basolateral surfaces of M cells have distinct features that enable them to rapidly and efficiently deliver mucosal Ags from the lumen to underlying leukocytes. For example, M cells lack the well-developed brush border and thick glycocalyx present on enterocytes (4). Consequently, M cell apical membranes are more accessible to particles, viruses, and bacteria than adjacent enterocytes (4, 5). The M cell basolat-

eral membrane is deeply invaginated to form a large intraepithelial "pocket" containing B and T lymphocytes (6) and occasional dendritic cells (7). The pocket brings the M cell basolateral surface to within a few microns of the apical surface, shortening the distance that transcytotic vesicles must travel to cross the epithelium.

There is evidence that neonatal and adult M cells express an IgR on their apical surfaces that functions in the binding and transepithelial transport of Abs from secretions. Roy and Varvayanis (8) first proposed the existence of such a receptor based on their observation that milk-derived IgA accumulated on the apical surfaces of suckling rabbit Peyer's patch M cells, but not on the apical surfaces of other intestinal epithelial cell types. Endogenous SIgA is also present on the luminal surfaces of M cells in adult rabbit Peyer's patch and appendix, indicating that the putative IgR is expressed throughout the lifetime of the animal (9, 10). The polymeric IgR (pIgR), which mediates basolateral to apical transport and secretion of polymeric IgA, is absent from the FAE, excluding this molecule as the M cell IgA receptor (11–15). Weltzin et al. (15) observed that colloidal gold particles coated with IgA injected into the mouse distal ileum adhered exclusively to Peyer's patch M cells. Furthermore, within minutes after injection IgA colloidal gold particles were detected within M cell cytoplasmic vesicles and in the extracellular space of M cell pockets, raising the possibility that M cells may mediate the transepithelial transport of Ab from the intestinal lumen to the O-MALT.

In this study we demonstrate that murine Peyer's patch M cells selectively bind IgA, with or without secretory component (SC), but not IgG or IgM Abs. IgA binding to M cells is not mediated by known lectin-like IgRs or by previously described Fc α Rs, including CD89 and the recently described Fc α μ R (16). Using human IgA subclasses and recombinant chimeric Igs we provide evidence that the M cell IgA receptor requires domains C α 1 and C α 2 of IgA, further differentiating it from other known IgRs. Finally, we observed that M cells in the human ileum are coated with SIgA, raising the possibility that human M cells, like those in the mouse,

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³ Abbreviations used in this paper: SIgA, secretory IgA; UEA-1, *Ulex europaeus* type I; TRITC, tetramethylrhodamine isothiocyanate; O-MALT, organized mucosa-associated lymphoid tissue; FAE, follicle-associated epithelium; pIgR, polymeric IgR; SC, secretory component; ASGPR, asialoglycoprotein receptor; Cy3, indocarbocyanine.

may express an IgA-specific receptor on their apical surfaces that mediates the transcytosis of SIgA and SIgA-Ag complexes.

Materials and Methods

Chemical and biological reagents

FITC- and tetramethylrhodamine isothiocyanate (TRITC)-conjugated streptavidin was obtained from Pierce (Rockford, IL). HRP conjugated to avidin was from Sigma-Aldrich (St. Louis, MO). The lectin *Ulex europaeus* type I (UEA-1) was obtained from Vector Laboratories (Burlingame, CA). Neoglycoproteins were purchased from EY Laboratories (San Mateo, CA). Paraformaldehyde (16% aqueous solution) and Epon 812-Araldite 502 embedding chemicals were purchased from Electron Microscopy Sciences (Warrington, PA). Mowiol was purchased from Calbiochem (San Diego, CA) and mounting medium containing 1,2-diazobicyclo-(2,2,2)-octane (Sigma-Aldrich) was made as described (17).

Igs and Ig labeling

Biotinylated mouse serum IgG was from Pierce. MOPC-315 (mouse IgA, λ), TEPC-15 (mouse IgA, κ), TEPC-183 (mouse IgM, κ), MOPC-104e (mouse IgM, λ), human colostral IgA, and human serum IgA were purchased from Sigma-Aldrich. Purified human myeloma IgA1 and IgA2 were from Calbiochem. The generation and purification of recombinant human IgA1, IgA2 of the m-1 allotype (IgA2 m1), human IgA1 lacking the hinge region, IgG2, and IgG2 with CH1 of IgA1 have been previously described (18, 19).

Igs were biotinylated using EZ-link sulfo-NHS-LC-biotin (Pierce) or EZ-link LC-biotin-hydrazide (Pierce) following instructions provided by the manufacturer. Biotinylation was confirmed by dot blot analysis using streptavidin-HRP and an ECL detection kit purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Protein concentrations were determined using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). Biotinylated Igs (0.8–1 mg/ml in PBS) were stored at -80°C until before use.

Mouse polymeric/dimeric IgA was purified from MB.2 hybridoma supernatant by size exclusion chromatography on a Sephacryl S300 column ($2.6 \times 100 \text{ cm}^2$) and conjugated to indocarbocyanine (Cy3) using Fluorolink (Amersham Pharmacia Biotech). Cy3-labeled polymeric/dimeric IgA were mixed with recombinant SC produced in Chinese hamster ovary cells⁴ to reconstitute SIgA *in vitro* (20).

