**Cell Reports**

**Essential role of TOSO/FAIM3 in intestinal IgM reverse transcytosis**

**Graphical abstract**

**Highlights**
- SIgM is taken up by TOSO+ M cells of the murine GALT and the NALT
- SIgM is retro-transported toward DC-SIGN+ DCs from lymphoid tissues
- SIgM-based complexes result in mucosal and systemic antigen-specific antibody responses

**Authors**
Nicolas Rochereau, Eva Michaud, Louis Waeckel, ..., Gilles Biolley, Blaise Corthesy, Stephane Paul

**Correspondence**
stephane.paul@chuv-st-etienne.fr

**In brief**
Rochereau et al. investigate the transport of SIgM across the murine nasal and gut mucosa. They provide evidence that IgM is taken up by mucosal M cells and then retro-transported toward cells in lymphoid tissues. This function of SIgM could play an important role in the regulation of mucosal immunity.
Essential role of TOSO/FAIM3 in intestinal IgM reverse transcytosis

Nicolas Rochereau, Eva Michaud, Louis Waeckel, Martin Killian, Rémi Gayet, Roman Goguyer-Deschaumes, Xavier Roblin, Gilles Biolley, Blaise Corthésy, and Stéphane Paul

1Centre International de Recherche en Infectiologie (CIRI), Team GIMAP, Université de Lyon, Université Claude Bernard Lyon 1, INSERM, U1111, CNRS, UMR530, CIC 1408 Vaccinology, 42023 Saint-Etienne, France
2R&D Laboratory of the Division of Immunology and Allergy, CHUV, Centre des Laboratoires d’Epalinges, 1066 Epalinges, Switzerland
3These authors contributed equally
*Correspondence: stephane.paul@chu-st-etienne.fr

SUMMARY

Secretory immunoglobulin A (SIgA) can travel to and from the lumen and transport antigen to subepithelial cells. However, IgM can also multimerize into functional secretory component-bound immunoglobulin. While it is already known that both SIgA and SlgM undergo transcytosis to be secreted at the mucosal surface, only SIgA has been shown to perform retrotranscytosis through microfold cells (M cells) of the Peyer’s patch. Here, we investigate whether SIgM could also be taken up by M cells via retrotranscytosis. This transport involves FcμR binding at the apical membrane of M cells. We then demonstrate that SlgM can be exploited by SlgM-p24 (HIV-capsid protein) complexes during immunization in the nasal- or gut-associated lymphoid tissue (NALT or GALT), conferring efficient immune responses against p24. Our data demonstrate a mucosal function of SlgM, which could play a role in the regulation of mucosal immunity.

INTRODUCTION

The concept of local or mucosal immunity has expanded dramatically over the past few decades. The mucosal wall is equipped with a tightly regulated defense system. The intestinal mucosa contains more than 80% of all immunoglobulin-producing cells in the human body. Gut-associated lymphoid tissue (GALT) is the primary inductive site for secretory immunoglobulins, of which secretory immunoglobulin A (SIgA) is the dominant entity. SIgA has long been recognized as the first line of defense in protecting the intestinal epithelium from enteric pathogens and toxins. It is generally assumed that SlgM acts primarily through receptor blockade, steric hindrance, and/or immune exclusion. Evidence has emerged indicating that SlgM promotes the uptake and delivery of antigens from the intestinal lumen to dendritic cell (DC) subsets located in the GALT. It thusly influences inflammatory responses normally associated with the uptake of highly pathogenic bacteria and potentially allergenic antigens. This particular feature of SlgA, called reverse transcytosis, is mediated by antigen-sampling microfold cells (M cells) (Rochereau et al., 2013a). Our and previous data have shown that SlgA selectively binds Dectin-1 and SIGLEC-5 (sialic acid-binding immunoglobulin-like lectin 5) receptors at the apical membrane of Peyer’s patches (PPs) in M cells. This triggers SlgA transport across the epithelium toward underlying DCs of the subepithelial dome (SED) region, inducing their maturation and migration to the interfollicular areas (Rochereau et al., 2013a). We also demonstrated that both the C31 region and glycosylation, particularly sialic acid residues, are involved in M-cell-mediated reverse transcytosis. Finally, oral and nasal administration of an SlgA-bound p24 antigen (HIV-capsid protein) vaccine candidate was shown to elicit antigen-specific immune responses in both mucosal and systemic compartments, ensuring protection against a recombinant vaccinia virus expressing the gag antigen (Rochereau et al., 2014, 2016). Although considerable emphasis has been devoted to the role of IgA because of its relative abundance in digestive tract secretions, mucosal functions of SlgM have been comparatively neglected.

Human secreted IgM (SlgM) is the first antibody to be produced during an immune response (Gong et al., 2018; Michaud et al., 2020). Bearing five N-linked glycosylation sites on each μ chain (Arnold et al., 2005), SlgM is a J-chain-bound pentamer. It is secreted at mucosal surfaces through polymeric immunoglobulin receptor (pIgR)-mediated transcytosis, similarly to SlgA (Brandtzaeg, 1981; Norderhaug et al., 1999). To date, two additional Fc receptors, Fcμ receptor (FcμR) and Fcγ/μ receptor (Fcγ/μR), have been shown to bind to IgM (Shibuya et al., 2000) in addition to the pIgR (Bournazos et al., 2009). Both Fcγ/μR and pIgR can bind both to IgA and IgM with intermediate affinity. FcμR, also known as TOSO/Fas apoptotic inhibitory molecule 3 (FAIM3), has been identified the latest as a highly specific receptor for IgM and data suggest that it may serve as an uptake receptor for IgM-opsonized antigens by B cells (Shima et al., 2010).

