M Cells in the Follicle-Associated Epithelium of the Rabbit Conjunctiva Preferentially Bind and Translocate Latex Beads

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Purpose. This study investigates the presence of M cells in the rabbit conjunctiva. Resolving whether the conjunctiva contains M cells is important, because at other mucosal sites, these antigen sampling cells are known to initiate the mucosal immune response and to act as a site of entry for opportunistic pathogens.

Methods. Fluorescent 0.2-μm polystyrene latex beads were either instilled into the conjunctival sac for 20 to 120 minutes in vivo or applied to flat mounts in vitro. Specimens were assessed by epi-fluorescence stereomicroscopy, widefield fluorescence microscopy, confocal scanning laser microscopy, and transmission and scanning electron microscopy.

Results. Latex beads preferentially bound to a subpopulation of cells in the epithelium overlying mucosal lymphoid follicles in the fornix region. At 4°C, the beads were associated with the apical surface of cells that had longer, more irregular microvilli than the surrounding epithelial cells. Within 20 minutes of an in vivo exposure, latex beads were internalized by the follicle-associated epithelial cells and with time moved into the underlying follicle region. After 120 minutes of in vivo exposure, latex beads could be found in cervical lymph nodes.

Conclusions. This study demonstrates that the follicle-associated epithelium of the rabbit conjunctiva contains a cell with morphologic characteristics and the ability to bind and translocate latex beads, which make it indistinguishable from antigen sampling M cells in the rabbit cecum and tonsils. Consistent with its hypothesized antigen sampling role, beads that have been translocated by this cell are rapidly transferred to cervical lymph nodes. (*Invest Ophthalmol Vis Sci.* 2005;46:4217–4223) DOI:10.1167/iovs.05-0280

It is widely accepted that lymphoid follicles underlying the epithelial layer are present in the conjunctival mucosal of the rabbit, the human, and many other mammals.[1–3] It is more controversial, however, whether the conjunctival follicle-associated epithelium (FAE) contains the unique antigen sampling M cells present in the FAE at other mucosal locations, such as intestinal Peyer's patches.[2,4] M cells have an unusual architecture and high endocytic abilities that allow them to sample soluble- and membrane-bound antigens from the external world and rapidly translocate them across the cell for release into intraepithelial pockets filled with lymphoid cells and antigen-presenting cells. The activated lymphoid cells can then migrate into the follicle and ultimately into regional lymph nodes to initiate an appropriate mucosal immune response. Passage across the M cell does not always lead to a positive immunologic response, however, because numerous bacterial and viral pathogens have been found to exploit the M cell as a means to breach the mucosal barrier and infect the host.[5–7]

One reason that the question of whether M cells are present in the conjunctival FAE has not been resolved is that the morphology of M cells and their expression of membrane markers vary widely not only between species, but often between different mucosal sites within a single species. The only unequivocal criterion for all M cells is the ability of an epithelial cell within the FAE to conduct transcytosis.[8] The recent demonstration that a subset of cells in the guinea pig conjunctival FAE could preferentially bind and transcytose the sialyllactose-binding lectin Maackia amurensis leukoagglutinin was the first report of M cells in the mammalian conjunctiva that met this standard.[9]

Intestinal M cells of multiple species and multiple mucosal locations have been shown to preferentially bind and transcytose hydrophobic polystyrene latex beads.[10–14] The present study examines the interaction of latex beads with the rabbit conjunctival FAE and surrounding regions.

Materials and Methods

Latex Beads

All studies in this article used 0.2-μm green-fluorescent polystyrene latex beads (Fluoresbrite YG microspheres; Polysciences, Warrington, PA) in sterile PBS.

Animals and Surgical Procedure

New Zealand White rabbits (n = 12), between 12 to 15 weeks old, were used in the study (Harlan, Indianapolis, IN). Rabbits were anesthetized with ketamine and xylazine and were given butorphanol for analgesia. Nembutal was used to euthanatize the rabbits. All experimental procedures used in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and University of Missouri Animal Care and Use Committee guidelines.

