Secretory IgA Mediates Bacterial Translocation to Dendritic Cells in Mouse Peyer’s Patches with Restriction to Mucosal Compartment

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In addition to fulfilling its function of immune exclusion at mucosal surfaces, secretory IgA (SIgA) Ab exhibits the striking feature to adhere selectively to M cells in the mouse and human intestinal Peyer’s patches (PPs). Subsequent uptake drives the SIgA Ab to dendritic cells (DCs), which become partially activated. Using freshly isolated mouse DCs, we found that the interaction with SIgA was tissue and DC subtype dependent. Only DCs isolated from PPs and mesenteric lymph nodes interacted with the Ab. CD11c⁺CD11b⁻ DCs internalized SIgA, while CD11c⁺CD19⁺ DCs only bound SIgA on their surface, and no interaction occurred with CD11c⁺CD8α⁺ DCs. We next examined whether SIgA could deliver a sizeable cargo to PP DCs in vivo by administering SIgA-Shigella flexneri immune complexes into a mouse ligated intestinal loop containing a PP. We found that such immune complexes entered the PPs and were internalized by subepithelial dome PP DCs, in contrast to S. flexneri alone that did not penetrate the intestinal epithelium in mice. Dissemination of intraepithelial S. flexneri delivered as immune complexes was limited to PPs and mesenteric lymph nodes. We propose that preexisting SIgA Abs associated with microbes contribute to mucosal defense by eliciting responses that prevent overreaction while maintaining productive immunity. The Journal of Immunology, 2007, 179: 7751–7757.

Mucosal surfaces are endowed with powerful defense mechanisms, which selectively handle harmful and innocuous Ags to ensure local homeostasis. Constitutive physicochemical and mechanical features (mucus, glycocalyx, lactoferrin, defensins, peristalsis) are backed up by a highly adaptive immune system, whose controlled action contributes to shape proper adaptive immunity (1, 2). An important activity of mucosal epithelia is the production of the special type of Abs referred to as secretory IgA (SIgA). SIgA is produced predominantly as a dimer containing the J chain and is complexed with a secretory component (SC) from epithelial origin. Bound SC is the cleaved extracellular portion of the polymeric Ig receptor, that transports dimers and larger polymers of IgA (collectively called pIgA) into mucosal secretions (4). The classical view is that SIgA reinforces the first line of defense against microorganisms by agglutinating potential invaders and facilitating their clearance by peristaltic and mucociliary movements, a mechanism called immune exclusion (5).

In the intestine, Peyer’s patches (PPs) and associated M cells represent the primary site for uptake and presentation of ingested Ags (6). In rabbit, exogenously administered SIgA has been observed in association with the apical surface and inside the intraepithelial pocket of M cells (7). SIgA injected into a mouse ligated ileal loop bound selectively to M cells, whereas IgG or IgM did not (8). Uptake of SIgA by PP DCs targeted this class of Ab to dendritic cells (DCs) in the subepithelial dome (SED) region and T cells in the interfollicular regions (IFRs), and this had raised the question of the immunological consequences of such an interaction in modulating ongoing mucosal immune responses and/or control of local homeostasis (9). In the mouse, oral delivery of exogenous SIgA comprising human SC and mouse IgA induced hSC-specific Ab and cellular responses in mucosal and peripheral tissues (10). This occurred in the absence of the prototype mucosal adjuvant cholera toxin. Specific immune responses were accompanied by sustained IL-10 and TGF-β expression in draining mesenteric lymph nodes (MLNs) and spleen.

Because DCs in the SED region are anatomically positioned to sample Ags from the intestinal lumen and contribute to the regulation of local immune responses (11), these observations provided a link between DC targeting and possible immune regulation by SIgA. This was further consistent with the phenotypic analysis of DCs within tissues of the gastrointestinal tract that had identified a plethora of distinct subtypes with different spatial distribution and effector functions (12). In the present study, we show specific interaction between SIgA and PP and MLN DCs, as compared with DCs recovered from peripheral lymph nodes and spleen. We demonstrate the capacity of SIgA to transport Ags across the intestinal epithelium comprising a PP using as a cargo Shigella flexneri not capable of spontaneously entering the mouse epithelium. The bacterium is rapidly recovered within PPs and MLNs, with no further spreading, and this takes place with fully preserved intestinal tissue. Our results suggest that selective microbe delivery mediated by SIgA at the level of PPs may serve to trigger mucosal immune responses under neutralizing, noninflammatory conditions.

