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Convergent and Divergent Development among M Cell Lineages in Mouse Mucosal Epithelium

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M cells are specialized epithelial cells of the airway and intestinal mucosa that play a primary role in mucosal immune surveillance (1–3). Captured microparticles are transported (transcytosed) across the epithelium for uptake by underlying dendritic cells (DC) and stimulation of mucosal immunity. Most M cells are found in follicle-associated epithelium overlying organized mucosal lymphoid aggregates such as intestinal Peyer’s patches (PPs) (4) and nasal-associated lymphoid tissues (NALT) (5). M cells are also present over inducible lymphoid aggregates such as isolated lymphoid follicles (ILF) (6) in the intestine and BALT (7) in the lung. In addition, an inducible M cell population has been described at the tips of intestinal villi, called villous M cells (8).

The regulation of M cell development and function is not well understood despite the assumed central role of M cells in mucosal immunity. In the constitutive mucosal lymphoid tissues, it would be reasonable to assume that lymphoid tissue inducer cells (LTi) responsible for the formation of PPs (9) and associated stromal cells are also involved in M cell development. Accordingly, LTi-type TNF and lymphotixin ligands have been reported to induce expression of genes in intestinal epithelium that were associated with follicle epithelium and M cells (10). Because the follicle epithelium turns over rapidly with a half-life of a few days (11), the regulation of M cell proportion among follicle epithelial cells requires dynamic regulation. Thus, the origin and induction of M cells has been subject to controversy. Various studies have supported the competing views that M cell induction is due to either a subset of crypt stem cells dedicated solely to M cell production (12–14) or transdifferentiation from existing mature enterocytes (15, 16). The situation becomes more complex in settings where immune stimulation by bacteria (e.g., *Streptococcus pneumoniae*) (17) or their components (e.g., flagellin or cholera toxin [CT]) (18) can induce rapid production of new M cells; in this study, the kinetics of induction challenge the idea that crypt stem cells directly account for all new M cell production.

Studies have suggested that M cell development in the intestine is dependent on B lymphocytes; indeed, a characteristic feature of PP follicle M cells is the presence of a basolateral pocket usually containing at least one B lymphocyte (19, 20). Cocultures between Caco-2 intestinal epithelium and B lymphocytes have been shown to induce an M cell phenotype (15, 21), and mice that lack B lymphocytes appear to lack M cell transcytosis function (20). However, in CD137-deficient mice that do not experience development of M cell basolateral pockets and transcytosis function, the initial lineage commitment of progenitors to the M cell lineage appeared intact (22). Thus, it is possible that M cell development is a two-step process with the first lineage commitment step being B cell independent, followed by a step that is B cell dependent.

The induction of the M cell phenotype is no less complex in the case of mouse NALT M cells. The apical membrane of intestinal M cells appears membranous (hence the name M cell) compared with neighboring cells with an actin-based brush border (4). In contrast, airway M cells, also with a membranous apical surface, are surrounded by cells with long, tubulin-based cilia (5). Instead of
a rapid turnover and crypt stem cell origin, airway epithelial cells are generated from a dispersed population of basal cells and have half-lives on the order of weeks to months (23–26). Despite their distinct origins and turnover, airway and intestinal M cells show strikingly similar phenotype and functions, suggesting that convergent or overlapping genetic programs are induced.