Eyelids were sutured and 300 μL of 1×10^{10} particles/mL latex beads in PBS (pH 7.4) were instilled into conjunctival sacs in vivo and incubated for 20 to 120 minutes. After exenteration of the orbits, the cornea was separated from the sclera, and the conjunctiva sac was opened and pinned out onto a flat sheet of dental wax, as previously described in detail.[15] The flat mount was rinsed with PBS and immersed in 2% freshly depolymerized paraformaldehyde in HEPES wash buffer (HWB; 70 mM NaCl, 30 mM HEPES, 2 mM $CaCl_2$, pH 7.4) at room temperature for 2 hours. To identify latex bead binding sites in the absence of uptake, some tissues were excised without prior exposure to latex beads, preincubated in ice-cold PBS for 3 minutes, and then exposed to 1×10^{10} particles/mL latex beads in PBS for 20 minutes at 4°C. These samples were rinsed in cold PBS and were fixed for 2 hours at 4°C in PFG fixative (2% freshly depolymerized paraformaldehyde + 2.5% glutaraldehyde in HWB). After fixation and rinsing in 50 mM glycine in HWB, all tissues were viewed by using a stereo-

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scope with epi-fluorescent illumination to aid dissection. All follicles used in the present study were isolated from the fornix region. No difference between follicles from the superior and inferior fornices was detected and therefore no distinction between these locations is made in the presentation of the data. Control tissues were collected from random locations in the palpebral and bulbar regions and nonfollicular regions of the fornix.

Tissue Processing

For cryomicrotomy, tissues were infiltrated with 30% sucrose and frozen in medium (Tissue-Tek OCT; Electron Microscopy Sciences, Hatfield, PA). Widefield and confocal fluorescence microscopy were used to view 10-μm cryosections.

Tissues for transmission and scanning electron microscopy (TEM and SEM) were post-fixed in 1% OsO_4 in HWB for 2 hours at room temperature, rinsed in deionized water, and dehydrated with an ethanol series. Tissues for TEM were embedded (EMbed 812; Electron Microscopy Sciences), and thin sections were counterstained with uranyl acetate and lead citrate. Tissues for SEM were critical point dried and platinum coated before viewing with an S-4700 field-emission scanning electron microscope (Hitachi; High Technologies America, Inc., Pleasanton, CA).

Morphometry

To quantify latex bead uptake, 9 randomly chosen follicles (3 from each of 3 rabbits) from each time point of the in vivo experiments were cryosectioned. The epithelial cells were stained for 4 hours with 10 μg/mL of a monoclonal antibody cocktail against keratins (AE1/AE3; Laboratory Vision, Fremont, CA) followed by 2 hours with a conjugated goat anti-mouse IgG antisera (Alexa568; Molecular Probes, Eugene, OR). Nuclei were counterstained with 3 nM 4′,6-diamino-2-phenylindole dihydrochloride (DAPI) during the secondary antibody-staining step. The entire width of the FAE in the central three sections of each follicle was photographed at $\times$400 using widefield fluorescence microscopy, and the beads within the epithelial layer and underlying follicle region were counted. An average length of 9664 μm of FAE was measured for each time point. Similarly, two randomly selected fields of view from three adjacent cryosections from three areas of the palpebral regions of the same animals were photographed and quantified; a total length of 11,556 μm of control tissue was examined for each time point. The Mann–Whitney *U* test was used to determine statistical significance.

Vimentin Immunocytochemistry

For immunocytochemical localization of vimentin, conjunctival tissue was either fixed and permeabilized by immersion into ice cold acetone or fixed in 2% formaldehyde in HWB and permeabilized by a 5 minutes exposure to 0.1% Triton X-100 in HWB. A goat anti-vimentin polyclonal antisera (Sigma Chemical Co., St. Louis, MO), mouse monoclonal VIM13.3 (Sigma) and mouse monoclonal AMF-17B developed by Alice B. Fulton (obtained from the Developmental Studies Hybridoma Bank at the University of Iowa, Iowa City, IA) gave similar results, although the goat antisera gave the strongest and cleanest signal in our protocols. Secondary labels included conjugated donkey anti-goat IgG (Alexa 568; Molecular Probes) and 10-nm colloidal gold-labeled goat anti-mouse IgG (Electron Microscopy Sciences). Secondary antibodies did not cross-react with sections of conjunctiva when applied alone or after nonimmune primary antibodies (data not shown). To visualize vimentin distribution in whole mounts by stereomicroscopy, acetone fixed tissues were rehydrated and incubated with 10 μg/mL of one of the monoclonal antibodies overnight at 4°C, rinsed extensively, and incubated in a 1:10 dilution of gold-conjugated goat anti-mouse immunoglobulins in 0.1% BSA for 4 hours. The size of the gold particles was subsequently increased by using a gold-enhancement kit for 20 minutes according to the manufacturer's protocol (Nanoprobes, Yaphank, NY). Labeling of whole mounts for examination by confocal microscopy or in wide-field fluorescence microscopic imaging of cryostat sections was performed by incubation incubated overnight in a 1:10 dilution of the goat anti-vimentin antiserum in 0.1% BSA overnight at room temperature. After extensive rinsing, the tissues were stained for 4 hours with 1 μg/mL Alexa 568 conjugated donkey anti-goat IgG. Nuclei were counterstained by inclusion of 300 nM DAPI in the secondary antibody incubation step. Cryosections of whole mount labeled follicles were prepared as described above.