Animals

Mice and rats used in this study were housed under conventional specific pathogen-free conditions and were treated in strict compliance with guidelines established by Children's Hospital (Boston, MA), Harvard Medical School (Boston, MA), and the National Institutes of Health (Bethesda, MD). Female BALB/c mice, 6–8 wk of age, were purchased from Charles River Breeding Laboratories (Wilmington, MA). Asialoglycoprotein receptor (ASGPR)-deficient mice carrying homozygous null mutations in the mouse hepatic lectin-2 subunit of the receptor (21) were purchased from The Jackson Laboratory (Bar Harbor, ME). IgA-deficient mice originally generated by Dr. G. Harriman (Baylor College of Medicine, Houston, TX) (22) were a gift from Dr. J. Nedrud (Case Western Reserve University, Cleveland, OH). Sprague Dawley mice were obtained from Taconic Farms (Germantown, NY).

Ligated ileal loop assays and processing of Peyer's patch tissues

Mouse ligated ileal loop assays were done as previously described (15). During the procedure mice were maintained under avertin (5 g tri-bromoethanol in 2.5 ml *t*-amyl alcohol; 200 mg/kg animal weight) anesthesia and kept warm on a 37°C warming pad. Biotinylated Abs were injected into ligated ileal loops at a concentration of 100 $\mu\text{g}/\text{ml}$, unless noted otherwise. M cells were labeled *in vivo* by coinjection of TRITC-labeled UEA-1 (20 $\mu\text{g}/\text{ml}$). At the completion of the experiment, the mice were sacrificed by cervical dislocation and Peyer's patches were removed, gently washed in PBS to remove unbound Abs, then immersed in paraformaldehyde (4% w/v in PBS). Whole, fixed Peyer's patches were labeled with streptavidin-FITC (20 $\mu\text{g}/\text{ml}$) to detect bound biotinylated Igs, then cryosectioned using a

Leica cryostat model CM3050 (Leica, Nussloch, Germany). Sections were captured on Superfrost microscope slides (Fisher, Pittsburgh, PA) and coverslips were mounted with using Mowiol. Alternatively, freshly isolated Peyer's patch tissues were fixed in paraformaldehyde (4% w/v in 0.1 M cacodylate buffer) and embedded in Epon-Araldite, as described previously (23). Epon-Araldite blocks were sectioned using glass knives mounted on a Leica Ultracut E microtome. Semithin plastic sections (0.5–1 μm) were etched with melting solution and labeled with streptavidin-FITC (2 $\mu\text{g}/\text{ml}$) and UEA-1 TRITC (2 $\mu\text{g}/\text{ml}$) (23).

Immunolabeling rat liver and intestinal tissues

Freshly excised rat Peyer's patch or liver tissue samples were embedded in Tissue-Tek O.C.T. (Sakura FineTek, Torrance, CA) in plastic base molds (Curtin Matheson Scientific, Houston, TX), then snap-frozen in liquid nitrogen-cooled isopentane. Acetone-fixed, frozen sections (5–7 μm) of rat liver or Peyer's patch were labeled with polyclonal rabbit anti-rat ASGPR antiserum (kindly provided by Dr. D. Alpers, Washington University, St. Louis, MO (24)) or control nonimmune serum, followed by biotinylated goat anti-rabbit IgG (Southern Biotechnology Associates, Birmingham, AL) and streptavidin-FITC.

Immunolabeling human intestinal tissues

Paraffin sections of pediatric ileal biopsies were obtained from the Children's Hospital Department of Pathology after approval from the Children's Hospital Internal Review Board. Sections (5 μm) were deparaffinized, blocked for 1 h in PBS containing goat serum (2% v/v), and stained with biotin-labeled, affinity-purified goat anti-human IgA, IgG, or IgM Abs (ICN Pharmaceuticals, Costa Mesa, CA) followed by streptavidin-FITC.

Microscopy

Tissue sections were viewed using a Zeiss Axiophot microscope (Carl Zeiss, Thornwood, NY) equipped for epifluorescence or a Bio-Rad MRC1024 confocal microscope (Bio-Rad). When using the Zeiss Axiophot, images were either photographed using a 35-mm camera and Kodak Elite Chrome 400 film (Kodak, Rochester, NY) or collected electronically using a SPOT digital camera (Diagnostic Instruments, Sterling Heights, CA). When using the MRC1024 confocal microscope, images were collected electronically using software provided by the manufacturer. Electronic images were edited and annotated using Adobe Photoshop (Adobe Systems, Mountain View, CA).

Results

Ig binding to the apical surfaces of mouse Peyer's patch M cells is IgA specific

We began by establishing a convenient model for studying the interaction of Igs with M cells. To confirm IgA binding in this model, mouse hybridoma IgA TEPC-15 (mouse IgA, κ) or MOPC-315 (mouse IgA, λ) were biotinylated and injected into BALB/c mouse ligated ileal loops containing Peyer's patches. After 40 min the Peyer's patches were removed, fixed, and frozen or embedded in EPON. Frozen thick sections or semithin EPON sections were labeled with streptavidin-FITC to detect biotinylated IgA. Both monoclonal IgA Abs irrespective of L chain identity bound to the apical surfaces of most M cells within the Peyer's patch FAE (Fig. 1B). M cells were identified by phase contrast microscopy (Fig. 1A) and by costaining with the lectin UEA-1 (Fig. 1, C and D). Neither monoclonal IgA Ab bound to adjacent FAE enterocytes or villus enterocytes, although both Abs occasionally associated with the mucus layer overlying the FAE and villus epithelium (data not shown).

