Distinct Gene Expression Profiles Characterize Cellular Phenotypes of Follicle-Associated Epithelium and M Cells

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Abstract

Follicle-associated epithelium (FAE) covering Peyer’s patches contains specialized epithelial M cells that take up ingested macromolecules and microorganisms from the lumen of the gut by transcytosis. Using high-density oligonucleotide microarrays, we analyzed the gene expression profiles of FAE and M cells in order to characterize their cellular phenotypes. The microarray data revealed that, among approximately 14,000 genes, 409 were expressed in FAE at twofold or higher levels compared to the intestinal epithelial cells (IECs) of the villi. These included genes involved in membrane traffic, host defense and transcriptional regulation, as well as uncharacterized genes. Real-time PCR and in situ hybridization analyses identified three molecules, ubiquitin D (Ub-D), tumor necrosis factor receptor superfamily 12a (TNFRsf12a), and transmembrane 4 superfamily 4 (Tm4sf4), which were predominantly distributed throughout FAE, but were expressed little, if at all, in IECs. By contrast, transcripts of secretory granule neuroendocrine protein 1 (Sgne-1) were scattered in FAE, and were co-localized with Ulex europaeus agglutinin-1 (UEA-1)-positive cells. This clearly suggests that expression of Sgne-1 in the gut is specific to M cells. Such a unique pattern of gene expression distinguishes FAE and M cells from IECs, and may reflect their cellular phenotype(s) associated with specific functional features.

Key words: FAE; M cells; Sgne-1; Affymetrix; microarray

1. Introduction

Gut-associated lymphoid tissues (GALT), such as Peyer’s patches (PPs) and isolated lymphoid follicles (ILFs), are important inductive sites for mucosal immune response. The mucosal surface of the body is exposed to a vast array of foreign antigens and microorganisms. Different from other secondary lymphoid tissues that filter out foreign antigens from the lymphatic fluid or the bloodstream, GALT respond to external antigens present on the gut mucosa. The mucosal antigens are transported from the gut lumen to organized lymphoid follicles (LFs) by follicle-associated epithelium (FAE) overlaying the LFs. FAE is structurally similar to normal intestinal epithelium, the major population of which is absorptive enterocytes having a tall columnar shape and a well-organized brush border. However, they differ markedly in cellular phenotype, namely, FAE contains limited numbers of goblet cells and enteroendocrine cells (EECs), which leads to reduced mucin production. The expression of brush border-integrated hydrolases in FAE is also downregulated compared to that in intestinal epithelial cells (IECs). Furthermore, the basal membrane of FAE is devoid of polymeric immunoglobulin receptors that are important for IgA secretion into the gut lumen.

The most striking feature of FAE is the presence of M cells, which play a pivotal role in immunosurveillance on the mucosal surface by recognizing and taking up foreign macromolecules through the active transepithelial vesicular transport called transcytosis. The transfer of transcytosed antigens to antigen presenting cells (APCs) accumulating beneath FAE leads to the onset of mucosal immune responses: secretory IgA (sIgA) induction by foreign pathogens as well as oral tolerance to food ingredients and commensal bacteria. The macromolecular transcytosis by M cells is, however, a double-edged sword. Certain pathogenic microbes (e.g., Salmonella, Yersinia and reovirus) exploit M cells as a portal for invasion. Although the mechanisms of bacterial internalization by M cells are largely unknown, proteins and/or oligosaccharides expressed on the M cell apical membrane are presumed to serve as adherent receptors for the pathogens. Such microbial-M cell interactions have
given rise to the idea of utilizing the transcytotic activity of M cells for the delivery of oral vaccines to the mucosal immune system. Indeed, attenuated live bacteria represented by Salmonella species are being tested for possible use as vectors for delivering external antigens or DNA vaccines. The elucidation of the molecular architecture of the M cell apical surface is therefore critical to understanding the mechanisms of pathogenic invasion as well as developing drug and vaccine delivery systems that target M cells.

Despite many years of research, the specific molecules for distinguishing FAE and M cells from IECs have yet to be fully clarified, because of limitations in the number of M cells as well as the method for isolating FAE. The results of an in vitro reporter assay suggest that FAE and villous IECs are differently regulated at the transcriptional level. Recently, Lo et al. have applied TOGA gene expression profiling to an in vitro M cell-like translation model using Caco-2 human IECs, and defined several molecules expressed throughout FAE. Among them, the short form splicing variant of peptidoglycan recognition protein (PGRP-S) was expressed only in M cells, whereas the long form was expressed only in FAE.