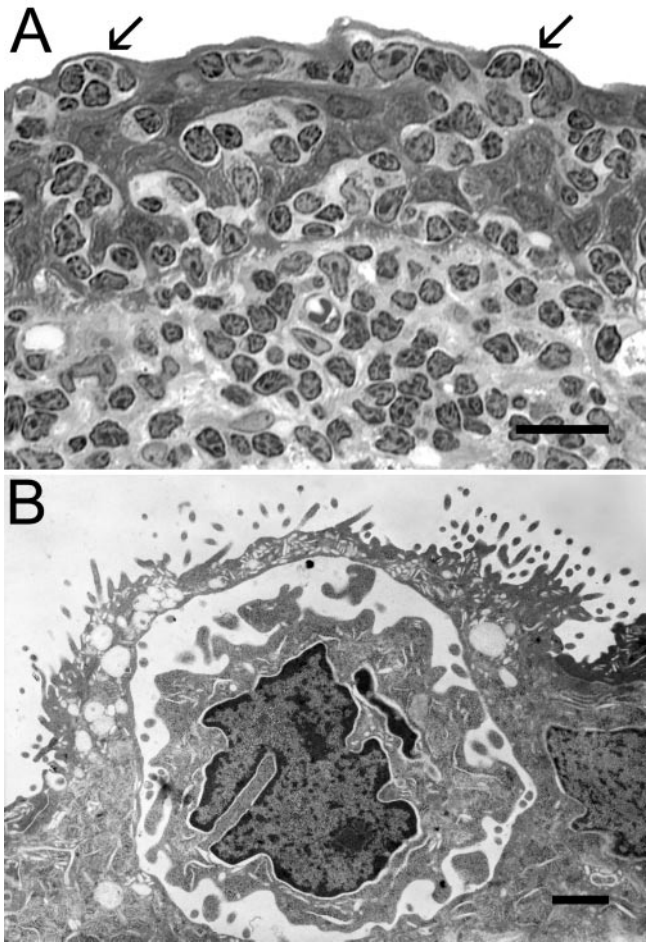

FIGURE 1. (**A**) Light microscopic view of a 0.5-μm section through a conjunctival follicle. Pockets of leukocytes can be seen pressing into the basolateral membranes of some of the FAE (*arrows*). A thin band of cytoplasm, a typical feature of membranous M cells, separates the ocular surface from the underlying follicle. The absence of goblet cells in the FAE presumably results in a reduced surface mucus coat and greater access of antigens and pathogens to the M cell surface. (**B**) TEM view of a rabbit conjunctival FAE cell with the typical morphologic characteristics of an M cell: a thin cytoplasmic bridge overlying a lymphoid cell, irregular microvilli and a cytoplasm filled with vesicles. (**A**) Bar = 25 μm; (**B**) Bar = 1 μm.

RESULTS

Morphologic Characterization of Conjunctival FAE

Lymphoid follicles were primarily restricted to the fornix region and only occasionally observed in the palpebral and bulbar regions. Follicles could be recognized under stereoscopic examination by their slight elevation and the distinctive branching pattern of blood vessels underlying the follicle region. Cross-sectional views of the follicles revealed that few or no goblet cells were present within the FAE (Fig. 1A). The FAE

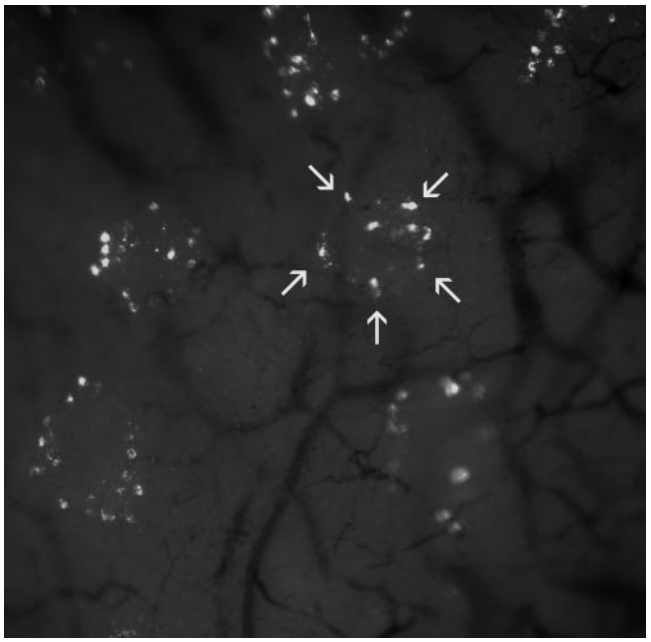

FIGURE 2. Fluorescent stereomicroscopy was used to image conjunctival whole mounts after a 60 minutes in vivo exposure to 0.2-μm fluorescent latex beads. Preferential labeling of six spherical follicles is evident in this view of the fornix. Clusters of latex beads (*arrows*) are more concentrated along the outer lateral edges of the follicular mounds.