SlgM represents up to 20% of mucosal antibodies in humans (Chen et al., 2020). Gut IgM* B cells have been shown to recall...
efficient anti-commensal responses via SIgM secretion and IgA class-switch recombination (CSR) (Magri et al., 2017). Studies in patients with selective IgA deficiency (SIgAD) show partial functional redundancy between IgA and IgM coating of commensal bacteria (Catanzaro et al., 2019; Fadlallah et al., 2018a), which is thought to limit the induction of dysbiosis. Although it does not recapitulate all typical SIgA functions, human SIgM binds a broader set of commensals than SIgA (Catanzaro et al., 2019; Fadlallah et al., 2018a), activates complement (Michaelsen et al., 2017) and extensive anti-GLCNAc SIgM repertoire protect the neonatal mucosa (Xia and Gildersleeve, 2019) during early bacterial colonization. Moreover, SIgM-deficient mice and humans have increased sensitivity to viral, bacterial, and protozoal infections (Louis and Gupta, 2014). As these studies suggest IgM samples bacterial antigens in the intestinal mucosa, we sought to study whether M cells also transport SIgM from the intestinal lumen to PPs through receptor-specific processes. We demonstrate that SIgMs were able to perform reverse transcytosis via M cells in both human in vitro models and murine in vivo models. We were then able to demonstrate that this relied on to the ability of SIgM to bind TOSO/FAIM3 on M cells. We then showed that SIgM-bound p24 HIV-1 antigen (human immunodeficiency virus-1) was taken up by M cells in vivo in both murine GALT and nasal-associated lymphoid tissue (NALT), allowing for the induction of a strong p24-specific immunity.

RESULTS

SIgMs are efficiently taken up and transported by murine M cells

One feature of M cells is their ability to transport a broad range of materials, including antibodies from the lumen to the underlying follicles. Specific retrotransport of IgM was evaluated in an in vitro model of human follicle-associated epithelium (FAE) containing M-like cells as previously described (Rochereau et al., 2013). Significant transport of IgM across the cell monolayer harboring M-like cells was observed (Figure 1A). No transport was detected in the absence of M cells. Similar results were obtained with either recombinant CD20- or tumor necrosis factor (TNF)-specific IgM (Figure 1A). Association with human SC (secretory component) did not increase IgM uptake by M-like cells. Uptake of IgM by M-like cells was also confirmed by colocalizing IgM with CA19-9 and GP2 M-cell markers (Figure 1B). Specificity of IgM reverse transcytosis was further confirmed in vivo after fluorescent IgM administration in ligated murine intestinal loop experiments (Figure 1C). The number of IgM+ cells were 8 times more abundant than that of IgG+ cells in PPs, thus indicating increased transport of IgM through the FAE (Figure 1E). NALT is separated from the nasal cavity by an epithelium containing M cells (Date et al., 2017). This anatomic similarity with the gastrointestinal tract led us to evaluate the specific retrotransport of IgM after nasal administration in mice (Figure 1D). Intranasal administration of fluorescent IgM showed colocalization between IgM and UEA-1, a typical murine M-cell marker (Mabbott et al., 2013). IgM+ cells were 10 times more abundant than IgG+ cells in the NALT (Figure 1E). These results demonstrate that IgM is taken up by M cells of the GALT and NALT before being retrotransported into lymphoid tissues. IgM reverse transcytosis is glycosylation independent

Influence of N-glycosylation on the uptake of IgA2 by M-like cells has been previously described (Rochereau et al., 2013b). To determine whether IgM N-glycosylations could influence their uptake and transport by M-like cells, we tested the in vitro reverse transcytosis of enzymatically digested IgM by PNGase (peptide:N-glycosidase) and neuraminidase. First, SDS-PAGE performed under reducing conditions confirmed the expected molecular weight for the light and heavy chains of deglycosylated IgM (Figure S1A). Surprisingly, the efficiency of IgM reverse transcytosis was not dependent on its glycosylation motifs in vitro. No decrease of the retrotransport was detected in the presence of M cells under either PNGase or neuraminidase treatment (Figure S1B), and no binding on M cells was detected by epifluorescence (Figure S1C). To further explore the possible involvement of sialoglycans in IgM trafficking, blocking experiments were performed using β-glucans, mono- or disaccharides, and sialic acids (Figure S1D). In contrast with IgA2 (Rochereau et al., 2013a), no inhibition was observed with any tested sugar families, independently of the concentration we used.