To better understand the molecular fingerprint of FAE, we compared the gene expression profiles of FAE and IECs using high-density oligonucleotide probe arrays. The results were further examined by quantitative PCR and in situ hybridization (ISH) analysis to identify the molecules specifically expressed in FAE and M cells.

2. MATERIALS AND METHODS

2.1. Animals

Male BALB/c mice between 7 and 10 weeks old were obtained from Japan Clea and maintained under specific pathogen-free conditions at RIKEN’s animal facility. All animal experiments were approved by the Animal Research Committee of RIKEN Yokohama Research Institute.

2.2. Isolation of epithelium and lymphocytes

FAE was isolated from mouse small intestine by modifying the method described by Imura et al. Briefly, PPs were dissected from the mouse small intestine and soaked in Hank’s balanced salt solution (HBSS; GIBCO) containing 30 mM EDTA. After incubation at room temperature for 20 min, FAE was isolated by manipulation with a fine needle under stereomicroscopic monitoring. IECs were also isolated in the same manner from small pieces of duodenum, jejunum and ileum after excluding PPs. The isolated epithelial cell sheets were kept in ice-cold HBSS until RNA extraction.

Mouse Peyer’s patch lymphocytes (PPLs) were prepared according to a method described previously with minor modifications. Briefly, PPs excised from the intestinal wall were dissociated in RPMI1640 medium (GIBCO) containing 0.5 mg/ml collagenase (Nitta gelatin, Osaka, Japan), 0.5 mg/ml DNase (Roche), 2% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 20 mM HEPES (pH 7.2), at 37°C for 20 min to obtain a single-cell suspension. The cell dissociation was repeated again in fresh collagenase solution. The cell suspensions were pooled and washed twice with RPMI1640, and subjected to Percoll gradient separation to remove epithelial cells. Immunofluorescent staining and flow cytometric analysis showed that the PPL preparation contained B cells (90%), T cells (8–10%) and dendritic cells (DCs; 1%).

2.3. Gene chip expression analysis

Mouse genome-wide gene expression was examined by using the Mouse Genome MOE430A Probe Array (Affymetrix, Santa Clara, CA) that contains oligonucleotide probe sets for approximately 14,000 full-length genes and expressed sequence tags (ESTs). Total RNA was extracted from FAE or IECs prepared from three mice using an RNeasy Mini Kit (QIAGEN, Valencia, CA). Double-stranded cDNA was synthesized from 5 µg of total RNA, and the cDNA was subjected to in vitro transcription in the presence of biotinylated nucleotide triphosphates. The biotinylated cRNA was hybridized with a probe array for 16 h at 45°C. The hybridization was performed one each for a pooled sample of FAE or IEC. The hybridized products were stained with streptavidin-phycocerythrin (PE), and then scanned with a Hewlett-Packard Gene Array Scanner (Palo Alto, CA). The fluorescence intensity of each probe was quantified using GeneChip Analysis Suite 5.0 software (Affymetrix). The level of gene expression was determined as the average difference (AD) using the GeneChip software. The level of gene expression was determined as the average difference (AD) using the GeneChip software. Data analysis was further performed with Genespring software version 6.1 (Silicon Genetics, San Carlos, CA). Measurement values less than 0.01 were set at 0.01, and per-chip normalization was performed to the median of all measurements. Expression data were considered significant when they differed by at least twofold between FAE and IECs, and contained at least one ‘present’ tag indicated by Affymetrix algorithm in two samples.

2.4. Quantitative PCR analysis

The genes predominantly expressed in FAE as determined by the microarray analysis were further confirmed by real-time PCR using the SYBR®-Green PCR assay and an ABI Prism 7900 Sequence Detection System, as described previously, with minor modifications. Briefly, the expression levels of selected genes, by FAE, IEC, PP and PPL, were calculated by extrapolating to a standard curve made with authentic samples containing large amounts of the target genes, and the calculated values were normalized to the expression level of GAPDH. Am-
plification of the expected single products was confirmed using 1% agarose gel electrophoresis and ethidium bromide staining. The specific primer sets for each target gene are available upon request.