To determine the isotype specificity of Ig recognition by M cells, mouse monoclonal IgM Abs TEPC-183 and MOPC-104e, and mouse polyclonal serum IgG, were biotinylated and tested in the ligated ileal loop assay. While IgA bound to Peyer's patch M cells (Fig. 2A), neither IgG nor IgM adhered to the FAE (Fig. 2, C and D), even when these Igs were injected into

⁴ A. Phalipon, A. Cardona, L. Edelman, J.-P. Kraehenbuhl, P. Sansonetti, and B. Corthésy. Secretory component: a new role in secretory IgA-mediated immune exclusion *in vivo*. Submitted for publication.

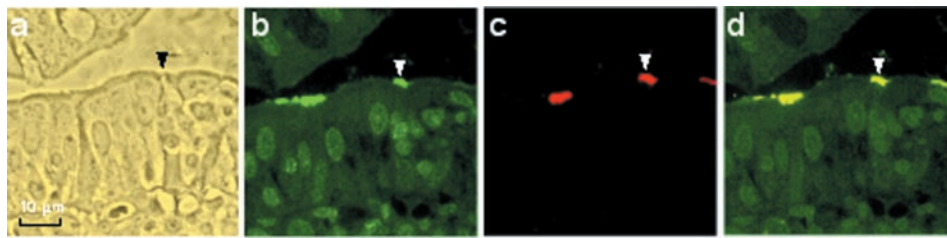


FIGURE 1. Selective adherence of IgA to the apical surfaces of mouse Peyer's patch M cells. Biotinylated mouse monoclonal IgA Ab TEPC-15 (100 $\mu\text{g}/\text{ml}$) and TRITC-labeled UEA-1 (20 $\mu\text{g}/\text{ml}$) were coinjected into a BALB/c ligated ileal loop containing a single Peyer's patch and incubated for 40 min. A plastic 1- μm section of Peyer's patch was labeled with streptavidin-FITC and visualized by phase contrast (A) and fluorescence (B–D) microscopy. A, Phase contrast image shows FAE and villus epithelium (upper left). When in the proper plane of section (arrowhead), M cells can be identified morphologically by their abbreviated apical membrane and the clusters of lymphocytes in their intraepithelial pockets. B, The same section viewed by fluorescence microscopy shows that IgA adhered to a subpopulation of cells within the FAE, but not to cells in villus epithelium. C, UEA-1 identifies M cells within the FAE. D, Merge of B and C demonstrates colocalization of IgA and UEA-1 on the apical surfaces of M cells (arrowhead).

ligated loops at concentrations exceeding 1 mg/ml. In competitive inhibition assays, adherence of biotinylated mouse monoclonal IgA to Peyer's patch M cells was reduced to undetectable levels by coinjection of 100-fold excess (w/w) nonbiotinylated IgA, but not by coinjection of equivalent molar excess of nonbiotinylated IgG or IgM. We considered the possibility that the M cell IgR may bind IgG or IgM with a lower affinity than IgA, and that the failure of IgG or IgM to bind to M cells could be due to competition with endogenous SIgA in the intestinal lumen. To examine this possibility, Ig binding assays were performed in IgA-deficient mice (22). In these mice IgA, but not IgM or IgG, adhered to Peyer's patch M cells (data not shown). Thus, the inability of IgM and IgG to bind to M cells is not due to competition with endogenous SIgA.

A previous report that latex beads coated with human colostrum IgA adhered to mouse Peyer's patch M cells better than beads coated with BSA (25) prompted us to test directly whether murine M cells recognize human IgA. Purified polyclonal human IgA Abs from serum and colostrum were biotinylated and examined for M cell binding using the murine ligated ileal loop assay. We found that both colostrum IgA (Fig. 2B) and serum IgA (data not shown) adhered to mouse Peyer's patch M cells. These data indicated that the structural features of IgA important for M cell binding are shared between mouse and human IgA. These data also confirm that IgA, with or without SC, can bind to M cells because human colostrum contains primarily SIgA, whereas serum IgA lacks SC.

Mouse Peyer's patch M cells bind and endocytose SIgA

We wished to test whether binding of SIgA to the apical surfaces of M cells results in Ab transcytosis, as was previously proposed by Weltzin et al. (15). To visualize IgA directly and with greater sensitivity, purified mouse monoclonal IgA consisting exclusively of dimers and higher m.w. polymers was labeled with Cy3 and associated in vitro with recombinant SC.⁴ Cy3-labeled SIgA Abs were injected into BALB/c mouse ligated ileal loops and visualized on frozen Peyer's patch tissue sections by confocal laser scanning microscopy. Forty minutes after inoculation, Cy3-SIgA Abs were detected on the apical surfaces of Peyer's patch M cells and within M cell intracellular vesicles (Fig. 3, A and B). Intracellular vesicles containing SIgA were located apically, clustered near the intraepithelial pocket membrane, and along the basolateral membranes. In some cases, SIgA was present in M cell basal processes that extend through the basal lamina (Fig. 3C) (23). These data indicate polymeric SIgA can adhere to M cell surfaces and be transported across the FAE.