TOSO is a receptor for SIgM expressed on M cells

To further explore the uptake of IgM in M cells, antibodies directed against IgA or IgM receptors that have been described as being involved in their transport were used in blocking experiments (Figure 2A). In contrast to IgA2 (Rochereau et al., 2013a), the use of an anti-DC3/1 and anti-SIGLEC-5 monoclonal antibody (mAb) did not influence IgM retrotransport. Similarly, targeting the human myeloid IgA Fc receptor (CD89) with a specific mAb, or the mannose receptor with an anti-CD206 mAb, did not block IgM transport through M-like cells. Other receptors described as being involved in IgA transport, such as transferrin receptor (CD71), plgR, and Fcα/I receptor (CD31), showed similar results. Oppositely, the use of an anti-TOSO mAb, a novel IgM receptor, led to a complete inhibition of IgM reverse transcytosis. Consistently, the binding of IgM on TOSO and its presence on M-like cells in vitro was observed by epifluorescence microscopy (Figure 2B) and flow cytometry (Figures 2C and 2D). These figures show colocalization of GP2+/glycoprotein 2) and TOSO+ cells and IgM+ and TOSO+ cells only on co-cultures, suggesting TOSO might be upregulated upon M-cell differentiation. To confirm these in vitro results, TOSO expression was also analyzed in vivo in the murine FAE (Figure 2E) and ex vivo in the human FAE (Figure 2F). Both panels show colocalization between TOSO and M cells markers. Moreover, we observed a significant 9-fold decrease in IgM transport when cells were treated with TOSO siRNA (small interfering RNA) in vitro, confirming its implication in reverse transcytosis (Figure 2G), as background levels of IgM retrotransport were not affected after transfection with siRNA knockdown. Indeed, either with or without M-like cells in the polarized monolayer, IgM retrotransport is comparable to that in Figure 1A (lanes control and random oligonucleotide). In additional control experiments, both the decrease in the level of targeted proteins and the monolayer integrity (trans-epithelial electrical resistance [TEER]) were systematically monitored after siRNA transfection (Figures S2A and S2B). No significant modifications among control, knockdown, and stimulated cells were observed. In order to confirm the role of TOSO in
IgM reverse transcytosis, we evaluated the retrotransport of IgM in mice PPs with or without the incubation of an anti-TOSO antibody into the loops. Immunofluorescence staining is shown in Figure 2H. We observe a significant decrease of retrotransported IgM in PP while blocking TOSO receptors (Figure 2I) and a significant decrease of bound IgM on M cells (Figure 2J).

Traditional transport (transcytosis) across polarized epithelial of secretory polymeric immunoglobulin into secretions requires polymeric immunoglobulin receptor and a plethora of evolutionarily well-conserved intracellular proteins, including EEA-1, Rab-5, Rab-9, Rab-11, Rab-17, and Rab-25 (Fung et al., 2018; Jing and Prekeris, 2009; Rojas and Apodaca, 2002). EEA-1, Rab-5, and Rab-17 siRNA knockdown led to a significant decrease of IgM transport relative to controls (Figure 2G).

Reverse-transcytosed SlgM targets mucosal DC-specific receptors in vitro and in vivo
To measure IgM transport from the intestinal lumen to DCs located in the SED region, we took advantage of previous data showing that SlgA is recognized by DCs via DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing non-integrin) receptor after their transport through M cells (Baumann et al., 2010; Rochereau et al., 2013a, 2014, 2016). IgM uptake by DCs was assessed in vivo in an intestinal ligated loop model and the nasal cavity from wild-type (WT) mice using an anti-DC-SIGN-fluorescein isothiocyanate (FITC) and IgM-(PE) (poly-erythrin) specific immunostaining. IgMs transported in the GALT and NALT were found in close association with murine DC-SIGN+ DCs (Figures 3A and 3B). In parallel, HeLa transfectants stably expressing human DC-SIGN added to the
Figure 2. TOSO receptor is involved in IgM reverse transcytosis

(A) Mono- (Caco-2 cells alone) and co-cultures (Caco-2 and Raji cells) were pre-incubated apically with 10 mg blocking Abs directed against potential receptors prior to addition of various IgM for 90 min at 37°C. The concentration of transported IgM was evaluated by ELISA (n = 4). Vertical bars show the mean value ± SEM. (B and C) One-way ANOVA followed by Bonferroni post hoc test was used. Detection of TOSO on M-like cells (GP2 cells) in vitro assessed by immunofluorescence (top view) (B) and flow cytometry (C). Scale bars represent 100 µm, except for (B), top and bottom left, where they represent 10 µm.

(D) TOSO (Alexa Fluor 488) expression increases under co-culture conditions and is bound by IgM-APC(allophycocyanin). Here, two commercial IgMs with different paratopes (Invitrogen) were used. Data are representative of three distinct experiments.

(E and F) Tissue section showing a PP obtained from an intestine in mice (E) and patient biopsy samples taken from terminal ileum (F). Tissues were immunolabeled with PE-UEA-1 and FITC-TOSO (E) and human PE-GP2 and FITC-TOSO (F) at room temperature (RT) for 2 h. Colocalization between GP2 or UEA-1 with TOSO was confirmed (n = 2). In all images, dotted lines delineate the FAE separating the intestinal lumen and the lymphoid tissue (side view). Scale bars, 100 µm.

(G) After siRNA knockdown, IgM transport was quantified in the inverted in vitro model of FAE, and transported IgM was evaluated by ELISA. Vertical bars show the mean value ± SEM; n = 3 independent experiments. One-way ANOVA followed by Bonferroni post hoc test was used (***p < 0.005).