Materials and Methods

Animals

B cell-deficient mutant mice on the BALB/c background (C.129S2-Igh-6m1Cgn/J; referred to as “Igh-6b” in this report), C57BL/1-EGFP knock-in and Foxj1-EGFP transgenic, and BALB/c mice were obtained from The Jackson Laboratory. CD117-deficient mice on the BALB/c background were provided by Dr. B.S. Kwon. All mice were bred in the University of California Santa Cruz genome Bioinformatics database (http://genome.ucsc.edu/), and screened for putative promoter elements using the TRANSFAC Transcription Factor Binding Sites Database (http://gene-regulation.com/cgi-bin/pub/databases/transfac/search.cgi), with results in Supplemental Fig. 1. A total of 4 kb of candidate promoter sequence was then cloned by PCR from mouse genomic DNA using these primers: 5′-TTCCCAAG-CTTGCTGATAGCCTTTCTAAAACTG-3′ and 5′-GGTC-TCAGTGCTATCCATGTTGACGTGC-CGCACAGG-3′. Based on conventional splice-site sequences and the size of the intron, the second construct from PGRP-S was also cloned using the primers 5′-GTAAGTACATCC-TGAGCTG-3′ and 5′-CTGCAAGGGGAGGGACAAGATA-CAGA-3′, and inserted into the dsRed expression2 coding sequence by overlap PCR. The insertion site of dsRed express2 was decided according to the conservative splice-site AG at 5′ exon and G at 3′ exon. The dsRed express2 fragment was obtained from the pCMV-dsRedExpress2 vector (Clontech) and was inserted downstream of the PGRP promoter fragment by overlap PCR. The BGH polyA site fragment, obtained from pCDNA 3.1(+) (Invitrogen), was added to the end of the dsRed coding sequence. Expression of dsRed with the PGRP-S intron (in pCDNA) was confirmed by transfection into Caco-2BBe cells before cloning downstream of the PGRP-S promoter. The 5.5-kb insert in pGEM was excised from the plasmid backbone by HindIII/KpnI digestion, gel purified, and injected into fertilized oocytes of CB6 F2 mice to generate transgenic mice. Founders were identified by PCR analysis of DNA isolated from tail clips using primers specific for DsRed express2 (5′-CGACATCCCGGA-CTACAAGAAC-3′ and 5′-CTTCAGCTTCAGGGC-CTTTGGGAT-3′). Founders were bred to BALB/c wild type mice.

CT administration

CT (Calbiochem) was reconstituted in PBS to a final concentration of 1 μg/μl. Mice were starved for 12 h before CT administration. The dose of CT (15 μg) was based on the studies of Terahara et al. (18) and was delivered either by gavage (in 200 μl PBS) or intranasally (in 20 μl PBS, 10 μl each side). Intranasal volume (10 μl per side) was chosen to ensure accurate dosing. After 48 h, mice were humanely killed and dissected for analysis.

Nucleoside analog labeling and staining

Mice were injected i.p. with 200 μg of the nucleoside analog 5-ethynyl-2′-deoxyuridine (EdU; Invitrogen) in 200 μl PBS at the same time CT was administered. Small intestine, PP, and NALT were harvested at 48 h after injection. Dissected tissues were fixed in 4% formaldehyde/PBS for 15 min, followed by washing with 3% BSA/PBS twice. Tissue was then permeabilized with 0.5% Triton X-100/PBS for 20 min and blocked with 6% BSA/PBS for another 30 min before staining with Alexa Fluor 488 azide Click it reaction mixture (Invitrogen) for 30 min at room temperature. Tissue was again washed with 3% BSA/PBS twice and used for immunohistochemistry staining as described later.

Immunohistochemistry and confocal microscopy

For cryostat sections, dissected NALT or PP tissue was fixed in 4% paraformaldehyde/PBS, flash-frozen for cryostat sections, and stained in sections. For whole-tissue mounts, dissected tissues were fixed in 4% paraformaldehyde/PBS and then stained in whole-tissue fragments. For both cryostat sections and whole mounts, tissues were then permeabilized in PBS, 0.1% Tween, and blocked in 0.1% Tween in casein solution. After that, tissues were stained with Abs to GP2 (Medical and Biological Laboratories) and rhodamine, fluorescein, or biotinylated Ulex europaeus agglutinin 1 (UEA-1) lectin (Vector), followed by secondary reagents Alexa Fluor 488-conjugated anti-rat Ab or streptavidin-conjugated Alexa Fluor 647. Post-fixation was done with 4% paraformaldehyde/PBS. Tissue was then mounted with Prolong Gold antifade reagent (Invitrogen) and DAPI as a nuclear counterstain. Images were obtained using a BD CARVII Confocal Imaging (BD Biosystems) on a Zeiss Axio Observer inverted microscope. Hardware control (microscope, confocal and digital camera; Diagnostic Instrument Xplorer–XS) used BD IPlab Imaging Software. Image Z resolution was further optimized with Volocity software (PerkinElmer). M cell numbers were counted for every image and then normalized by surface area, measured by Volocity software.