IgA binding to Peyer's patch M cells is not mediated by the ASGPR

Two receptors with IgA binding capacity have been reported on rodent intestinal epithelium in vivo: the pIgR and the ASGPR. Expression of pIgR is largely restricted to crypt epithelial cells and is not present on the FAE (11–15). The ASGPR, first identified on the sinusoidal membranes of hepatocytes, is a lectin-like receptor that can bind IgA via oligosaccharide side chains bearing terminal galactose residues (26, 27). ASGPR has been detected on the apical surfaces of certain human colonic epithelial cell lines in vitro (28) and on the luminal surfaces of rat neonatal (but not adult) enterocytes in vivo (24), although its function on enterocytes remains unknown. Because it has been previously reported that rat Peyer's patch M cells bind IgA (15), we used available rabbit anti-rat ASGPR antiserum and immunofluorescence microscopy to examine whether the ASGPR is expressed on the apical surfaces of M cells in the FAE of Peyer's patches. Anti-ASGPR antiserum failed to label either the villus epithelium or FAE on frozen sections of rat Peyer's patch (Fig. 4). The specificity and activity of the anti-ASGPR antiserum was confirmed on sections of rat liver where the antiserum labeled hepatocyte sinusoidal membranes, consistent with the known distribution of ASGPR (Fig. 4).

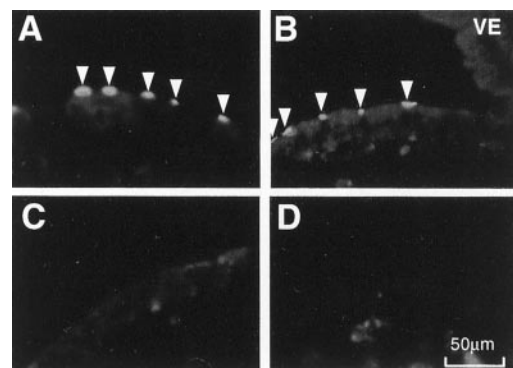


FIGURE 2. Ig binding to M cells is IgA specific. Biotinylated Igs (100 $\mu\text{g}/\text{ml}$) were assayed for M cells binding in the BALB/c ligated ileal loop assay. Whole, fixed Peyer's patches were stained with streptavidin-FITC and frozen sections (5–7 μm) were visualized by fluorescence microscopy. Mouse monoclonal IgA TEPC-15 (A) and human colostrum IgA (B) adhered to M cells within the FAE (arrowheads) but not villus epithelium (VE). Neither mouse monoclonal IgM TEPC-183 (C) nor mouse polyclonal serum IgG (D) adhered to the FAE.

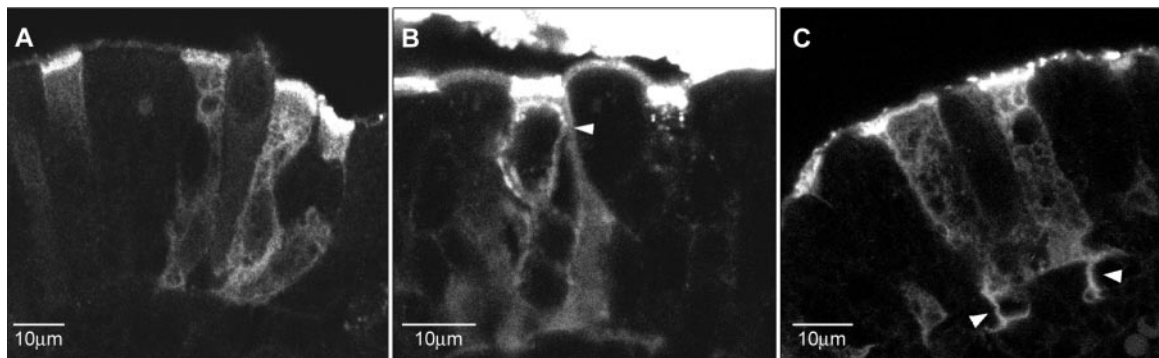


FIGURE 3. Binding and transepithelial transport of SIgA by Peyer's patch M cells. Cy3-labeled mouse monoclonal SIgA (100 $\mu\text{g}/\text{ml}$) was injected into BALB/c ligated ileal loops containing a single Peyer's patch and incubated for 40 min. Frozen sections (5–7 μm) of fixed tissue were viewed by confocal microscopy. *A*, SIgA was present within M cells and concentrated on M cell apical surfaces. *B*, In some M cells, SIgA was visible in apical vesicles, on lateral membranes (arrowhead), and on the membrane lining the intraepithelial pocket. *C*, SIgA was also detected in M cell processes (arrowheads) that extend through the basal lamina.