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Materials and Methods

**Protein production, purification, and characterization**

Polymeric IgA Ab from hybridoma clone IgAC5 specific for \( S. \) _flexneri_ serotype 5a LPS (13) was obtained as previously described (14). Purified free hSC was prepared from Chinese hamster ovary cells (15). SlaG molecules were obtained by combining in PBS pIgA molecules with a 2-fold excess of hSC for 2 h at room temperature according to the conditions described in the study of Rindisbacher et al. (16). Hamster anti-mouse CD11c IgG was produced from N418 hybridoma cells (17) and purified by affinity chromatography using protein G-Sepharose beads (Amersham Biosciences). ELISAs for quantification of IgA and SC was performed as described in the study of Rindisbacher et al. (16, 18). The biphenichonic acid assay protein assay kit (Pierce) was used for protein measurement.

**Hamster anti-mouse CD11c IgG Ab.**

Hamster anti-mouse CD11c IgG was produced from N418 hybridoma cells (17) and purified by affinity chromatography using protein G-Sepharose beads (Amersham Biosciences). ELISA for quantification of IgA and SC was performed as described in the study of Rindisbacher et al. (16, 18). The biphenichonic acid assay kit (Pierce) was used for protein measurement. Cy3-IgA and Cy3-SlaG molecules were obtained by conjugation to indocarbocyanine-Cy3 using the FluoroLink mAb-Cy3 labeling kit (Amersham Biosciences) according to the procedure provided by the manufacturer. The same protocol was used for the coupling of the Cy5 fluorochrome (Amersham Biosciences) to purified hamster anti-mouse CD11c IgG Ab.

**Mice**

Four-week-old female BALB/c mice were obtained from Harlan Breeders and used at the age of 8–10 wk. They were housed in the animal facility of the Centre Hospitalier Universitaire Vaudois under standard conditions. All experiments were approved by the State Veterinary Office.

**Purification of DCs from mouse tissues**

Mice were killed by euthanasia, the abdomen was incised, and the small intestine was removed and thoroughly rinsed with cold PBS. Macroposphically visible PPs were excised using curved surgery scissors and kept in cold, plain MEM-α for no longer than 30 min. PPs were digested with prewarmed (37°C) collagen IV (0.5 mg/ml in plain MEM-α; Sigma-Aldrich) for 15 min at room temperature under gentle shaking. After enzymatic treatment, PPs were crushed and forced with a 5-ml syringe pestle through a 70-μm pore nylon mesh cell strainer (BD Biosciences). After centrifugation for 5 min at 400 x g at 4°C, cells were then passed through a 40-μm pore nylon mesh cell strainer and resuspended in cold buffer A (PBS pH 7.3, 5% FCS, and 5 mM EDTA) at a concentration of 5 x 10^6 cells/100 μl. Total cells were recovered from mouse spleen, MLNs, bronchial lymph nodes (BLNs), and inguinal lymph nodes (ILNs) using the same procedure as for PPs, with the exception that no collagenase was added. Freshly isolated cells (10–25 x 10^6 cells) were positively selected by two successive magnetic separations (MACS) using anti-CD11c magnetic beads (Miltenyi Biotec).