Scanning electron microscopy

For scanning electron microscopy (EM), tissues were dissected and fixed in 2.5% glutaraldehyde (Electron Microscopy Sciences) for 3 h and 1% OSO4 (Thermo) in PBS for another 3 h. Tissue was then prepared by critical point drying and gold sputter coating. Samples were examined with an FEI XL30 field emission scanning EM at 5 kV.

Statistics

Comparison of M cell numbers between CT-treated and nontreated mice were performed using two-tailed, nonparametric Mann–Whitney U test. The nonparametric test was chosen because counts were presented as numbers per defined area rather than absolute counts, though t tests gave similar results. Comparison of M cell numbers among nontreated, CT gavaged, and CT intranasally treated mice were performed using one-way ANOVA (nonparametric) Kruskal–Wallis test followed by Dunn’s multiple-comparison post test. All statistical analyses were performed using Prism software (GraphPad).

Results

Expression of PGRP-S–dsRed transgene in neutrophils and M cells of PP follicle epithelium and NALT

One challenge to the study of M cell development and function has been the lack of genetic markers of M cell differentiation. In the mouse, identification has depended on the lectin UEA-1 specific for fucose displayed largely on the apical surface of M cells. The association with a specific fucosyltransferase gene has been described (18), though it is not clear that this gene’s expression is unique to M cells. We recently identified the peptidoglycan recognition protein, PGRP-S, as expressed specifically by PP follicle-associated M cells (27), in addition to its known expression by neutrophils (28–30). After an analysis of upstream genomic sequences, we identified a candidate promoter fragment, which we used to drive expression of a reporter red fluorescent protein transgene, called PGRP-S-dsRed (see Supplemental Figs. 1, 2).

PGRP-S-dsRed transgenic mice expressed red fluorescence in all neutrophils as expected, but also in a small population of F4/80+ CD11b+ cells presumably in the macrophage/myeloid lineage (see Supplemental material). Most importantly, red fluorescence was strong in UEA-1–stained epithelial cells in PP follicle epithelium (Fig. 1A, 1B). Although neutrophils can be found within normal mucosal lymphoid tissues, they are not usually found within follicle epithelium; because the morphology of the bone marrow–derived cells can be clearly distinguished from M cells, dsRed expression appears to be a promising marker for follicle epithelium M cells.

Among cells in the PP follicle epithelium, expression of dsRed showed a specific pattern of coincidence with UEA-1+ epithelial cells suggesting a consistent association with late commitment to the M cell lineage. UEA-1 staining is predominantly at the apical membrane of M cells, whereas the dsRed is cytoplasmic; therefore, in whole mounts of PPs, the UEA-1 and dsRed fluorescence within the same cell are sufficiently separated so that a coincident fluorescence signal (e.g., green + red = yellow) depends on z-stack...
movies to illustrate the correlation between apical UEA-1 and cytoplasmic dsRed (see Supplemental Video 1). At the margins of the follicle epithelium, newly generated M cells (identified by UEA-1 binding) migrate from the adjacent intestinal crypts, so early M cell lineage commitment and maturation likely occurs in this zone. By quantifying the coincidence of apical UEA-1 staining with cytoplasmic dsRed expression, we found that in the outer third of the follicle dome, <50% of the UEA-1* cells also expressed dsRed. By contrast, UEA-1* cells within the central two thirds of the dome of the follicle epithelium showed >99% coincidence with cytoplasmic dsRed expression (Fig. 1C). Thus, once PP M cell commitment has already occurred at the margin of the follicle dome, dsRed expression highly correlated with M cell differentiation thereafter. UEA-1 staining consistently preceded expression of dsRed; in turn, dsRed expression coincided with or slightly preceded the expression of another M cell-associated gene, gp2 (31, 32) (Fig. 1D). Together, these results suggest that lineage commitment (defined by UEA-1 lectin binding) and the two genetic markers of M cell development (PGRP-S/dsRed and gp2) are sequentially coordinated at the margin of the follicle epithelium.