However, we could not exclude the possibility that the ASGPR is present on the apical surfaces of M cells at low levels not detectable by immunohistochemistry. We predicted that if the ASGPR is the IgA receptor on Peyer's patch M cells, then IgA binding to M cells would be abolished in ASGPR-deficient mice (21). Using the ligated ileal loop assay in ASGPR knockout mice, we found that IgA, but not IgM, accumulated on the luminal surfaces of Peyer's patch FAE with a pattern indistinguishable from that observed in normal mice (data not shown). Thus, we conclude that the ASGPR is not the receptor that mediates binding of IgA to the apical surfaces of M cells.

We also tested the possibility that IgA may bind via its carbohydrate moieties to other lectin-like receptors on M cells. Although no specific lectins have been identified on M cells, lectin-like receptors are widespread on mammalian cells (29). Mouse IgA contains two *N*-linked oligosaccharide side chains terminating in mannose, galactose, *N*-glycolylneuraminic acid, or *N*-acetylneuraminic acid (30). To test whether IgA oligosaccharides are important for M cell binding, IgA was biotinylated

either before or after treatment with sodium periodate to disrupt monosaccharides containing vicinal hydroxyl groups and destroy most carbohydrate epitopes (31). Periodate-treated IgA bound to the M cells in the BALB/c mouse with a pattern identical to untreated IgA (data not shown). To detect the possible presence of lectin-like receptors on M cells, we examined whether other glycoproteins with terminal galactose or mannose residues, including asialofetuin, asialoorosomucoid, BSA-galactose, BSA-mannose, or BSA-glucose, bound to M cells. No binding of any of these glycoproteins (tested at concentrations ranging from 10 to 1000 $\mu\text{g}/\text{ml}$) to M cells was detected in the BALB/c ligated ileal loop assay (data not shown). These data argue against the presence of a lectin-like receptor on the apical surfaces of M cells involved in binding SIgA.

Domains C α 1 and C α 2 are important for IgA binding to mouse Peyer's patch M cells

Identification of the domains of IgA that are important for M cell binding could provide clues as to the nature of the IgA receptor. Human IgA is comprised of two subclasses, IgA1 and IgA2 (32). The primary difference between the two subclasses is that IgA1 has a heavily *O*-glycosylated 16-aa hinge region located between domains C α 1 and C α 2, whereas IgA2 has a 3-aa hinge that is not glycosylated. Excluding the hinge, the protein sequence of human IgA1 and IgA2 differ in only 14 aa scattered along the polypeptide (32). To determine whether both classes of human IgA bind to mouse M cells, purified myeloma IgA1 and IgA2 were biotinylated and tested in the ligated ileal loop assay. We observed that human IgA2 bound to mouse M cells but human IgA1 did not (Fig. 5, *A* and *B*). The same result was obtained when recombinant human glycosylated IgA1 and IgA2 produced by mouse Sp2/O myeloma cells were tested (data not shown). This differentiates the M cell IgA receptor from CD89 (Fc α RI), a receptor on human myeloid-derived cells that recognizes IgA1 and IgA2 with equal affinity (33).

We hypothesized that the inability of IgA1 to bind to mouse M cells could be due to the presence of the 16-aa hinge located between C α 1 and C α 2. To test this we used recombinant IgA1 molecule lacking the hinge (IgA1 Δ hinge) that was otherwise fully glycosylated (18). In the BALB/c ligated ileal loop assay, IgA1 Δ hinge adhered to the apical surfaces of Peyer's patch M cells (Fig. 5, *C–F*). Furthermore, a recombinant Ig containing C α 1 of IgA2 fused to the IgG2 hinge and C γ 2 and C γ 3 domains did not bind (Fig. 6), indicating that the IgA C α 1 domain alone is not

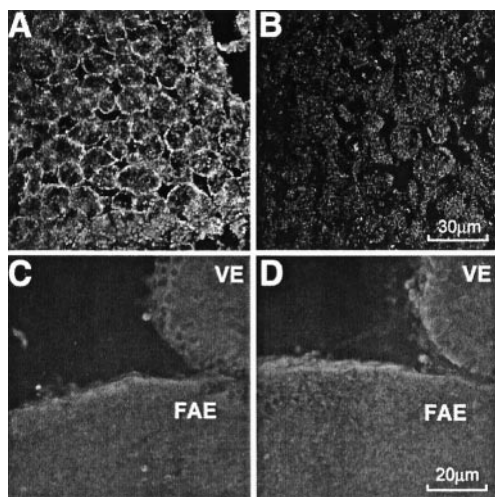


FIGURE 4. ASGPR is expressed on rat hepatocytes but not on Peyer's patch FAE. Frozen sections of rat liver (*A* and *B*) or Peyer's patch (*C* and *D*) were labeled with rabbit anti-rat ASGPR antiserum (*A* and *C*) or control nonimmune serum (*B* and *D*) as described in *Materials and Methods* and viewed by confocal microscopy. *A*, Anti-ASGPR Abs labeled sinusoidal membranes in liver sections. *B*, Control serum did not label hepatocytes. Neither anti-ASGPR antiserum (*C*) nor control serum (*D*) labeled Peyer's patch FAE or villus epithelium (VE).