2.5. In situ hybridization (ISH)

One cDNA fragment each of ubiquitin D (Ub-D), tumor necrosis factor receptor superfamily 12a (TNFRsf12a), transmembrane 4 superfamily 4 (Tm4sf4) and secretory granule neuroendocrine protein 1 (Sgne-1) was amplified from FAE-derived cDNA by PCR. The specific primers used were as follows: 5′- ACTGGACTCCACAGCTTCTGCT -3′ (forward) and 5′- ATCTCGAGATTCCTTGTGCAGATGCT -3′ (reverse) for TNFRsf12a; 5′- CTTGGATCCGCACCTGTGTCGCTCC -3′ (forward) and 5′- CAGCTCGAGCAACTGCCACTTTGA -3′ (reverse) for Ub-D; 5′- TAATCTCGAGCTTGTGCAGATGCT -3′ (forward) and 5′- CCATCTGGATCCCTCCGATGA -3′ (reverse) for Tm4sf4; 5′- ATTGGATCCAGCATCCGTATATGCC -3′ (forward) and 5′- TATCTCGGAGATGGGGGACAGAT TTC -3′ (reverse) for Sgne-1. The PCR product was digested with BamHI and XhoI, and subcloned into pcDNA3.0 (Invitrogen, Carlsbad, CA). Digoxigenin-labeled RNA probe was prepared by in vitro transcription with T7 or SP6 RNA polymerase (Roche Molecular Biochemicals, Indianapolis, IN) using the above plasmid vector digested with XhoI or BamHI as a template, respectively. ISH was performed with a Discovery Automated ISH System and a Ribomap Kit following the manufacturer’s instructions (Ventana Japan, Yokohama, Japan). Briefly, 4% formaldehyde-fixed sections of mouse PPs were deparaffinized, treated with protease, and hybridized with 10–50 ng of the specific antisense riboprobe or the control sense probe for 6 h at 65°C. The sections were then incubated with biotin-labeled anti-digoxigenin antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) for 20 min at 37°C, followed by streptavidin-alkaline phosphatase conjugate incubation for 16 min at 37°C. The signal was detected with a BlueMap NBT/BCIP Substrate Kit (Ventana Japan), and the sections were counterstained with nuclear fast red.

In a separate experiment, the sections subjected to ISH analysis with Sgne-1-specific probe were further stained with PBS containing 10 μg/ml rhodamine-labeled Ulex europaeus agglutinin-1 (UEA-1) lectin for 10 min at room temperature.

3. RESULTS

3.1. Gene expression profiling of FAE and IECs

We performed microarray analysis to search for candidate molecules that are specifically expressed in FAE and not in IECs. The accuracy of the results obtained with this approach depends on the purity and the viability of the cells. Dome-like structures of FAE were surrounded by crowded villi on the luminal surface of PPs dissected from the mouse small intestine (Fig. 1A, labeled by f). Therefore, it was quite difficult to remove villous IECs from the FAE preparation when conventional methods for epithelial preparation were used, i.e., EDTA or collagenase incubation at 37°C. Recently, Iimura et al. developed a new approach to dissect fresh epithelial monolayers from human colonic biopsies. We modified this method for FAE purification, as described in Materials and Methods, so that pure FAE domes connected with FAE-associated crypts were obtained (Fig. 1B). A single sheet of IECs containing an entire unit of villus and crypt was also dissected in the same manner (Fig. 1C). Hema-toxylin and eosin (H&E) staining of the sections confirmed that the isolated FAE and IECs were almost exclusively composed of epithelial monolayers with a trace amount of lymphocytes (Fig. 1D,E). BALB/c mice possess approximately 8–10 PPs in the small intestine, and each of them contains four or more LFs, namely, around 30 to 50 FAE domes are present in the small intestine. The yield of our FAE preparation was 50% to 70%, which gave rise to 2–3 μg of total RNA per mouse.

Total RNAs prepared from FAE or IECs were used for microarray analysis. The scattered plots of the normalized data clearly suggest similar gene expression profiles between FAE and IECs, in which the difference in the expression levels of most of the genes was within twofold (Fig. 2). On the other hand, the expression levels of 409 genes were upregulated by at least twofold in FAE compared to those in IECs. One-fourth of them were housekeeping genes, including metabolic enzymes, cytoskeleton proteins, and membrane traffic molecules. The remaining genes were involved in cell adhesion, host defense (e.g. pathogen recognition proteins, cytokines/chemokines and their receptors), signaling, transcription and cell cycle regulation, and other genes encoded membrane-associated molecules (Fig. 3, Supplemental Table 1, http://www.dna- res.kazusa.or.jp/12/2/05/supplement/supplement1.xls). Furthermore, nearly one-fourth were uncharacterized genes, such as the EST clones found in the RIKEN FANTOM project. By contrast, the expression levels of 466 genes were downregulated in FAE, most of which were housekeeping genes, including brush border enzymes, alkaline phosphatase and dipeptidylpeptidase 4. This is consistent with the results of a previous immunohistochemical analysis.