(H) Immunofluorescence staining of murine PPs after intestinal loop experiments with and without anti-TOSO antibody injection into the loops. The white line marks the edges of the PP. Images are representative of observations made from three distinct experiments. Scale bar, 100 µm.

(I) Quantification of Cy3 signal in PPs using ImageJ (Fiji). Four fields were acquired per section, and three to four sections per loop were imaged for each mouse. Data are shown as means ± SEM. *p < 0.05 (two-way ANOVA with Tukey correction).

(J) Quantification of double-positive Cy3-AF488 signal in PPs. Four fields were acquired per section, and three to four sections per loop were imaged for each mouse. Data are shown as means ± SEM. *p < 0.05 (two-way ANOVA with Tukey correction).
basolateral chamber in Caco-2 cells co-cultures were used as surrogates to human DCs populating the SED region of PPs (Rochereau et al., 2011). The binding of IgM that had previously crossed the monolayer containing M-like cells was observed by immunostaining of HeLa-DC-SIGN (Figure 3C), but not with WT HeLa cells. SIgM is first taken up by M cells via TOSO receptor and is subsequently targeted to DC-SIGN+ DCs located in the SED region. We have also confirmed DC-SIGN binding to IgM in an in vitro models of human monocyte-derived DCs (MoDCs) using two commercial IgMs with distinct paratopes (anti-TNF and anti-CD20 IgM). To avoid a potential effect of residual endotoxins present in IgM preparations, all experiments were performed in the presence of polymyxin B. In addition, while IgA1 and IgA2 have been shown to induce mild and highly activated pro-inflammatory profiles in a similar model (Gayet et al., 2020), both IgMs strikingly dampened surface activation marker expression relative to cytokine-stimulated controls (Figure 3E). Compared to the transcriptomic profile of unstimulated MoDCs, cytokine-stimulated cells exhibited heightened expression of pro-inflammatory chemokines (CXCL1, ~136-fold; CXCL13, ~126-fold; CCL19: ~490-fold; CCL20, ~77-fold) and cytokines (IL1a: ~69-fold; IL-1b, ~230-fold; IL-12b, ~149-fold; IL-8, ~90-fold; TNFSF11, ~37-fold; BMP7, ~86-fold) involved in innate cells attraction and adaptive effector maturation/activation and...
more generally acting as TNF-α activators or downstream effectors (Figure 3F). In contrast, IgM-treated MoDCs presented a stark transcriptional decrease for most of these markers, suggesting potential tolerogenic properties that have not been explored in the mucosal compartment.

Altogether, these results indicate that IgM stimulation reprograms gene expression in MoDCs. We next investigated whether oral and nasal immunization with p24-SIgM complexes could induce subsequent p24-specific immune responses.

**Oral and nasal administration of p24-SIgM complexes is highly immunogenic in vivo**

BALB/c mice were immunized as described in STAR Methods. Mucosal and systemic p24-specific humoral immune responses were measured by ELISA in serum and various mucosal compartments, including feces and vaginal lavage samples collected 1 week after the last immunization (Figure 4A). Immunization with p24-SIgM induced significantly higher p24-specific IgG and IgA titers in serum and feces samples after oral administration compared to p24 alone. p24-IgG and IgA titers were also significantly higher than for p24 alone in vaginal lavages after nasal administration (Figure 4A). Splenic B lymphocytes recovered from immunized mice were co-cultured with p24 in ELISpot assays. In both oral or nasal immunization routes, B cell populations included significantly higher levels of IgA- and IgG-secreting plasma cells than p24 control samples, indicating specific activation and migration of locally primed cells to distant effector sites (Figure 4B).

Induction of interferon-γ (IFN-γ)-secreting Th1 cells in the process of fighting mucosal infections, particularly viral infections, is highly relevant. To assess this response, splenocytes from immunized mice were co-cultured with p24 in ELISpot assays. Nasal and oral immunizations with p24-SIgM both promoted significantly more elevated IFN-γ production in response to p24 (Figure 4B). Altogether, these results highlight the potential of IgM as a functional and potent vaccine carrier in promoting both humoral and cellular immunity in vivo.

**DISCUSSION**

M cells possess a high transcytosis capacity, allowing for a wide range of materials to be transported into the mucosa, including particulate antigens, soluble macromolecules, and pathogens. The latter are delivered from the intestinal lumen to inductive sites of the mucosal immune system. M cells are also the primary route through which SlgA promotes GALT immunity. We previously found that SlgA-bound Dectin-1 on M cells and that the ensuing transport of SlgA-bound antigen into nasal and intestinal lymphoid tissue targeted DC-SIGN+ DCs. In the current study, we investigated the transport of SlgM across the murine GALT and NALT. We provide evidence that IgM is taken up by TOSO+ M cells of both the GALT and NALT. We also demonstrate that it is then retrotransported toward DC-SIGN+ DCs from lymphoid tissues. Finally, we validate these *in vitro* results using *in vivo* analyses of murine tissues, ultimately demonstrating that TOSO-mediated uptake of SlgM-based complexes...
results in productive mucosal and systemic antigen-specific antibody responses.