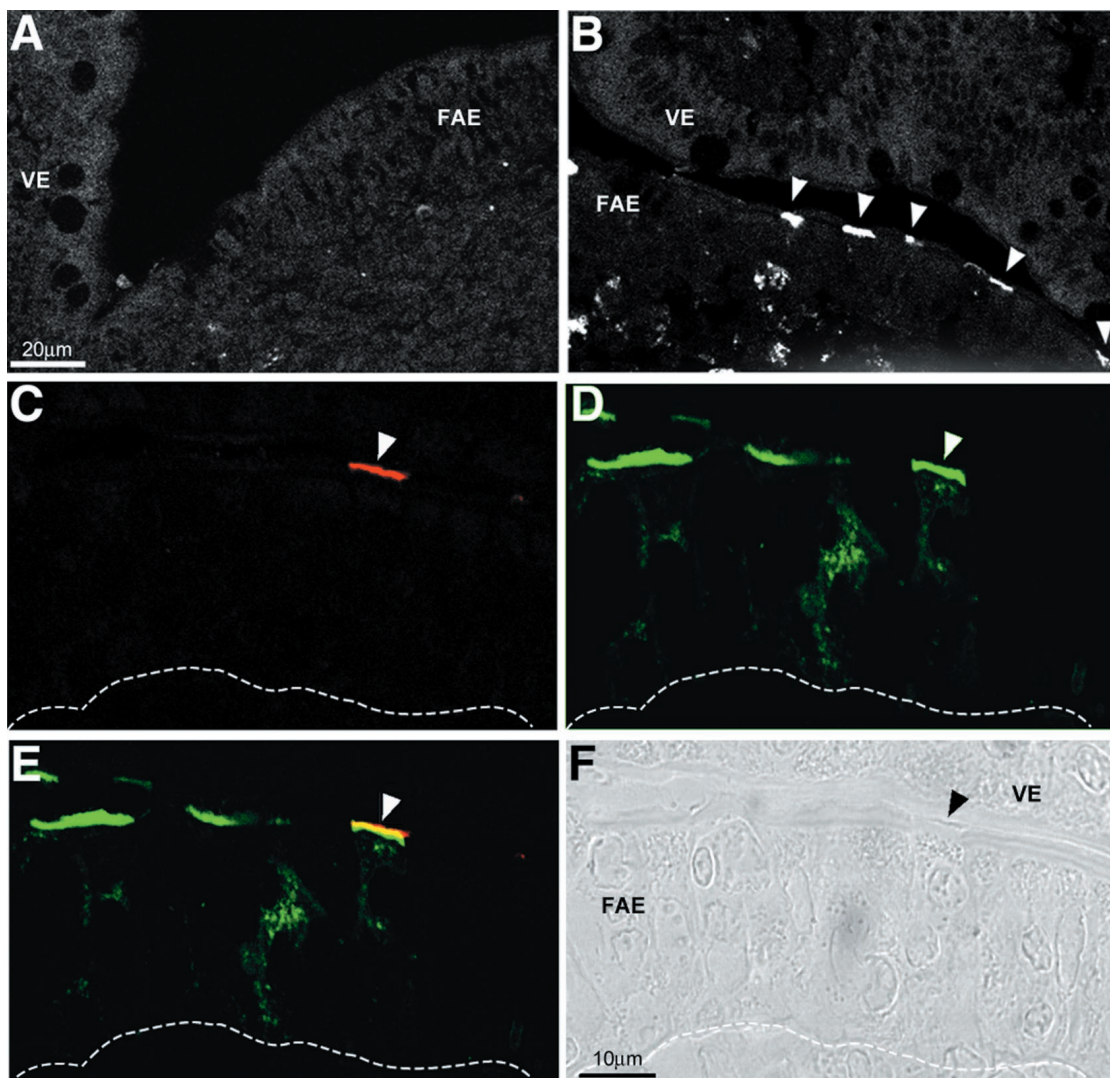


FIGURE 5. Adherence of natural variants and recombinant human IgA Abs to mouse Peyer's patch M cells. Human myeloma IgA1, human myeloma IgA2, or recombinant human IgA1 Δ hinge Abs were tested for M cell binding in the BALB/c ligated ileal loop assay. *A* and *B*, Mouse Peyer's patches were exposed to biotinylated human IgA1 (*A*) or biotinylated human IgA2 (*B*) and frozen sections were stained with streptavidin-TRITC and viewed by fluorescence microscopy. Human IgA1 (*A*) did not adhere to mouse Peyer's patch M cells, whereas human IgA2 (*B*; arrowheads) did. VE, Villus epithelium. *C–F*, A mouse Peyer's patch exposed to biotinylated human IgA1 Δ hinge in a ligated ileal loop assay was embedded in plastic and a thin section (1 μ m) was labeled with streptavidin-TRITC and costained with UEA-1-FITC to detect M cells. *C*, Biotin-IgA1 Δ hinge adhered to the FAE (arrowhead). *D*, UEA-1-FITC labels the apical and basolateral membranes of M cells. *E*, A merge of *C* and *D* indicates overlap between IgA1 Δ hinge binding and UEA-1 binding on the apical surface of one of the three UEA-1-positive M cells on this section (yellow; arrowhead). *F*, Phase contrast microscopy of the section in shown in *C–E*. Arrowhead in *F* corresponds to those in shown in *C–E*. The dashed line shown in *C–F* delineates the epithelial basal lamina.

sufficient to mediate Ig binding to M cells. Taken together these results suggest that the IgA-M cell interaction requires both C α 1 and C α 2 domains in a well-defined spatial conformation, and that the inability of human IgA1 to bind to mouse M cells is due to the extended hinge.