contained morphologically distinct cells with thin apical bridges that formed as a result of lymphoid cells pressing into their lateral membranes forming intraepithelial pockets. By TEM, the epithelial cells in direct contact with the lymphoid cells frequently had irregular microvilli and an extensive tubulovesicular network within the cytoplasm (Fig. 1B).

Exposure to Latex Beads

The binding and the uptake of fluorescently tagged latex beads were examined in flat mounts by fluorescent stereomicroscopy and SEM, and in cryostat sections by using widefield fluorescent microscopy after either an in vivo exposure or in vitro incubation at 4°C.

In both in vitro and in vivo exposed conjunctiva, fluorescent stereomicroscopy revealed that latex beads were selectively associated with lymphoid follicles in the fornix region (Fig. 2). The beads were preferentially associated with the lateral flanks of the follicles. Few follicles were observed in the palpebral or bulbar regions. It was not possible when using stereomicroscopy to tell whether the latex beads were on the apical surface or had been internalized.

When SEM was used to examine lymphoid follicles after in vivo exposure to latex beads for 20 minutes or longer, few beads were found associated with the apical surface. In contrast, when conjunctival flat mounts were exposed to latex beads for 20 minutes at 4°C, significant numbers of beads remained associated with the surface. Binding of latex beads was primarily restricted to FAE cells that had microvilli that were much longer and more irregular than those on adjacent cells (Fig. 3). As many as 153 beads were found associated with the apical surface of a single cell. The consistent failure to observe latex beads on the surface of conjunctival tissues exposed in vivo indicates rapid endocytosis of the particles. The few beads found in the palpebral and bulbar regions were often associated with strands of extracellular mucus.

Morphometric analysis of cryostat sections showed that, at all time points, more latex beads were found in the FAE and underlying follicle than in the palpebral region (Fig. 4A). At 20 minutes (Fig. 5A), there were 12.97 ± 8.8 beads per 100 μm in the FAE/follicle region, while there were significantly fewer in the palpebral region (0.48 ± 0.51 beads per 100 μm; $P < 0.001$). By 60 minutes (Fig. 5B), the number of beads in the FAE/follicle had increased to 30.67 ± 22.3 per 100 μm and remained low (0.72 ± 0.57; $P < 0.001$) in the palpebral zone. At 120 minutes (Fig. 5C), the number of beads in the FAE/follicle had dropped to 5.37 ± 2.75 per 100 μm but was still significantly higher than the 0.44 ± 0.81 beads per 100 μm found in the palpebral region ($P < 0.001$). The relative proportion of beads in the FAE/follicle region present within the subepithelial follicle itself increased at each time point (Fig. 4B). At 20 minutes, most beads remained within the FAE zone, and only 1.3% of the beads had migrated to the follicle. By 60 minutes, the proportion of beads in the follicle had climbed to 9.9% and, by 120 minutes, to 33%. The rise in the proportion of beads in the follicle region over time, combined with the decrease in the total number of beads in the FAE/follicle region that occurred between the 60 and 120 minutes time points, suggested that the beads were first transported to the follicle and then rapidly exited the follicle via the lymphatic drainage. Consistent with this hypothesis, we found fluorescent beads in cryostat cross-sections of cervical lymph nodes after a 120-minute conjunctival exposure (Fig. 5D).