3.2. Quantitative PCR analysis of selected genes

The above gene expression profiling by microarray analysis provided the candidate molecules for FAE and M cell function as well as their specific biomarkers. To narrow down the number of candidates, we focused on
Figure 1. Epithelial monolayer isolated from mouse Peyer’s patches (PPs). A: The luminal side of PP dissected from mouse small intestine. FAE (f) surrounded by villous epithelium is clearly observed. B–C: FAE (B) and villous epithelium (C) separated from adjacent mesenchymal tissue. D–F: H&E staining of isolated villous epithelium (D) and FAE (E, F). Epithelia derived from villi or crypts are indicated by arrows or arrowheads, respectively (D). Higher magnification of the FAE sheet shows blank M cell pockets (F, arrowheads). Original magnifications: ×50 (A), ×150 (B), ×90 (C). Bar scale: 800 µm (A), 300 µm (B, C), 200 µm (D, E) or 100 µm (F).

Figure 2. Comparison of gene expression profiling between FAE and IECs. Normalized data obtained by microarray analysis are drawn in a scatter plot. The upper and lower lines indicate the threshold levels of twofold higher and lower expression, respectively.
the subset of genes encoding membrane-bound molecules, vesicular traffic-related proteins, transcription factors, and uncharacterized molecules. The function and cellular localization of each molecule were referenced to the gene ontology of UniGene and Genbank databases, and the specific domains were predicted by referring to the Protein Families Database of Alignments and HMMs (Pfam; Sanger Institute). Finally, 102 genes were selected and examined by quantitative PCR analysis to confirm their expression levels in FAE. The expression of several of those genes might be also upregulated not only in FAE but also in PPLs and/or mesenchymal tissue, even though their expression was low in IECs. In addition, there are recent reports that DCs are integrated into the intestinal epithelial monolayer. It is difficult to exclude the possibility that the molecules derived from epithelial DCs could bias the microarray results. To exclude such molecules, total RNA samples from purified PPLs that contained B cells, T cells and DCs as well as whole PP preparation that contained mesenchymal tissue in addition to PPLs were also subjected to the PCR analysis for comparison. The expression levels of most genes tested here were higher in FAE than in IECs, which coincided with the microarray analysis data. Moreover, as listed in Table 1, 38 genes showed the highest expression level in FAE.

3.3. Identification of FAE-specific molecules by in situ hybridization analysis

In view of the above results, the 38 selected genes were found to be promising candidates for encoding FAE-specific molecules. However, their individual Ct values, which are negatively correlated with their expression levels, were widely distributed between 19.6 and 33.6. Only the genes that were highly expressed were analyzed by ISH to identify the localization of cells expressing them, because those with low expression levels might not be detected by the ISH. The ISH analysis revealed that positive signals of several molecules were observed to be either diffuse throughout FAE and IECs or restricted to the bottom of crypts with little or no expression at the villi or FAE surface (data not shown). In contrast, Ub-D, Tm4sf4 and TNFR12a were predominantly expressed in FAE (Fig. 4). On the other hand, Sgne-1 revealed an interesting expression pattern. Its transcripts were distributed in FAE but not in IECs, and positive signals were diffusely scattered on FAE (Fig. 5), indicating that only a subpopulation of FAE cells highly expressed this gene.

3.4. M cell-specific expression of Sgne-1

The expression pattern of Sgne-1 reminded us of the distribution of M cells: they are scattered in FAE, accumulating specifically around the peripheral region of the FAE dome and gradually disappearing toward the apical region. To confirm this, we next examined the colocalization of Sgne-1 expression by staining with UEA-1 lectin. This lectin binds to the α(1,2)-fucose moiety on the cell surface, as well as inside the cell although to a relatively low extent, and has been used for tracing M cells in FAE of BALB/c mice. After probing the Sgne-1 mRNA expression by ISH (Fig. 5C), the same section was stained with UEA-1 lectin (Fig. 5D). Sgne-1 expression was predominantly observed in UEA-1+ cells and not in UEA-1- cells (Fig. 5E). Similar results were obtained in two other PPs prepared from different individuals (data not shown), demonstrating the M cell-specific expression of Sgne-1 in the small intestine.
### Table 1. Identification of genes expressed in FAE at the highest level by quantitative PCR analysis.