**Flow cytometry analysis of DCs**

**Whole DC labeling.** Freshly isolated DCs (5,000–20,000 cells) were resuspended in 200 μl of cold buffer B (PBS pH 7.3, 0.5% BSA, and 5 mM EDTA) and incubated with FITC-conjugated anti-CD11c mAb (clone HL3, hamster IgG1, 1/100 dilution in buffer B; BD Biosciences) for 25 min on ice. FcγRII/III-Rs were blocked for 15 min at 4°C using rat anti-mouse CD16/CD32 mAbs (clone 2.4G2, 1/100 dilution; BD Biosciences). After washing with buffer B, flow cytometry analysis (FACScan flow cytometer; BD Biosciences) was conducted after exclusion of propidium iodide-positive dead cells, and live cells were used to determine the background of fluorescence. Data were processed using the CellQuest software (BD Biosciences).

**DC subtype-specific labeling.** To evaluate the percentage of myeloid and lymphoid DC subtypes or possible CD19^+ B cell contaminants after purification, DCs were labeled for 25 min on ice with a combination of either FITC-conjugated anti-CD11c mAb and PE-conjugated anti-CD11b mAb (clone M1/70, rat IgG2b, 1/200 dilution; BD Biosciences), PE-conjugated anti-CD8α mAb (clone 53-6.7, rat IgG2a, 1/200 dilution; BD Biosciences), or PE-conjugated anti-CD19 mAb (clone 1D3, rat IgG2a, 1/200 dilution; BD Biosciences), respectively. All Abs were diluted in buffer B. Propidium iodide staining excluded dead cells from the analysis. Data were processed using the CellQuest software.

**Association of SlgA with DCs isolated from PPs**

Freshly isolated DCs (15,000 cells) were resuspended in 50 μl of buffer B and incubated with 100 ng or 1 μg of Cy3:SlgA (80–800 ng of Cy3:SlgA per 10^6 cells) at room temperature for 2 h with shaking. Cy3:SlgA, a 5-fold molar excess of pIgA, hSC, or polymeric IgM was added to the DCs 5 min before incubation with the Cy3:SlgA. Similarly, dilutions of anti-FcγRα receptor (1/50) (19) or anti-transferrin receptor (1/50) (20) mAb capable of labeling cells positive for these two receptors were added 5 min before incubation with the Cy3:SlgA. Cells were then incubated for 25 min at room temperature with a 1/50 dilution of Cy5-conjugated anti-CD11c mAb. DC subtypes with Cy3:SlgA were determined as indicated above, with the exception that 1/50 dilutions of each FITC-labeled mAb was used in combination for 25 min at 4°C. Cells were spread onto glass slides, fixed in PBS/2% paraformaldehyde, and mounted with Vectashield (Vector Laboratories) for observation by laser scanning confocal (LSC) microscopy.

**LSC microscopy**

LSC microscopy pictures were obtained using a Leica TCS NT microscope (Leica Microsystems). Excitation was obtained with an argon-krypton laser, with lines set at 488, 568, and 647 nm for FITC, Cy3, and Cy5 fluorochromes, respectively. Images were taken with a 20 x or ×63 objective and processed using the Leica TCS NT software (Leica Microsystems).

**S. flexneri culture conditions**

M9OT, an invasive isolate from _S. flexneri_ serotype 5a LPS (21, 22) expressing GFP, was a gift from Dr. A. Phalipon (Pasteur Institute, Paris, France). Bacteria were grown overnight at 37°C on BBL-agar plates (3% BBL-trypticase soy broth, 1.5% Bacto-agar (BD Biosciences), 0.1% Congo Red, and 50 μg/ml ampicillin (Sigma-Aldrich)). Three colonies were picked and cultured in Luria-Bertani (LB) medium (1% Bacto-tryptone, 0.5% Bacto-yeast extracts (Sigma-Aldrich), 1% NaCl, and 50 μg/ml ampicillin) for 45 min at 37°C before being spread onto LB agar plates (1.5% Bacto-agar in LB medium). Bacteria grown overnight as a lawn were recovered in 0.9% NaCl, and their density was calculated using formula: 1 OD₆₀₀ corresponds to 5 x 10^8 bacteria/ml (15).