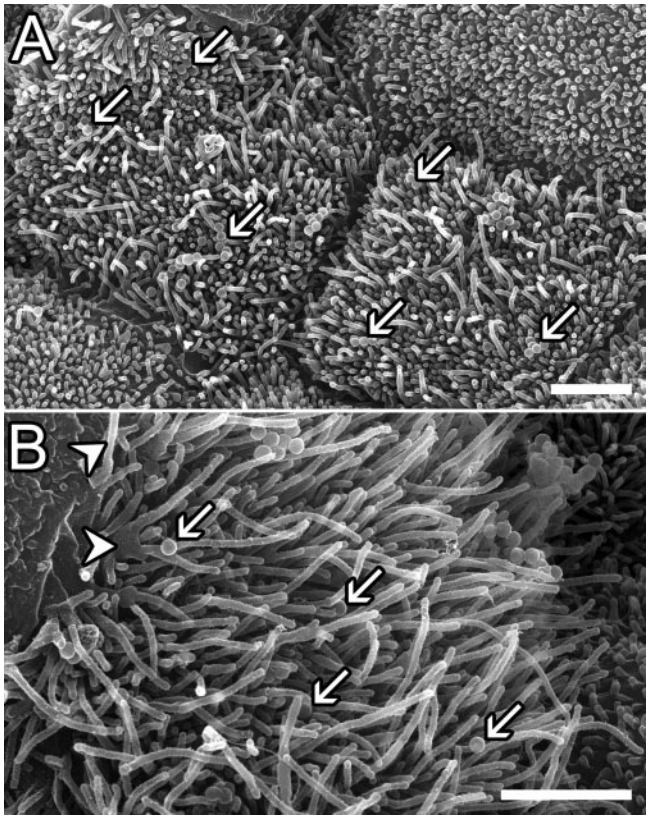

FIGURE 3. SEM images showing FAE cells in the fornix after a 20-minute in vitro incubation of conjunctival whole mounts with 0.2-μm green fluorescent latex beads at 4°C. Latex beads (*arrows*) are preferentially associated with cells that have longer, more pleiomorphic, and less densely spaced microvilli than the surrounding cells. Some microvilli on these cells branch (*arrowheads*). (**A**) Bar = 2 μm; (**B**) Bar = 1 μm.

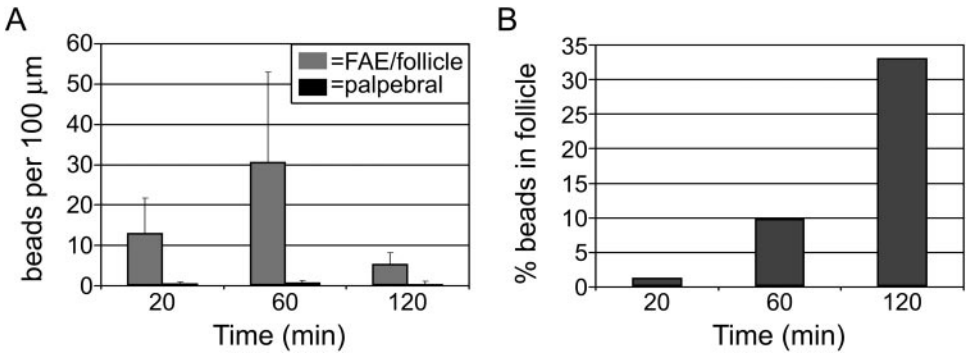

FIGURE 4. (**A**) The number of beads in the FAE and follicle region (*gray bars*) was compared with the number in a similar length of a palpebral region (*black bars*) after in vivo exposures for 20, 60, and 120 minutes At all time points, the number of beads in the FAE/follicle region was significantly higher ($P < 0.001$) than the corresponding palpebral region. (**B**) The relative proportion of beads in the follicle region compared with the total number of beads in both the FAE and follicle increases with time after the in vivo application.

Vimentin Immunolocalization

Immunocytochemical labeling of the intermediate filament vimentin has been found to be a marker for M cells at other mucosal sites in the rabbit.[16,17] Stereoscopic imaging of gold-labeled antivimentin stained whole mounts showed that labeling was predominately found along the lateral margins of most conjunctival follicles in a distribution pattern similar to that seen after latex bead binding (Fig. 6A). In some follicles, however, vimentin labeling was limited to more centrally located cells. In cryostat sections of whole mount labeled conjunctiva after a 30-minute in vivo exposure to latex beads, the strongest labeling by the antivimentin antiserum was found in FAE cells with M-cell-like morphology and which frequently contained fluorescent latex beads (Figs. 6B, 6C). Although we could continue to identify vimentin-positive cells with an M-cell morphology in paraffin or acrylic resin cross-sections of aldehyde-fixed conjunctiva, there was equal or stronger labeling of lymphocytes in both the follicle and FAE regions, subepithelial fibroblasts, follicular endothelial cells, and occasional epithelial cells outside the FAE zone that prevented our use of this label as an unequivocal marker in cross-sectioned materials (data not shown). Exposure to ethanol during fixation or dehydration before embedding seemed to contribute to this loss of selectivity.