Reverse transcytosis of IgM across human M-like cells using a cell culture model that reproduces features of the FAE tissue has been measured. We confirmed that human IgM, with or without SCs, selectively bound the apical surface of in-vitro-differentiated human M-like cells. In contrast to IgA (Rochereau et al., 2013a), IgM glycosylation does not appear to impact this retrotransport. This difference can be explained by the involvement of another receptor for IgM than that of IgA reverse transcytosis. A recent study shows that IgM-TOSO interaction is critically dependent on the Cμ4 domain, specifically on the glutamine at position 510. Although glutamates can be glycosylated, N-glycosylation of IgM is borne by asparagines and regulates post-translational protein folding (Giannone et al., 2017). There is, however, evidence of atypical glutamine N-glycosylation in human IgG2 that suggests further characterization of StgM N-glycans might be required (Valliere-Douglass et al., 2019). Sialic acids and SC-borne glycosylation are also involved in StgA binding to SIGLEC-5, a co-receptor for reverse transcytosis (Mathias and Corthe´sy, 2011; Rochereau et al., 2013b). SIGLECs are abundantly expressed at the apical membrane of M cells (Mabbert et al., 2013) and may also serve as co-receptors for StgM transport. Among them, CD22 is a likely candidate, as it has already been characterized as an IgM receptor (Adachi et al., 2012).

Our work evidences TOSO expression by M-like cells, together with its involvement in the reverse transcytosis of StgM. TOSO holds a critical role IgM homeostasis, B cell survival, and humoral responses. It is expressed by CD19+ cells, CD4+ and CD8+ T cells, and CD56+ CD3− natural killer (NK) cells in both human (Honjo et al., 2013; Kubagawa et al., 2000) and murine B cells (Honjo et al., 2012) and is rapidly internalized upon IgM binding (Ouchida et al., 2012; Vire et al., 2011), suggesting enhanced responsiveness of the mucosa that is yet to be demonstrated.

Transcytosis across M cells is known to enable the selective transport of particulate antigens in the absence of any assessable damage (Neutra et al., 1996; Owen and Ermak, 1990). This holds true for soluble StgM, as the transcytosed antibody released by M cells in the human in vitro and murine in vivo models was still able to specifically target cells expressing DC-SIGN in the basolateral environment (Figures 3A and 3B). In mucosal tissues such as the rectum, uterus, and cervix, DC-SIGN is abundantly expressed by DCs of the lamina propria and PPs, further substantiating the importance of the localization of DC-SIGN+ DCs as a first line of defense against viruses and pathogens. Moreover, DC-SIGN, by binding to ICAM-3 (intercellular adhesion molecule 3), favors the generation of antigen-specific suppressive CD4+ T cells, which produce interleukin-10 (IL-10) (Li et al., 2012), a regulatory cytokine that intervenes in both intestinal homeostasis and the production of local secretory immunoglobulin to promote commensal mutualism. Besides bringing information on the mechanism involved in IgM retrotransport, deciphering the identity of such receptor may lead to the further development of mucosal vaccines targeting M cells. Indeed, delivery in the form of StgM-based immune complexes promoted antigen-specific responses (Figure 4). Another recent report provides similar information on the prevention of SHIV (simian-human immunodeficiency virus) with a mucosally administered anti-HIV 33C6-IgM (Gong et al., 2018). In this study, the majority of the passively immunized rhesus macaques was completely protected.

When considering gut immunity, the microbiota will play a defining role in educating immune mucosal responses. As StgM dually binds IgA+ commensal flora (Fadlallah et al., 2018a) Magri et al., 2017; Sterlin et al., 2020), it is worth investigating whether they engage similar immune pathways upon antigen retrotransport. Notably, there is a higher diversity of anti-commensal mucosal B cell IgM repertoires compared to IgA or IgG (Li et al., 2020). While the IgA repertoire narrows with increased commensal exposure at the mucosal level, that of IgM further diversifies (Li et al., 2020), suggesting functional redundancy to IgA is not the only role mucosal IgM plays in the gut. In addition, human gut IgM plasma cells coexist with an extensive anti-commensal memory IgM+ B cell repertoire (Magri et al., 2017). Further elucidation of StgM functions, specifically regarding antigen selection and immune response polarization after mucosal antigen delivery, would fill this gap in knowledge and allow for efficient adaptation of oral vaccination approaches using StgM as cargo systems.

STAR METHODS

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Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.110006.