**Association of immune complexes with freshly isolated PP DCs**

Immune complexes were formed by incubating 10^9 GFP:_S. flexneri_ with 3.75 pmol of either Cy3:SlgAC5 or Cy3:lgG2C02 in PBS for 25 min on ice, corresponding to 200,000 molecules per bacterium. Freshly isolated DCs were resuspended in buffer B and incubated for 2 h at 37°C with GFP:_S. flexneri_-SlgA immune complexes (DC:bacterium ratio of 1:20). In control experiments, DCs were incubated with 10^3 Fluoresbrite Yellow Green microspheres (diameter 0.2 μm; Polysciences) per cell.

**Administration into mouse ligated intestinal loops**

Surgery and injection of 10^8 GFP:_S. flexneri_ or immune complexes in 100 μl of PBS were performed as previously described (9).

**Preparation of tissue sections and immunolabeling**

Intestinal portions containing one PP were fixed in PBS/4% paraformaldehyde for 2 h at 4°C, with subsequent embedding in PBS/12% sucrose for 90 min at 4°C, followed by overnight incubation in PBS/18% sucrose at 4°C. Intestine portions were flushed into the lumen with OCT (Sakura Finetek), followed by complete immersion. Sections cut at a 7-μm thickness were obtained, and blocking was conducted in PBS containing 5% mouse serum and 2% FCS. DCs were labeled using biotinylated anti-CD11c mAb (1/50 dilution) in PBS/2% FCS and Cy3-streptavidin or Cy5-streptavidin (1/1000 dilution) for 30 min each at room temperature and finally mounted with Vectashield (Vector Laboratories).

**Staining with hematoxylin-erythrosin B**

Slides were washed for 5 min in H₂O and then stained in hematoxylin (Sigma-Aldrich) for 45 s. After a rapid washing in H₂O, slides were soaked in 70% ethanol/0.1 M HCl for 5 s. Slides were extensively washed in H₂O, then colored in 0.25% erythrosin B (Merck) for 10 s. Dehydration steps were performed by washing slides successively in 70% ethanol, 95% ethanol, 100% ethanol, and xyloïl during 5 s for each step. Slides were then mounted with Eukitt medium (Sigma-Aldrich) and observed by light microscopy (Leica Microsystems).

**Delivery of immune complexes by the oral route**

GFP:_S. flexneri_ (10^8) alone or immune complexes were resuspended in 150 μl of PBS and administered intragastrically using a metal feeding tube (Harvard Apparatus) to starved mice. Sixteen hours after oral gavage, intestinal PPs, MLNs, spleen, and liver were aseptically removed, cropped, and resuspended in 1 ml of inhibition buffer containing 50 μg/ml gentamicin to kill extracellular bacteria. The tissues were washed twice with antibiotic-free cold PBS. Residual liquid was drained off, the tissue was smashed through a 40-μm pore nylon mesh cell strainer, and live cells were counted.
following staining with trypan blue. Culturable bacterial counts were determined by plating dilutions of cell suspension on agar plates containing 50 μg/ml ampicillin.

Results

Selective binding of SIgA to freshly isolated PP DCs

Tracking of exogenously delivered SIgA in the intestinal lumen has previously shown selective binding to M cells and subsequent uptake by underlying DCs located in the SED region (8, 9). We first sought to characterize the DC features responsible for SIgA capture using CD11c+ DCs freshly isolated from mouse PPs. Upon incubation with Cy3-labeled pIgA or SIgA, we confirmed that DCs in vitro display the same pattern of association and internalization as DCs in the tissue, as assessed by LSC microscopy (Fig. 1, A and B). Uptake and/or surface binding took place over a large range of pIgA (80–800 ng) or SIgA (100 ng to 1 μg) amounts tested and was completely prevented upon incubation at 4°C (our unpublished observations). Specificity of DC-IgA association was further established in competition experiments by preincubation with 5-fold molar excess of unlabeled pIgA (Fig. 1C). In contrast, neither free SC (Fig. 1C) nor IgG specific for FcγII and III receptors (our unpublished observations) used in equivalent molar excess competed for SIgA binding, arguing for the presence of a receptor selective for the IgA moiety of SIgA on DCs. Furthermore, no blocking of SIgA binding and internalization was observed by competition with IgM or after addition of mAb specific for either the FcαRI or the transferrin receptor (Fig. 1D). The FcαRI not existing in mice and not involved in human DC binding of IgA (23) can similarly be excluded.