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<th>Gene name</th>
<th>Relative expression against IEC</th>
<th>Ct Value</th>
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* Gene expression levels are normalized to that of GAPDH. Data are expressed as fold change in mRNA expression levels relative to IEC. 

** Ct value of FAE that negatively correlates to the mRNA expression level of each gene.

4. DISCUSSION

In the present study, we characterized the cellular phenotype of FAE by gene expression profiling using a microarray technique. The data revealed that hundreds of genes in FAE and IECs are differently regulated at the transcriptional level. Such a difference most likely reflects not only their difference in cell composition but also the changes in intracellular status. Whereas the expression of housekeeping genes, including brush border enzymes and cytoskeletal molecules, is lowered in FAE, many host defense molecules are upregulated in FAE. For instance, the expression levels of pepti-doglycan recognition protein 2 (Pglyrp2) and mannose receptor C type 1 (MRC1) are much higher in FAE than in IECs, consistent with a previous study. These molecules recognize pathogen-associated molecular patterns (PAMPs) and contribute to innate immunity in response to microbial infection. MRC1 serves as an endocytic and a phagocytic receptor for a wide variety of microbes and other endogenous glycoproteins. It is intriguing to ask whether MRC1 is involved in the internalization of macromolecules in M cells. Besides PAMP recognition molecules, the expression of subsets of chemokines (e.g., CCL9, CCL20 and CXCL16) is upregulated in FAE (Supplemental Table 1, http://www.dna-
Although IECs can also secrete chemokines, the secretion takes place mostly upon stimulation with bacterial infection or inflammatory cytokines. Given that FAE is closely associated with immune cells in LFs, this specialized epithelium may be immunologically activated under physiological conditions. This idea is further supported by our ISH results that Ub-D (also termed FAT10) and TNFRsf12a (also termed TweakR/Fn14) are highly expressed in FAE. The former molecule is encoded in the MHC class I locus and is inducible with interferon-γ and tumor necrosis factor-α, suggesting a potential role in antigen presentation. TNFRsf12a is also involved in the immune response. Stimulation of this molecule with the ligand Tweak induces NF-κB activation, leading to the cell surface expression of such cell adhesion molecules as ICAM-1 and the secretion of chemokines. Similarly, the expression of cell adhesion molecules (i.e., ICAM-1 and VCAM-1) and chemokines is upregulated in FAE. Although lymphotoxin β receptor signaling has been reported to induce CCL20 in IECs, Tweak/TNFRsf12a signaling may also mediate the cell communication of hematopoietic cells with FAE. Among the chemokines upregulated in FAE, the physiological roles of CCL9 and CCL20 in immature DC migration have been well documented, however, the functions of the other chemokines produced by FAE remain to be elucidated.

We have also described here that Tm4sf4 is highly expressed in FAE. Tm4sf4 (also termed il-TMP) was originally regarded as a member of the tetraspanin family, based on the similarity in amino acid sequence and its structure that features four transmembrane domains. At present, however, Tm4sf4 is assigned to the recently emerging L6 membrane protein family because of the absence of conserved motifs characteristic of tetraspanins. Human Tm4sf4 is expressed in the small intestine and the liver, where the expression is observed in the plasma membrane of terminally differentiated epithelial cells and hepatocytes. The forced expression of Tm4sf4 in a human colon adenocarcinoma cell line led to the inhibition of cell proliferation, suggesting its role in epithelial cell growth and differentiation. Of note is the observation that the transcripts of tetraspanin Tm4sf3 were present in human FAE. These molecules may contribute to the regulation of cell differentiation in FAE.