**PP M cell lineage commitment and PGRP-S reporter expression is B lymphocyte and CD137 independent**

Although late expression of PGRP-S and the dsRed transgene suggests association with commitment to the M cell lineage, it is not necessarily associated with M cell function despite the implicit utility of the peptidoglycan binding of PGRP-S for M cell capture of bacteria. Indeed, we recently reported that in CD137-deficient mice, M cell lineage commitment (i.e., appearance of UEA-1*) appeared to be intact, but M cell transcytosis function in both NALT and PP was absent. Associated with this defect was the failure to develop the characteristic B lymphocyte basolateral pockets, suggesting a requirement for a CD137-CD137L signal between M cells and B lymphocytes for pocket formation and M cell functional development. This was also consistent with the observation that in B cell-deficient mice, M cell function was absent (19, 20).

Therefore, to test whether PGRP-S and dsRed expression are specific to lineage commitment and not M cell function, we backcrossed the PGRP-S–dsRed transgene to CD137-deficient (Fig. 2A) and B lymphocyte-deficient Igh-6 (Fig. 2B) mice. In both backcrosses, we confirmed previous studies (19, 20) that UEA-1* cells were still present in PP follicle epithelium. Because of the lack of B lymphocyte formation of basolateral pockets in both backcrosses, the UEA-1* cells were unusually narrow; however, more importantly, all of them were positive for cytoplasmic dsRed expression. Thus, the M cell-specific PGRP-S gene was still induced in UEA-1* cells despite the lack of basolateral pocket formation and M cell transcytosis function in both knockout (KO) models. This result suggests that the M cell genetic program that is initiated before basolateral pocket formation is fixed during initial lineage commitment regardless of whether transcytosis function develops. In addition, it supports the interesting possibility that acquisition of transcytosis function is dependent more on cytoskeletal changes associated with the formation of the basolateral pocket rather than any M cell-specific genetic program.

**M cell–DC interactions suggest functional units in PP**

Although M cell function may be dependent on basolateral pocket B lymphocytes, their critical cellular interaction in immune surveillance is their delivery of lumenal Ags to subepithelial APCs. Therefore, to examine the relationship between M cells and underlying DC, we crossed the PGRP-S–dsRed transgene to the CX3CR1-EGFP reporter transgene that expresses EGFP in...
myeloid cells such as DC (33). Because the dsRed and EGFP proteins fill the cytoplasm of the M cells and DC, their full morphology can be visualized by confocal microscopy. Interestingly, most red fluorescent PP M cells appeared to have a close association with an underlying green fluorescent DC, and in many cases, dendritic processes can be identified extending up along the basolateral pocket of the M cells, where the local contact points between the cells appear as yellow fluorescence (Fig. 3). This one-to-one association suggests the formation of an M cell–DC functional unit, though it may also reflect the high density of myeloid cells in the PP subepithelial zone.

CT induces de novo M cell transdifferentiation from enterocytes on PP

Several immune stimuli can activate the generation of new M cells within hours to days (16–18, 34). This phenomenon has been controversial, with various studies arguing that the induction is due to: 1) rapid activation of M cell production from crypt stem cells; 2) acute transdifferentiation from mature enterocytes; or 3) alternatively, increased activity of existing M cells (35). It is possible that under different experimental conditions, one or more of these mechanisms may be acting at the same time. In our studies, we focused only on the actual numbers of UEA-1+ M cells across the PP follicle epithelium, and we found that CT induced the development of up to 30% more M cells across the PP follicle epithelium within 48 h (Fig. 4A–C). Despite the increased numbers of M cells in the follicle epithelium, they maintained a dispersed distribution across the epithelium, in contrast with the clustering of M cells seen with villous M cells (see later). The effect of CT on intestinal M cells was similar whether the CT was administered intranasally or orally (Fig. 4C); this could be because of oral ingestion of CT draining from nasal administration, although a direct or indirect systemic effect through cytokines such as TNF-α (10) or RANKL (36) may also be responsible.

The induction of higher numbers of M cells by CT was not significant in the context of the CD137KO; however, in this study, the nontreated M cell levels were already considerably higher than the background levels on wild type BALB/c (Fig. 4D), suggesting that the KO phenotype was associated with some chronic stimulation of M cells. Indeed, Lee et al. (37) reported that CD137KO mice show evidence for some immune hyperreactivity, which might result in an increased basal level of mucosal stimulation of M cell production. In the case of B cell-deficient Ig-6 mice, CT induction of new M cells over follicle epithelium was clearly evident despite the absence of B cells or CD137 (Fig. 4E). Thus, CT induction of new M cell lineage cells was largely along the lines of first step lineage commitment, independent of B cells.