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AUTHOR CONTRIBUTIONS

N.R., E.M., L.W., M.K., R.G., and R.G.-D. carried out the experiment. N.R. wrote the manuscript with support from X.R., G.B., B.C., and S.P. B.C. supervised the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCE TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Anti-human Dectin-1/CLEC 7A polyclonal Ab (pAb) (goat IgG) | R&D Systems | Cat# MAB1859; RRID:AB_2081791 |
| anti-human plgR Ab (goat IgG) | R&D Systems | Cat# AF2717; RRID:AB_2164821 |
| anti-human CD170 mAb (SIGLEC-5) (mouse IgG1) | R&D Systems | Cat# MAB1072; RRID:AB_2239301 |
| Anti-human CD71 mAb (mouse IgG1) | Clinisciences | Cat# 11-235-C100; RRID:AB_10755321 |
| Anti-FAIM3 Ab (rabbit IgG) | LifeSpan BioSciences | Cat# LS-C375684-50 |
| anti-human FAIM3 (TOSO) | Biolegend | Cat# 398102; RRID:AB_2814380 |
| Anti-CD351 antibody | Abcam | Cat# ab170491 |
| anti-human GP2 mAb | MBLbio | Cat# D277-3; RRID:AB_10598500 |
| anti-human GP-2-PE | MBLbio | Cat# D277-5; RRID:AB_11160953 |
| mouse anti-human CA19.9 | Thermo Scientific | Cat# MA5-12421; RRID:AB_10980397 |
| anti-DC-SIGN BV421 | BD Biosciences | Cat# 564127; RRID:AB_2738610 |
| anti-CD1a APC-Vio770 | Miltenyi Biotec | Cat# 130-100-224; RRID:AB_2656026 |
| anti-CD14 PE | Miltenyi Biotec | Cat# 130-113-149; RRID:AB_2725977 |
| anti-CD11b PE-Vio770 | Miltenyi Biotec | Cat# 130-113-803; RRID:AB_2819369 |
| anti-HLA-DR BV510 | BD Biosciences | Cat# 740173; RRID:AB_2739926 |
| anti-CD80-PE-Vio770 | Miltenyi Biotec | Cat# 130-102-885; RRID:AB_2659270 |
| anti-CD86-FITC | Miltenyi Biotec | Cat# 130-113-571; RRID:AB_2751132 |
| anti-CD40-PE | Miltenyi Biotec | Cat# 130-105-376; RRID:AB_2660764 |
| anti-iGM-APC | Southern Biotech | Cat# 9020-11; RRID:AB_2687522 |

**Chemical compound**

Matrigel | BD Biosciences | Cat# 356234 |
Pomzymkin B | InvivoGen | Cat# trl-pmb |
Halt™ protease inhibitor cocktail | Thermo Scientific | Cat# 11824111 |
ON-TARGETplus SMARTpool siRNAs | Thermo Scientific | Cat# NC1593972 |
heterobifunctional crosslinker Sulfo-KMUS | Thermo Scientific | Cat# 21111 |
ELISPOTplus kit for mouse IFN-γ | Mabtech | Cat# 3321-4HPW-2 |

**Experimental models:**

Mouse:
- 8-week-old female BALB/c mice | Charles River | Strain Code: 028 |

Cell lines:
- Caco-2 cells | obtained from Dr. Maria Rescigno | Rescigno et al., 2001 |
- human Burkitt’s lymphoma cell line Raji B | American Type Culture Collection | Raji, CCL-86 |

**Resource availability**

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact: Pr Stéphane Paul (stephane.paul@chu-st-etienne.fr)

**Materials availability**
This study did not generate new unique reagents

**Data and code availability**
- All data reported in this paper will be shared by the lead contact upon request.
This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL DETAILS

Cell culture
Both the human intestinal cell line Caco-2 cell (clone 1) (obtained from Dr. Maria Rescigno, University of Milan-Bicocca, Milan, Italy) (Rescigno et al., 2001) and CHO cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (PAA) supplemented with 10\% (v/v) fetal bovine serum (FBS, Thermo-Fisher), 1\% (v/v) non-essential amino-acids (PAA), and 1\% (v/v) penicillin-streptomycin (PAA). The human Burkitt’s lymphoma cell line Raji B (American Type Culture Collection) was cultured in RPMI 1640 supplemented with 10\% (v/v) FBS, 1\% (v/v) non-essential amino-acids, 1\% (v/v) L-glutamine and 1\% (v/v) penicillin-streptomycin.

Mice
Mouse strains used in this study consisted of 8-week-old female BALB/c mice (Charles River). All animals were housed at the Unité hospitalo-universitaire d’expérimentation animale (Saint-Etienne, France). The protocol was approved by the Ethics Committee of CREEA (Permit number: No. 69387487).

METHOD DETAILS

Key reagents
Pullulan from *Aureobasidium pullulans*, mannann from *Saccharomyces cerevisiae*, Lactose, L-fructose, glycogen from bovine liver, sucrose, curdlan from *Alcaligenes faecalis*, laminarin from *Laminaria digitata* and zymosan from *Saccharomyces cerevisiae* were all purchased from Sigma-Aldrich. Anti-human Dectin-1/CLEC 7A polyclonal Ab (goat IgG), anti-human pIgR Ab (goat IgG) and anti-human CD170 mAb (SIGLEC-5) (mouse IgG1) were purchased from R&D Systems. Anti-human CD206 mAb (mouse IgG1) (mannose receptor) was purchased from Ozyme. Anti-human CD71 mAb (mouse IgG1) was purchased from Clinisciences. Anti-FAIM3 Ab (rabbit IgG) were purchased from LifeSpan BioSciences and Anti-CD351 antibody were purchased from Abcam. All were blocking antibodies and were used according to manufacturers’ instructions.