DISCUSSION

Although M cells frequently have distinctive morphologic phenotypes, the only unequivocal criterion for an M cell is dem-

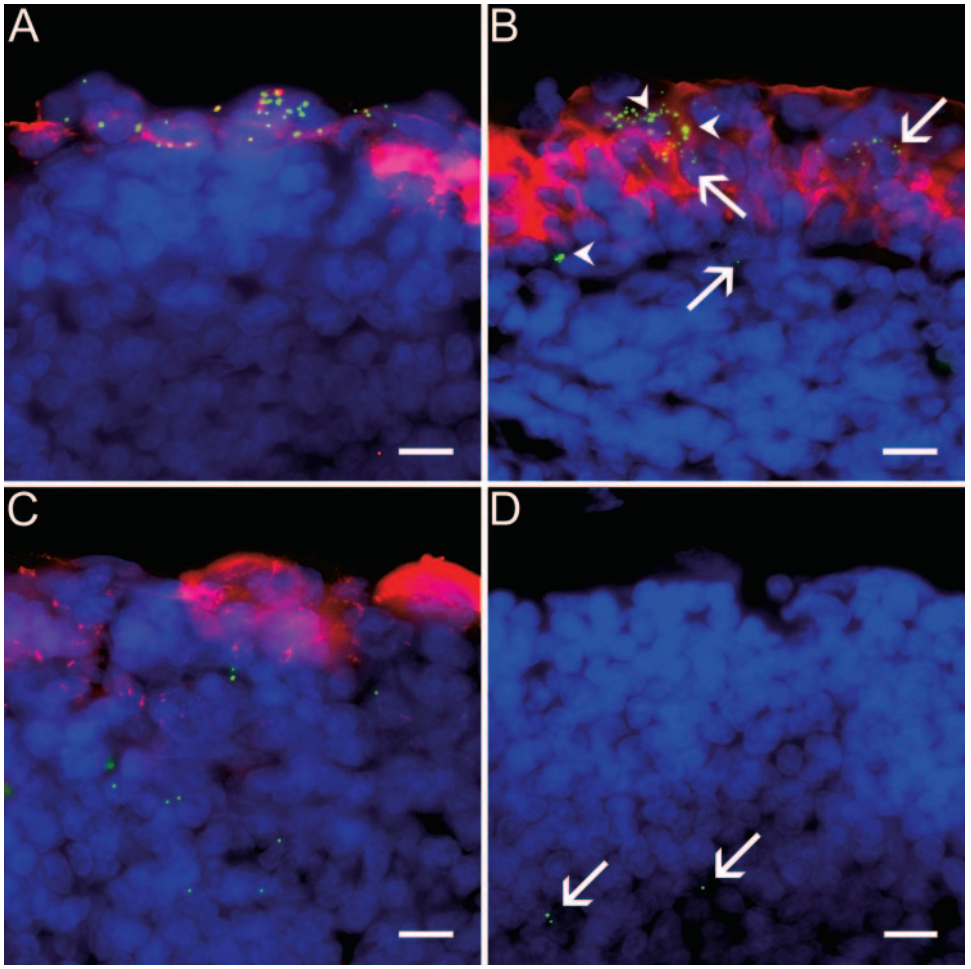

FIGURE 5. Cryostat sections of conjunctival lymphoid follicles (**A**, **B**, and **C**) and cervical lymph node (**D**) after in vivo exposure to 0.2-µm green fluorescent latex beads. The epithelium was been counterstained with anticytokeratin antibodies (*red*) and the nuclei with DAPI (*blue*). (**A**) After a 20-minute exposure, most beads are located in the superficial epithelial layers. (**B**) After 60 minutes, both single beads (*arrows*), as well as conglomerations (*arrowheads*) are found deeper in the epithelial layer or in lymphoid pockets within the epithelial layer and have begun to be more common in the follicular region. (**C**) At 120 minutes, the relative proportion of beads in the subepithelial follicle increases, and there are fewer beads in the FAE and follicle region overall compared with the 60-minute time point. (**D**) Beads are present in the subcapsular sinus (not shown) and parenchyma of cervical lymph nodes (*arrows*) within 120 minutes of instillation of beads into the conjunctival sac. (**A**, **B**, **C**) Bar = 10 µm; (**D**) Bar = 20 µm.