The induction of new M cells was rapid, but sustained, consistent with the normal half-life of intestinal epithelium. The kinetics of this rapid increase suggested that the new M cells were generated from mature follicle enterocytes rather than from new cells emerging from the nearby crypts. We examined this question in two ways. First, we administered CT along with the nucleoside analog EdU. Within the 48 h of the M cell induction, EdU uptake was clearly evident within the neighboring intestinal crypts, but not in any of the follicle epithelial cells farther from the crypts, including the UEA-1+ dsRed+ cells (Fig. 4F). Thus, any acutely differentiated M cells appear to arise by direct transdifferentiation from mature enterocytes. Second, we looked at where the new M cells appeared on the follicle dome. Consistent with the EdU study, the increased density of M cells was similar when quantified separately for both the outer third of the follicle and the inner two thirds, indicating that the new M cells appeared uniformly across the dome and not just among newly formed cells coming from the crypts (Fig. 4C).

**Phenotypic and functional differences in CT-induced villous M cells**

The apparent transdifferentiation of mature enterocytes to an M cell phenotype is especially prominent in the case of villous M cell development. In untreated mice, villous M cells are infrequently present at the tips of the intestinal villi (Fig. 5A), but in response to CT, villi with clusters of UEA-1+ M cells are more easily identified (Fig. 5B). Considering their location exclusively at the tips of villi, it was unlikely that they were newly generated from crypt stem cell precursors; accordingly, CT-induced villous M cells did not label with EdU during the induction period (Fig. 5B), suggesting that these are also the product of transdifferentiation from mature enterocytes. Scanning EM confirmed the loss of brush-border microvilli (Fig. 5A, 5B). In contrast with PP follicle M cells, they were consistently found in clusters rather than dispersed as single M cells. Some individual villous M cells showed contacts with underlying DC (Fig. 5C, arrows), though this was much less frequent compared with PP. Consistent with the differences in gene expression phenotype, the PGRPS–dsRed transgene was also not expressed in these M cells (Fig. 5C). As with follicle M cells, CT induction was not dependent on the presence of B cells or CD137 (not shown).

**ILF M cells resemble PP M cells in PGRPS–dsRed expression**

Because villous M cells are inducible by acute stimuli such as CT, other mucosal lymphoid tissues such as ILF can also be induced by immunological stimulation. In contrast with villous M cells, ILF have organized lymphoid tissues below the epithelium (38, 39), are dependent on LTi (40, 41), and appear to be the consequence of chronic (rather than acute) stimulation, so they are found more frequently in older animals (42). Thus, it would be expected that ILF should develop epithelial M cells that are more similar to PP M cells rather than villous M cells. This was confirmed in older (5–12 mo) PGRPS–dsRed mice, where the small number of UEA-1+ epithelial cells overlying ILF (found in sections of intestine after PP were removed) also expressed dsRed (Fig. 6A, 6B).
NALT M cells express PGRP-S–dsRed transgene and can also develop from Foxj1+ ciliated progenitors

M cells in the mouse are also are present in airway epithelium over NALT. In contrast with intestinal M cells residing among enterocytes with apical brush-border microvilli (formed by actin bundles), NALT M cells are found among epithelium with apical cilia (formed by tubulin filaments). Despite these distinct origins, airway and intestinal M cell populations could show similarities in their development and genetic program. For example, similar to intestinal M cells, we found that NALT M cell initial lineage commitment is also independent of B cells and CD137 (22). However, in view of the different developmental origins of M cell populations in the airway versus intestine, the M cell phenotype may be alternatively viewed as a convergent development toward a common functional phenotype. For example, in contrast with intestinal epithelium, airway epithelial cells are generated from basal cell precursors (23, 24), which give rise to both ciliated airway epithelium and Clara cells. Ciliated airway epithelium expresses the transcription factor Foxj1, associated with terminal differentiation, and they have a very long t1/2, on the order of weeks or months (23, 25, 26, 43). In response to chemical injury, new Clara cells are quickly formed, and evidence suggests that they come from the basal cells and not from transdifferentiation of mature Foxj1+ ciliated epithelium. Although this has not been directly studied in the case of NALT epithelium, we expected similarities in Foxj1 expression and epithelial cell turnover.