Subtype and tissue specificity of DCs associating with SIgA

Closer examination of LSC microscopy photographs led us to observe three main patterns of association between PP DCs and Cy3-labeled SIgA or pIgA: 1) DCs that internalize SIgA, 2) DCs that do not interact with SIgA, and 3) DCs that display binding of SIgA limited to their surface. In the mouse, PPs contain three predominant populations of DCs expressing the CD11c surface integrin (17): the myeloid DC subtype coexpressing the CD11b surface marker (CD11c+CD11b+), the lymphoid DC subtype coexpressing the α-chain of the CD8 receptor (CD11c+CD8α+), and the double-negative subtype (CD11c+CD11b−CD8α−) (24). We speculated that the SIgA-binding properties of DCs are explained by the nature of the three DC subtypes previously identified in PPs. BALB/c mice used in the study yielded 38 ± 10.6% of CD11c+/CD11b+ DCs, 36% ± 16.6 of CD11c+CD8α+ DCs and 45 ± 26.5% of CD11c−CD11b−CD8α− DCs, consistent with previous reports (25, 26). We found that myeloid DCs bind and internalize SIgA, whereas lymphoid DCs did not interact with the SIgA (Fig. 2, A and B). In control experiments aimed at excluding the presence of copurified B cells, we identified the existence of a DC subtype expressing the CD19 marker that is capable of binding SIgA and pIgA at their surface only (Fig. 2C). The same pattern of association with DCs was obtained with other preparations of SIgA and pIgA obtained from different hybridomas with various profiles of glycosylation, arguing for a limited, if not absent, role of carbohydrates in recognition between the Ab and cell partners (our unpublished observations).

In addition to subtype specificity, we found that the tissue origin of DCs represents another essential variable that contributes to the specificity of the association with SIgA/pIgA. As for PPs, DCs isolated from MLNs interacted with both SIgA and pIgA, whereas BLNs, NLNs, and spleen DCs did not bind pIgA or SIgA under identical experimental conditions (Fig. 3). The results strongly suggest that the interaction with SIgA is limited to DCs from lymphoid tissues associated with the gut.
firmed by the appearance of yellow spots, was observed on the cell microscopy (Fig. 4 reflecting formation of immune complexes was assessed by LSC microscopy. Surface binding of yellow SIgA-GFP: S. flexneri along with internalization of free SIgA (A) was observed by incubating freshly isolated DCs. A representative image of at least 50 observed fields is depicted.

Uptake of S. flexneri-SIgA immune complexes by freshly isolated PP DCs

Having established that DCs isolated from PPs specifically bind SIgA/pIgA, we next investigated whether immune complexes consisting of S. flexneri and SIgA in our experimental setting are similarly taken up. Two different types of immune complexes were obtained by incubating S. flexneri-expressing GFP (GFP:S. flexneri) with Cy3:SIgAC5 or Cy3:IgGC20 (both specific for S. flexneri serotype 5a LPS (13, 27)). SIgA Ab coating of GFP:S. flexneri reflecting formation of immune complexes was assessed by LSC microscopy (Fig. 4A). The presence of immune complexes, as confirmed by the appearance of yellow spots, was observed on the cell surface only (Fig. 4B). Surface binding was identically detected using 0.2-μm latex beads (Fig. 4C), yet they also localized within the cytoplasm of DCs in the SED region in vivo (10). This supports the notion that although PP DCs maintain their capability to internalize SIgA/pIgA in vitro, their phagocytic properties are lost for larger Ags after isolation from the tissue. These unexpected practical limitations prompted us to examine the outcome of immune complexes in vivo.