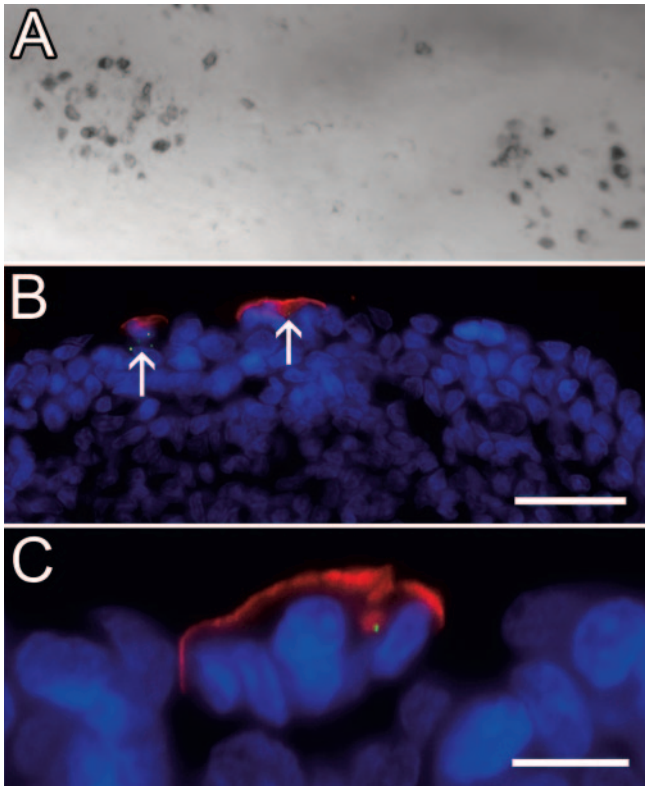

FIGURE 6. (**A**) Conjunctiva stained with antivimentin monoclonal AMF-17B and colloidal gold-conjugated secondary antibodies. Stereoscopic imaging of the whole mount after gold intensification shows vimentin labeling is strongest along the lateral flanks of the follicles. (**B**) In cryostat sections of conjunctiva stained with the goat polyclonal antisera against vimentin after a 30 minutes in vivo exposure to 0.2-μm green fluorescent latex beads. Beads (*arrows*) colocalized with vimentin-positive FAE cells (*red*). (**C**) A higher power view of a vimentin-labeled cell that has engulfed a fluorescent latex bead. The cell has the classic M-cell morphologic feature of an intraepithelial pocket filled with several cells. Nuclei have been stained blue by using DAPI in both **B** and **C**. (**B**) Bar = 30 μm; (**C**) Bar = 10 μm.

onstration of transcytosis by an epithelial cell within the FAE.[8] The present study shows selective transcytosis of latex particles by M cells in the rabbit conjunctiva and for the first time demonstrates subsequent rapid transport of the transcytosed material to local lymph nodes.

The ability of the guinea pig conjunctiva to take up latex beads was originally explored with TEM by Latkovic and Nilsson.[18,19] After a 24-hour in vivo exposure during which the beads were replenished every 8 hours, the investigators reported beads were present in the epithelial layer throughout the conjunctiva but did not describe any in the underlying substantia propria. This earlier study was designed simply to demonstrate the phagocytic capacity of conjunctival epithelium, and no attempt was made to distinguish between phagocytosis and transcytosis. Furthermore, the level of uptake and differences between regions were not examined in this earlier work. The results of the present study, demonstrating rapid exit of latex beads once they have been transcytosed across the epithelial barrier, make it difficult to compare our findings with the older studies that used repeated exposures over 24 hours.[19]

Latex beads have been widely used as a functional marker of M cells in other locations. The original observation that polystyrene latex beads selectively bind to M cells was made in the rabbit Peyer's patch.[10] Subsequent studies have shown selective binding and bead translocation by M cells in the rabbit nasal mucosa,[11] rat Peyer's patches,[13] mouse Peyer's patches,[14] and a human in vitro M cell model.[20] The reason that latex microspheres preferentially associate with and are internalized by M cells is still somewhat unclear but is likely because of their physical properties, as well as the unique apical surface of the M cell.[21] The efficiency of microsphere internalization can be optimized by selecting the appropriate size, charge, and hydrophobicity.[22,23] Interest in the interactions between microspheres and M cells is not simply restricted to identifying cells that translocate particles. The use of antigen-loaded or coated microspheres as a means to stimulate mucosal immunity or to deliver drugs is currently being studied by several groups.[24–26]

The origin of the "M cell" name came from the initial descriptions of these cells in the human intestine, where their apical membranes were covered with irregular microfolds rather than the more typical homogeneous brush border of microvilli on neighboring enterocytes.[27] Subsequent studies showed that M cells in other locations frequently had microvilli, therefore, the name has evolved to refer to the thin "membranous" rim of cytoplasm that separates the external environment from the underlying lymphoid cells. The presence or the absence of microvilli and their relative length cannot, therefore, be used as a reliable marker for the identification of M cells. It is safe to say that, consistent with their unique function, the apical membrane specializations of M cells are generally different from the surrounding cells. M cells in the rabbit Peyer's patch have shorter microvilli than the neighboring enterocytes.[28] In contrast, M cells in the conjunctiva, like their counterparts in the rabbit tonsils and cecum, have irregular, frequently branching, microvilli that are longer than those found on surrounding cells.[29–32]