As with the intestine, the PGRP-S–dsRed reporter was expressed in a subset of airway epithelium, but unlike PP, expression was not limited to UEA-1+ cells (Fig. 7A). CT (both intranasal or gavage administration) can also induce rapid development of new M cells in the NALT epithelium (Fig. 7B), and transdifferentiation from neighboring mature ciliated epithelial cells also may be involved. In Foxj1-EGFP transgenic mice (26), NALT ciliated epithelial cells expressed the EGFP reporter, whereas UEA-1+ M cells were all negative for EGFP (Fig. 7C). CT treatment significantly increased the number of NALT M cells, and they were all EGFP+ (Fig. 7D). Expression of the PGRP-S–dsRed transgene in the NALT was different from the pattern seen in the intestine, as many dsRed+ cells
The results of our studies, together with other recent reports, support the identification of at least three distinct mucosal M cell subsets: PP/ILF follicle epithelium M cells, inducible villous M cells, and NALT M cells. A recent report identified a fourth type of M cell, a “respiratory” M cell (44), present in the upper airway, which is likely to be an airway analog of the villous M cell. Given the distinct morphological and functional differences among the subsets, we propose to define broad categories of M cells as “organized” (PP, ILF, and NALT) versus “sporadic” (villous and respiratory). The organized M cells are found in epithelium overlaying organized mucosal lymphoid tissues, and they appear to form functional units with underlying lymphoid tissue DC. Despite the differences in their developmental origins and life span, one characteristic shared by these cells is their dispersed single-cell distribution across the face of the follicle epithelium. In addition, they express a subset of specific genes that appear helpful to function in microbial uptake, including PGRP-S (27) and gp2 (31).

By contrast, the sporadic subsets are scattered in various sites along the mucosal epithelium, not associated with underlying organized lymphoid tissues. Because of their scattered distribution, it can be difficult to provide consistent quantification, but it appears that inducible M cells are dependent on immune stimuli to develop in significant numbers. This may be driven, in part, by the inducible stromal cell expression of RANKL, as direct administration of RANKL was effective in inducing significant numbers of villous M cells (36). Because of the appearance of inducible villous M cells away from lymphoid tissues, any associations with underlying DC appeared random at best. Interestingly, in contrast with the dispersion of organized M cells, sporadic M cells could be found in dense clusters, such as at the tips of intestinal villi. Finally, function-associated genes PGRP-S and gp2 were not expressed by these cells, which appears to correlate with some reduced transcytosis function.

The situation becomes more complex as we consider the acute induction of new M cells by CT treatment. The numbers of M cells belonging to both the organized and sporadic subsets increased significantly. However, the striking result in this study was that the...
phenotype of the new PP and NALT M cells conformed strictly to the local phenotype; thus, new PP and NALT M cells all expressed the PGRP-S–dsRed transgene, whereas new villous M cells were all negative for the reporter transgene expression. Similarly, the dispersed single-cell distribution of M cells across the face of the PP and NALT epithelium was retained despite the increased density.

The CT-inducible M cells in both airway and intestine all appeared to be rapidly produced without evidence for cell division (i.e., EdU uptake) or recent crypt origin. The simplest explanation for the development of these cells is that they are the product of transdifferentiation from mature enterocytes or ciliated airway epithelium. Interestingly, the finding of double-expressing Foxj1–EGFP/PGRP-S–dsRed cells in the NALT epithelium raises the possibility that latent M cell precursors may already exist among mature mucosal epithelium. That is, a subset of differentiated ciliated airway epithelium may specifically retain the ability to convert to an M cell phenotype, losing their apical cilia. Unfortunately, there is no evidence for a similar follicle epithelium subset on PP, because all PGRP-S–dsRed–expressing epithelial cells were already also UEA-1+.