IgA is present on the apical surfaces of M cells from the human ileum

In adult and neonatal rabbit Peyer's patches and appendices, endogenous SIgA can be detected on the apical surfaces of M cells by immunohistochemistry (8–10). To determine whether endogenous SIgA is present on the apical surfaces of human M cells, paraffin sections of normal pediatric terminal ileum containing organized lymphoid follicles were stained with affinity-purified, goat anti-human IgA Abs. Anti-IgA Abs labeled plasma cells in the lamina propria (Fig. 7A) and occasionally in lymphoid follicles

(data not shown). Anti-IgA Abs also stained some (but not all) M cell membranes in biopsies from three different individuals (Fig. 7, B and C). M cells were identified by morphological criteria. Affinity-purified anti-IgM Abs or irrelevant control Abs did not label M cells (data not shown). These results suggest that human M cells, like those in rabbits and rodents, express a receptor for IgA.

Discussion

M cells are the primary route through which particulate Ags, soluble macromolecules, and pathogens are delivered from the intestinal lumen to inductive sites of the mucosal immune system. Adherence to the apical surfaces of these specialized epithelial cells is a prerequisite for efficient transepithelial transport. Therefore, identification of potential mediators of M cell adherence is central to understanding mucosal immune responses. In this paper we confirmed that IgA, with or without associated SC, selectively adheres

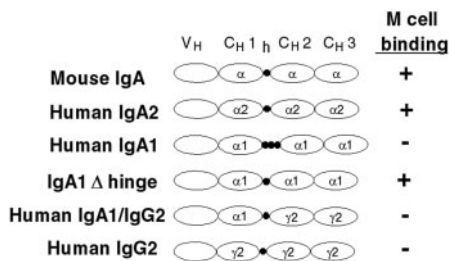


FIGURE 6. Summary of Ig binding to mouse Peyer's patch M cells. Cartoon depicting a single H chain of the natural and recombinant IgA, IgG, and chimeric IgA/IgG Abs that were tested for M cell binding in this study. The variable domains (V) of the H chains are aligned on the left, attached to the constant domains of human or mouse IgA (α) or IgG (γ). ●, The hinge regions between constant domains 1 and 2.

to the apical surfaces of mouse Peyer's patch M cells but not to other intestinal epithelial cell types. We found that the IgR on M cells is specific for IgA. This is in agreement with what was observed in suckling rabbits: maternal milk-derived SIgA, but not IgG or IgM, Abs accumulate on M cell apical surfaces (8). However, it is in contrast to a previous study from this laboratory (15) that reported IgG binding to mouse Peyer's patch M cells. This discrepancy is most likely due to differences in techniques used to trace Igs. Whereas the previous study relied on adsorbing Igs to colloidal gold particles (an association that is both reversible and difficult to quantitate), this study examined binding directly using biotinylated and fluorescent Abs.

At least four physiologically relevant receptors with IgA binding capability have been described on either intestinal epithelium or intestinal epithelial cell lines. These receptors include pIgR, ASGPR, β -1,4-galactosyl transferase, and an as-yet-undefined IgA receptor described on the colonic carcinoma cell line HT-29 (34). Through direct and indirect evidence presented in this paper, we can exclude all of these proteins as being the IgA receptor on Peyer's patch M cells. For example, ASGPR was not detectable on the FAE by immunofluorescence microscopy, and IgA bound to Peyer's patch M cells in ASGPR-deficient mice. β -1,4-galactosyl transferase recognizes IgA, IgG, and IgM Abs (albeit with varying affinity) (35), while we have shown in this study that the IgR on M cells is selective for IgA. Finally, the novel IgA receptor on HT-29 cells is unable to bind SIgA (34), whereas the M cell receptor binds IgA with or without associated SC (15). Generally, IgA receptors are classified into two groups: those that bind IgA via its oligosaccharide side chains and those that bind IgA via Fc domains (36). The observation that periodate treatment of IgA or biotinylation of IgA on carbohydrate moieties did not affect binding to M cells suggests that the receptor on M cells may recognize the polypeptide backbone of the α H chains rather than oligosaccharide side chains.