Although M cells are classically found in the simple columnar epithelium of the intestines, their presence in the stratified epithelium of the conjunctiva is similar to their occurrence in the stratified squamous epithelium of the rabbit's palatine tonsil.[29] As in the rabbit tonsil, cecum, appendix, and sacculus rotondus, M cells in the conjunctiva are predominately on the lateral flanks of the follicle.[17,21,31,33]

Although we consistently saw surface binding of latex beads to a distinctive subpopulation of FAE after in vitro incubation at 4°C, we only saw rare examples of beads binding to the FAE surface after in vivo exposure. This suggests the beads are rapidly internalized soon after they bind. The absence of any additional bead binding once the first wave of beads had been internalized is in agreement with the original report of bead uptake by M cells in the rabbit Peyer's patch.[10] Similarly, M cells in the mouse Peyer's patch do not show surface binding at the same time they are transporting beads.[14]

The rapid exit of latex beads from the conjunctiva and transport to cervical lymph nodes is similar to what has previously been observed in studies of the rat intestinal Peyer's patches, where latex beads can be detected in the mesenteric lymph within 5 to 10 minutes of instillation into the intestinal lumen.[34]

Vimentin has previously been shown to selectively label M cells in the FAE of Peyer's patch, appendix, cecum, tonsil, and bronchus of the rabbit but not in other species.[16,17,35,36] Consistent with our evidence for transcytosis by M cells in the rabbit conjunctiva, vimentin positive cells were found in ring-like distributions along the peripheral flanks of the follicles in acetone-fixed whole mounts. In cryostat cross-sections of aldehyde-fixed conjunctiva exposed in vivo to latex beads, vimentin immunolabeling coincided with latex bead distribution in the FAE. Although the functional significance of vimentin expression in M cells has not been established, the presence of this rabbit M cell marker is further evidence that the conjunc-

tival FAE has M cells equivalent to those found in other mucosal locations.

Mucosa associated lymphoid tissue (MALT) has been well established in the gastrointestinal, respiratory, and genital tract, and now there is growing evidence for the presence of MALT at the normal ocular surface.[2,3,37] Ocular MALT has been more controversial because of an absence of evidence of the specialized antigen sampling cells in this tissue.[38,39] A key question has been which animal species would be the best model to address this issue. Although conjunctival lymphoid follicles are present in humans and a wide range of mammals,[1–3] they are not found in mice and rats.[2] The inability to use these two common laboratory species led us to examine guinea pigs[9] and rabbits as models more closely resembling the human conjunctiva. Guinea pigs offer the advantage that they have been widely used in modeling ocular allergies. This will allow future studies to investigate the impact of allergic reactions and pharmacological treatments on conjunctival M cells, as well as testing whether M-cell-directed immunization can trigger IgA-mediated immune responses that can ameliorate IgE-mediated allergic reactions. Rabbits, on the other hand, have a better characterized immune system, and antibodies are available against CD markers on inflammatory cells, which will allow for characterization of these cell types in the mucosal immune response. Furthermore, because it is possible to make class-specific rabbit hybridomas,[40] future studies will be able to address whether IgA promotes antigen uptake by conjunctival M cells as previously described for M cells in the mouse intestine.[41] Another advantage of the rabbit is the availability of vimentin immunostaining as an M cell marker, which allowed for an additional confirmatory test for these cells in the conjunctiva.

Despite their beneficial role in initiating the mucosal immune response, M cells in other mucosal locations have also repeatedly been shown to be an entry point for opportunistic bacterial and viral pathogens.[4–6] It will be essential for future studies to address whether conjunctival M cells can be a similar entry point for pathogens. Furthermore, an intriguing hypothesis linking conjunctival and tear-duct-associated lymphoid tissue in the pathogenesis of dry eye has been proposed.[42] The identification of animal models with functional conjunctival M cells opens the possibility of testing this hypothesis by targeting antigens to these cells to determine whether the inappropriate stimulation of the underlying lymphoid tissue can trigger onset or exacerbation of dry eye.

The mechanism by which antigens are taken up by the conjunctiva has been elusive until a recently published study showed that the M cells selectively bind and transcytose a sialyllactose-binding lectin in the guinea pig conjunctiva.[9] The present study provides further evidence for a functional ocular MALT by demonstrating that the rabbit lymphoepithelium contains M cells with the ability to translocate particulates and, for the first time, demonstrates subsequent tracer transport to local lymph nodes. The rabbit is, therefore, a useful model to study the role of mucosal immunity in the maintenance of the delicate epithelial barrier of the ocular surface.

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