An important implication of the distinct phenotypes and functions of the organized versus sporadic M cell subsets is that the differential uptake of specific types of microbes will change as the balance of M cell subsets changes (8). Because various inflammatory and infectious conditions (such as the microbial components described earlier) can trigger the production of the sporadic phenotype, the nature of the mucosal immune response can change as local tissue responses change. There are two principal direct differences in the contributions of organized versus sporadic M cells. First, because sporadic M cells are less often associated with underlying DC and organized lymphoid tissues, uptake through these cells is far less likely to promote the production of secretory IgA, because the isotype switching of B cells is mainly associated with the organized mucosal lymphoid tissues (41, 45, 46). Second, the sporadic M cells do not express the sets of genes associated with selective microbe uptake (e.g., gp2 and PGRP-S); therefore, together with their relative inefficiency, they will enable a different array of microbes to cross the mucosal barrier. Because any potential immune response is more likely to be initiated in draining lymphoid tissues such as the mesenteric lymph node,

FIGURE 7. NALT M cells can develop from Foxj1+ progenitors. A, NALT from PGRP-S–dsRed transgenic mice shows UEA-1+ NALT M cells (cyan) among epithelial cells expressing dsRed (red). B, NALT M cell density in control (n = 22), CT gavaged (n = 20), and CT intranasally delivered (n = 19). Each count represents one image, three mice per treatment group. M cell density was significantly increased in the treated groups (***p < 0.001). C, NALT from Foxj1-GFP/PGRP-S–dsRed double-transgenic mice. All the UEA-1+ Foxj1-NALT M cells (cyan) and some of the UEA-1+ Foxj1+ ciliated epithelial cells (green) expressed dsRed (red, arrow). D, NALT from Foxj1-GFP/PGRP-S–dsRed double-transgenic mice treated with intranasal CT. After treatment, UEA-1+ NALT M cell numbers increased; moreover, there were no longer UEA-1+ Foxj1+ ciliated epithelial cells (green) expressing dsRed fluorescence. E, NALT M cell density count in Foxj1-GFP/PGRP-S–dsRed double-transgenic mice showing that UEA-1+ Foxj1-GFP/PGRP-S–dsRed double-expressing cells in control mice were similar in number to the increased number of new M cells in the CT-treated mice. Counts were from individual confocal images (n = 4 for UEA-1+ and UEA-1–untreated mice; n = 6 for CT-treated mice), two mice per group. ***p < 0.001.
production of secretory IgA is likely to be less prominent, with more dependence on IgG and T cell responses.

Although our studies were concerned with microbial (e.g., CT) triggers of M cell development, other settings involving mucosal inflammation (and TNF/lymphotixin cytokines) should also have an effect on the various M cell subsets present. However, because clear genetic markers for M cells have not been available until recently (at least for mouse), this question has not been closely studied in situations such as models of inflammatory bowel disease or ileitis. Here, the PGRP-S-dsRed transgenic strain should be useful in identifying differential induction of M cell subsets in vivo. The differential microbial uptake by M cell subsets may have an important effect on the progression or persistence of intestinal inflammation, in part through the differences in the induced mucosal immune response. As the relation between M cell function and mucosal immunity becomes more clearly defined, these questions will begin to be addressed.

Finally, we found that the PGRP-S promoter provided a useful reporter of M cell lineage commitment, corresponding to the expression of the PGRP in M cells. It is therefore interesting to consider the more narrow question of whether peptidoglycan has any direct influence on M cell development. PGRP-S is a secreted protein known for its potential antimicrobial activity (47, 48) but it has no known signaling function; a PGRP-S–deficient mouse has not been examined for M cell development or function (28). Importantly, several other proteins expressed in mucosal epithelium can signal in response to peptidoglycan, including TLR2, NOD2, and other PGRP family members such as PGRP-L, PGRP-Io, and PGRP-IB (30). Chabot et al. (49) found that peptidoglycan could induce a very rapid (45–90 min) recruitment of DC into follicle epithelium and an increase in M cell particle uptake. This effect was absent in TLR2-deficient mice, so no other PGRPs appear to be involved in this response. Although not directly examined by Chabot et al., the effect may be transient; we studied M cell numbers and particle uptake in TLR2–deficient mice and found no significant differences (V. Gusti and D. Lo, unpublished observations). Thus, at least in this context, peptidoglycan and TLR2 signaling might not influence M cell lineage commitment or long-term function.

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Disclosures
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