We observed that human IgA2 but not IgA1 bound to mouse Peyer's patch M cells. This finding is not physiologically relevant for mice that have only one class of IgA. Rather, this result provides new information about the domains of IgA that may be important for receptor recognition. The primary difference between the two human IgA subclasses is that IgA1 has a heavily *O*-glycosylated 16-aa hinge region located between domains C α 1 and C α 2, whereas IgA2 has a 3-aa hinge that is not glycosylated (32). Indeed, recombinant IgA1 lacking the hinge region bound to M cells. Considering that C α 1 alone was not sufficient to mediate Ig binding to M cells, we propose that the M cell IgA receptor spans the IgA hinge region and makes contact with both C α 1 and C α 2 domains. This further distin-

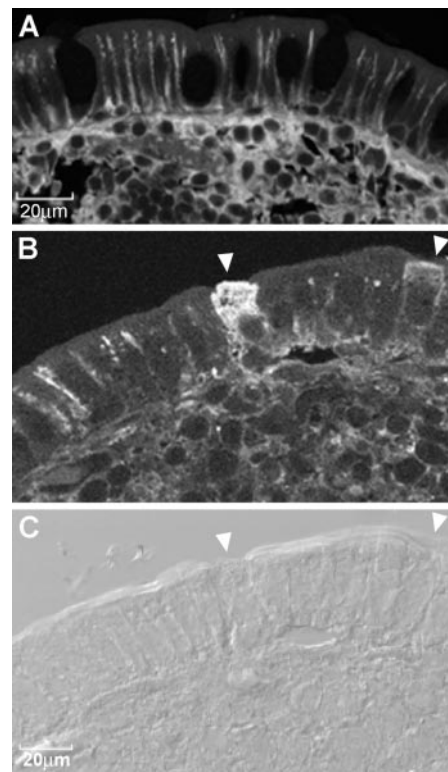


FIGURE 7. Endogenous IgA is present on the apical surfaces of M cells in the human terminal ileum. Paraffin sections of normal pediatric ileum were stained with biotin-labeled, affinity-purified anti-human IgA Abs and streptavidin-FITC, then visualized by confocal (A and B) or DIC (C) microscopy. A, A section of villus epithelium shows IgA-positive cells within the lamina propria. IgA staining is also visible along the basolateral surfaces of enterocytes. B, A section of FAE that shows M cells (arrowheads) have IgA on their apical surfaces and intracellularly. C, The same section as in B viewed by differential interference microscopy. The epithelial cells that stain positive for IgA in B correspond to M cells (arrowheads), as determined by their lack of a well-defined brush border.

guishes the M cell receptor from CD89 (Fc α RI), which recognizes the C terminus of C α 2 and N terminus of C α 3 (37, 38). We postulate that the extended hinge of IgA1 may interfere with binding by altering the spatial arrangement between C α 1 and C α 2 relative to each other such that the M cell receptor cannot simultaneously contact both domains. Alternatively, the *O*-linked oligosaccharide side chains branching from the IgA1 hinge (32) could potentially mask adjacent amino acids on C α 1 or C α 2 that are important for receptor recognition.

Although the data presented in this paper suggest that the protein responsible for binding SIgA on the apical surfaces of M cells is novel and distinct from previously described human and murine Fc α Rs, this conclusion is premature in the absence of the additional molecular information. For example, a recently described Fc α μ R has been shown by *in situ* hybridization and RT-PCR to be expressed at high levels in the small intestine (16, 39). Although the expression of this receptor was originally thought to be restricted to B cells and macrophages, it has been now reported to be present on mesangial cells (40). In light of these studies we cannot exclude the possibility that an alternative form of this receptor (which binds IgA but not IgM) could be expressed on M cells. Future studies in this laboratory will be aimed at identifying the M cell IgA receptor using molecular genetic and biochemical techniques.

The function of the IgA receptor on M cells may be to mediate the delivery of SIgA from the intestinal lumen to underlying O-MALT. Consistent with this, we observed that SIgA applied to the apical surfaces of M cells accumulated within vesicles clustered near the intraepithelial pocket membranes. Presumably these vesicles were released into the pocket. SIgA also accumulated in M cell basolateral processes that have been proposed to make direct contact with subepithelial leukocytes (23). Following M cell transcytosis, SIgA-Ag complexes could be sampled by pocket B lymphocytes (6) and/or subepithelial dendritic cells (7). Brandtzaeg et al. (41) have hypothesized that memory B cells in the M cell pocket present luminal Ags to neighboring resident T cells, promoting T cell survival and B cell differentiation. Human and murine mature B cells express an Fc α μ R that can mediate endocytosis of both IgA- and IgM-immune complexes (39), although it has not been determined whether B cells within the M cell pocket express this receptor. SIgA-immune complexes could also interact with dendritic cells that form a dense network below the FAE and occasionally migrate into the M cell pocket (7). In vitro, human monocyte-derived DC can bind and internalize SIgA (42, 43).

In this report we detected IgA on the apical surfaces of M cells in the human pediatric ileum, suggesting that human M cells, like those in the rabbit and mouse, bind and endocytose SIgA. This could have implications for understanding the regulation of mucosal immune responses in humans and potential applications for the development of oral vaccine delivery strategies. In humans a significant proportion of the intestinal microflora is coated with SIgA (44). This raises the possibility that M cells may "sample" IgA-coated commensal bacteria, promoting the maintenance of anticomensal immune responses that could control the luminal microflora and clear microorganisms from the mucosa (2). There is current interest in targeting vaccines to the apical surfaces of M cells in the human small intestine, colon, and rectum. In mice, exogenous IgA has been used as an Ag delivery vehicle, apparently promoting the sampling of oral or rectal vaccines by M cells and more efficient delivery to the mucosal immune system (45, 46). A better understanding of the interaction of SIgA with M cells and the resulting immune response is needed to assess the feasibility of such a vaccine strategy in humans.

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