Inverted in vitro model of the human FAE
The inverted FAE model has been previously published (des Rieux et al., 2007; Rochereau et al., 2013a). Inverted Transwell® polycarbonate inserts (12 wells, pore diameter of 3.0 \( \mu \text{m} \), Corning) were coated with Matrigel (BD Biosciences) prepared in pure DMEM to a final protein concentration of 100 \( \mu \text{g/mL} \) for 1 h at room temperature (RT). The coating solution was removed and inverted inserts washed with 300 \( \mu \text{L} \) of DMEM. Caco-2 cells (3 \( \times \) 10\(^5\)), resuspended in 300 \( \mu \text{L} \) of supplemented DMEM, were seeded on the lower insert side and cultured overnight. The inserts were then inverted and placed in a 12-well culture dish and kept for 9 days. Raji B cells (5 \( \times \) 10\(^5\)), were resuspended in supplemented DMEM, added to the basolateral compartment of the Caco-2 cells, and maintained for 5 days (named co-culture in the main text). Cultures of Caco-2 cells, cultivated as above but without the Raji B cells (named monoculture in the main text), were used as controls. Both co- and mono-cultures were controlled by measurement of TEER using an EndohmTM tissue resistance chamber (Endohm-12, World Precision Instruments) connected to a Millipellelectrical resistance (Millipore). The resistance of medium alone (9 \( \mathrm{U} \times \text{cm}^2 \)) was considered as background resistance and subtracted from each TEER value. Finally, the inserts were inverted in 6-well plates, and a piece of silicon tubing (14 \( \times \) 20 mm, Labomoderne) was placed on the basolateral side of each insert.

Immunolabelling of in vitro model of the human follicle-associated epithelium (FAE)

Immunofluorescence
Inserts were washed in HBSS to eliminate residual medium, incubated in 4\% paraformaldehyde for 30 min, permeabilized with 0.1\% Triton X-100 (Sigma-Aldrich), and blocked with PBS containing 5\% FBS for 15 min at room temperature. Immunolabeling was performed using a combination of PE-IgM, FITC-IgM, anti-human GP2 mAb (MBL), Anti-FAIM3 Ab (Abcam), and mouse anti-human CA19.9 (Dako). Each reagent was diluted to 1/100, and incubated for 2 h at RT. 1/200 dilutions of secondary antibodies labeled with a fluorochrome were incubated for 1 h at room temperature. After two washes, inserts were air-dried, mounted with Fluoprep (BioMerieux), and observed by epifluorescence microscopy (Eclipse Ti, Nikon).

Flow cytometry
Inserts were incubated for 10 minutes at 37 °C with PBS EDTA 2mM. Cells were gently washed off the insert by pipetting directly on the filter with PBS-EDTA and then washed once in FACS buffer (PBS, EDTA 1mM, FBS 2%). Cells were then stained first with anti-human FAIM3 (TOSO)- (Biolegend) for 20 minutes in the dark at 4 °C. After washing, they were then stained with AF488-conjugated rat anti-mouse IgG2 (BD Bioscience) 1/500 for 20 minutes. After 2 washes, cells were incubated with anti-human IgM-APC (Southern Biotech), and anti-human GP-2-PE (MBLbio) for 20 minutes at 4 °C in the dark. Unless specified, all antibodies were diluted...
Transport experiments were performed in HBSS at 37°C for 90 min with 10 mg of IgM (Sigma). Basolateral solutions were then recovered and the number of retro-transcytosed Ab measured by ELISA. Ninety-six-well Maxisorp plates were coated overnight with 50 μL of anti-human IgM (Abliance) (10 μg/mL in sterile PBS). Transported IgM were detected by means of incubation (for 1 hour at 37°C) with horseradish peroxidase–conjugated goat anti-human IgM (Oaris) for 1 hour at 37°C. Results are presented as mean ± SEM concentrations.

Inhibition experiments
cell monolayers were first pre-incubated apically with 5 mg of inhibitor in HBSS for 90 min at 37°C, and washed with HBSS, before adding the IgM suspension. All transport experiments were carried out in triplicate.

SirNA assays
ON-TARGETplus SMARTpool siRNAs (Dharmacon) were transfected at final concentration 5 nM using Silentfect reagent (Bio-Rad) in the inverted in vitro model of FAE according to the procedure provided by the manufacturer. The genes targeted in this study were TosFAIM3 (L-004451-00-0005), Dectin-1 (L-021476-00-0005), SIGLEC-5 (L-019522-02-0005), EEA-1 (L-004012-00-0005), plgR (L-017729-00-0005), Rab6 (L-004009-00-0005), Rab7 (L-010388-00-0005), Rab9 (L-004177-00-0005), Rab11 (L-004726-00-0005), Rab17 (L-006474-00-0005), Rab25 (L-010366-00-0005) (Dharmacon)

Flow cytometry
All wells were co-cultured with 50 μg/mL of polymyxin B (InvivoGen). Reagents were then added at indicated concentrations for moDC stimulation during 24 h: cytokines activator cocktail comprising 25 ng/mL human IL-1β (Miltenyi), 100 ng/mL human IL-6 (Miltenyi), 50 ng/mL of human TNF-α (Miltenyi), anti-TNF the various IgA at 12 μg/mL.

RNA from frozen pellets of 1 × 10^6 activated moDC were extracted using Quick-RNA MicroPrep Kit (Zymo Research). Total RNA quantification was performed on a 2000c nanodrop (Thermos scientific) and RNA quality was evaluated via the Experion™ system (Biorad, France). RT-qPCRs for the human cytokines and chemokines panel (QIAGEN, PAHS-150Z) were run on the Applied Biosystems 7500 system. Quantification of gene expression was performed via the online QIAGEN data Analysis Center following manufacturer guidelines.