The identification of M cell-specific molecules has been
a subject of current interest. Previous biochemical and histochemical studies have shown features characteristic of M cells. For instance, the apical membrane of M cells possesses spare and irregular microfolds instead of the rigid and closely packed microvilli observed in normal absorptive enterocytes. This is further characterized by a thin filamentous glycoprotein coat and the decreased expression of hydrolytic enzymes.\textsuperscript{22,40,41} The apical surfaces of M cells bind selectively to IgA and not to other classes of immunoglobulins, indicating the presence of an undefined IgA Fc receptor on M cells.\textsuperscript{42} The basolateral membrane of M cells is deeply invaginated, allowing the migration of lymphocytes, macrophages and DCs into the intraepithelial microdomain, called M-cell pockets.\textsuperscript{43,44} The M-cell pockets effectively shorten the distance from the apical to the basal membranes of M cells. Such morphological features of M cells facilitate the adhesion and transport of mucosal antigens. Apart from such well-documented morphological studies, however, little is known about the key molecules regulating antigen transcytosis in M cells.

Our ISH analysis revealed that the Sgne-1 molecule is highly expressed in FAE. This is an unexpected observation, because this molecule is considered to be a neuroendocrine marker.\textsuperscript{45} The mRNA or the protein of Sgne-1 is found in either neuronal tissues such as the anterior pituitary or in endocrine tissues such as the adrenal medulla and pancreas.\textsuperscript{46,47} In addition, its expression is observed in a subpopulation of neuroendocrine cells in the gastrointestinal tract, albeit at a low level.\textsuperscript{48–50} The possibility that enteroendocrine cells (EECs) in FAE express Sgne-1 is excluded for the following reasons. First, the distribution of EECs is restricted to the intestinal villi and is very rarely found in the FAE region.\textsuperscript{6} Second, the Sgne-1-expressing cells are UEA-1 positive. Although this lectin labels goblet cells and Paneth cells as well, these cells are scarcely found in FAE and, if present, are easily distinguishable because of their characteristic staining patterns that differ from M cells. It is, therefore, concluded that Sgne-1 is predominantly expressed by M cells in FAE. To our knowledge, this is the first observation of Sgne-1 expression by non-neuroendocrine cells.

Sgne-1 is an acidic protein residing in secretory granules, and is widely observed in both vertebrates and invertebrates. Its amino acid sequence is highly conserved among mammals, suggesting a fundamental role in homeostasis.\textsuperscript{45} The best-known function of Sgne-1 is as a specific molecular chaperone for proprotein convertase (PC) 2. PC2 catalyzes the conversion of β-lipotropic hormone (β-LPH) into β-melanocyte-stimulating hormone and β-endorphin. PC2 is biosynthesized in the endo-
plasmic reticulum (ER) as an inactive precursor. The maturation and the subsequent activation of PC2 take place in post-Golgi compartments, namely, the trans-Golgi network (TGN) and the secretory granules. Sgne-1 binds to an inactivated proPC2 at the ER, facilitating its transport through the Golgi apparatus and TGN to the secretory granules. However, the interaction with PC2 may not be the only cellular function of Sgne-1, because some lineages of cells contain the Sgne-1 transcript alone without PC2. In addition, unlike PC2 deficient mice that survive, Sgne-1-deficient mice die early after birth from a Cushing’s disease-like disorder with ACTH hypersecretion. Yeast two-hybrid analysis has raised the possibility that Sgne-1 interacts with molecules other than PC2 and has unexpected functions. Whereas Sgne-1 functions as a molecular chaperone of human insulin-like growth factor, it exerts intrinsic transcriptional activity by interacting with TATA box-binding protein. Sgne-1 also binds to the tumor suppressor protein p53, which suggests the involvement of Sgne-1 in cell cycle regulation. These studies imply the multifunctional properties of Sgne-1. The biological function of Sgne-1 in M cells remains unknown; however, it is surmised to contribute to the intracellular membrane trafficking of the mucosal antigens of M cells. In addition, this molecule may participate in chemokine secretion by M cells, as our recent immunohistochemical study revealed that M cells are a major source of FAE-derived chemokines, CCL9 and CCL20 (K. Hase and H. Ohno, unpublished data). Further investigation is required to verify this notion.

We identified three molecules that are specifically expressed throughout FAE, and one molecule that is expressed only by M cells in the mouse gut. These molecules are expected to function as the biochemical markers of FAE and M cells. Further studies are under way to elucidate the master molecules associated with the differentiation or the transcytosis function of M cells.

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References


Seidah, N. G., Hsi, K. L., De Serres, G. et al. 1983, Isolation and NH2-terminal sequence of a highly conserved human and porcine pituitary protein belonging to a new superfamily, *Immunocytochemical localization in pars distalis and pars nervosa of the pituitary and in the pars distalis and pars nervosa of the pituitary and in the...