Production of p24–SIgM complexes
Polymeric IgM from the IgMHF5 hybridoma was obtained from culture in CELLline flasks (Integra, 900010) in RPMI 1640 medium supplemented with 10% fetal bovine serum (SVF), 2mM L-glutamine (Sigma, G7513), 1% non-essential amino acids (Sigma, M7145), 10 mM HEPES (Sigma, H0887), and 1mM sodium pyruvate (Sigma, S8636). Supernatant containing IgM was purified on a POROS CaptureSelect IgM Affinity Matrix resin (Invitrogen) every week. Purified free human SC was produced in Chinese hamster ovary cells (Phalipon et al., 2002). SIgM were obtained by combining plgM molecules in PBS with a twofold excess of human SC for 1 h at room temperature as previously described (Rindisbacher et al., 1995). SIgM complexes were then purified before being covalently linked to p24 at equimolar ratio, p24 antigen was produced in CHO cells (Px Therapeutics, Lyon, France). Covalent association between p24 and SIgM was carried out with the heterobifunctional crosslinker Sulfo-KMUS according to the protocol provided by the manufacturer (Thermo Scientific, Waltham, MA, USA).
GALT and NALT uptake of IgM using intestinal ligated loop model and nasal administration

For ileal loop preparation, mice were anesthetized by intraperitoneal injection of a mix of ketamine and xylazine. A total of 100 μL of a 1 mg/mL solution of SIgM or Cy3-conjugated IgM diluted in PBS was administered into a 1.5-cm ileal loop containing a PP. Upon completion of the experiment, mice were sacrificed, the loop was removed, embedded in optimal cutting tissue solution (OCT) and placed on microscope slides.

An identical SIgM solution was administered intranasally and NALT dissection was performed as previously described (Rochereau et al., 2016). Processed tissue was embedded in OCT and mounted on microscope slides. Seven micrometer sections (Leica cryostat model CM1950; Leica Microsystems, Wetzlar, Germany) were mounted on Ultra Superfrost microscope slides (VWR International, Radnor, Pa). Slides were washed in PBS to eliminate residual OCT and blocked with PBS containing 5% FBS for 30 minutes at room temperature. Immunolabeling was performed with the following primary antibodies (1:200 dilution): anti-human DC-SIGN and anti-Ulex europaeus agglutinin 1 (UEA-1), both FITC-conjugated. Slides were examined on an epifluorescence microscope (Eclipse; Nikon, Tokyo, Japan).

Immunization with p24–SIgM complexes

Mouse oral and nasal immunizations were performed as previously described (Rochereau et al., 2014, 2016). Immunizations consisted of three administrations of 100 μL each at 1-week intervals, followed by a last boosting immunization 3 weeks later. Groups of five mice were immunized with either PBS, 50 μg of p24 or 50 μg of p24–SIgM at each time point.

Recovery of blood, feces, and vaginal lavages

Biological fluids were recovered prior to- and 1 week after each- immunization to examine their Ab content. Sera were obtained from whole blood recovered by retro-orbital collection, followed by centrifugation at 16,000x g and stored at −20°C until use. Vaginal lavages were taken up following injection of 100 μL of PBS with adapted tips (CP100, Gilson). 5 fresh feces from separate stools were collected from each animal and resuspended in PBS in order to obtain a same final concentration of 100mg/ml. Feces and vaginal lavages were incubated with Halt™ protease inhibitor cocktail (Thermo Scientific), centrifuged at 16,000 x g and stored at −20°C until use.

Quantification of p24-specific IgG and IgA

Abs in biological fluids p24-specific IgG/IgA in serum, vaginal lavages, feces and saliva samples were measured by quantitative ELISA. 96-well Maxisorp™ plates were coated with 50 μL of p24 antigen solution (5 μg/mL in sterile PBS). Bound or captured Igs were detected by incubation (1 h, 37°C) with HRP-conjugated goat anti-mouse IgG, and bound IgA was detected with biotinylated goat anti mouse IgA followed by streptavidin- HRP. Both assays were normalized using pre-titrated monoclonal p24-specific IgG and IgA (IBL). Results are given as the means of concentrations ± SEM and background Ab concentration measured on pre-immune samples was subtracted for each animal.

Detection of p24-specific Th1 response by ELISPOT

Mice were sacrificed by cervical dislocation under anesthesia 1 week after the booster immunization. ELISPOT assays were performed with splenocytes as described (Carvalho et al., 2001; Lefrançois and Lycke, 2001). ELISPOT assays were performed with the ELISPOTplus kit for mouse IFN-γ according to the procedure provided by the manufacturer (Mabtech, Cincinnati, OH, USA). Splenocytes were stimulated with recombinant p24 antigen à 1 μg/ml.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed with the InStat version 5.02 software from GraphPad Software. A nonparametric Mann–Whitney U-test or one-way ANOVA followed by Bonferroni post hoc test was used where appropriate (Clark et al., 2013; Xu and Südhof, 2013). The p values less than 0.05 (marked by *), or less than 0.01 (**), or less than 0.005 (***”) were considered as significant. Statistically significant differences between groups are emphasized by bars connecting the relevant columns. All statistical details of experiments can be found in the figure legends.