SIgA Ab delivers S. flexneri to the SED region of PPs

In contrast to rabbit and monkeys, intestinal Shigella infection in mice does not develop after oral administration. The mouse model is therefore perfectly suited to examine whether SIgA can mediate the entry of the bacterium in the form of immune complexes. We thus administered the immune complexes formed by GFP:S. flexneri and Cy3:SIgAC5 in a ligated intestinal loop containing a PP. Incubation in mice was allowed to proceed for 45 min. Multiple intense green spots indicative of the translocation of the bacteria were seen in the SED region of PPs and the IFRs when frozen sections were analyzed by LSC microscopy. (Fig. 5A, white arrowheads). In support of the concept that S. flexneri alone does not cross spontaneously the mouse epithelium, we could not detect any bacterium-associated fluorescence in PPs and neighboring epithelium (Fig. 5B, white arrowhead). Administration of immune complexes formed by GFP:S. flexneri and Cy3-labeled IgGC20 resulted in the same absence of bacterial translocation across the epithelium (Fig. 5C). Consistent with the observation that IgG does not bind to M cells (8), this indicated moreover selective Ag delivery properties for the SIgA Ab.

This represents the first demonstration that SIgA Ab can deliver a sizeable cargo, i.e., whole bacteria, into PPs in vivo. In accordance with the migratory properties of DCs originating from the PPs (28), GFP-labeled bacteria were recovered in the paracortical, T cell-rich region of draining MLNs 4 h after administration in the ligated intestinal loop (Fig. 5D, white arrowheads). Although not a direct demonstration that immune complexes are brought along by DCs, this was in keeping with the in vitro data showing that SIgA binds to DCs isolated from MLNs.

We then sought to determine whether this holds true when SIgA Ab is bound in immune complexes with S. flexneri. After uptake from the intestinal lumen, we observed colocalization of blue CD11c+ DCs in the SED region with yellow spots indicative of preserved immune complexes (Fig. 5E). In many instances, white spots resulting from the superimposition of red SIgA, green bacteria, and blue DCs were observed throughout the SED region. Sustained, although more diffuse, turquoise fluorescence coloring the cytoplasm of DCs was additionally detected, indicating that GFP:S. flexneri was indeed internalized, possibly along degradative pathways. Whether transepithelial transport of S. flexneri through the concerted action of SIgA and M cells affects the integrity of the mucosa was next analyzed.

Entry of S. flexneri-SIgA immune complexes into PPs does not result from tissue lesion and prevents systemic dissemination

In the rabbit ligated intestinal loop shigellosis infection model, massive leukocyte recruitment caused tissue damage within 4 h (29). In contrast, mice did not develop shigellosis and associated intestinal lesions because no entry of S. flexneri occurred. However, to rule out that artifactual passage across the epithelium takes place because of physical damages induced by surgery at the level of the intestinal loops, S. flexneri-SIgA immune complexes were administered by intragastric gavage, and both PPs and adjacent intestinal villus samples were prepared time-wise. Staining of frozen sections at 24 h with H&E showed no histopathological lesion...
FIGURE 5. Uptake of SIgA-S. flexneri immune complexes by PP DCs in vivo. A, Immune complexes made of GFP:S. flexneri and Cy3:SIgAC5 are observed in the SED region of PPs after incubation for 45 min in a ligated intestinal loop. No entry of GFP:S. flexneri alone (B) or GFP:S. flexneri-Cy3:IgGC20 immune complexes (C) occurred. Yellow lines indicate the interface between the intestinal lumen and tissue. D, GFP:S. flexneri are found in association with the deep paracortical region of the MLNs. E, Colocalization in PP sections of GFP:S. flexneri-Cy3:SIgAC5 immune complexes and Cy5-conjugated anti-CD11c mAb indicates targeting to DCs in the SED region. V, Villus. F, H&E coloration of a section of a PP and adjacent villi 24 h after delivery of $10^9$ immune complexes by intragastric gavage. G, Assessment of the penetration of GFP:S. flexneri-SlgAC5 immune complexes into PPs, MLNs, spleen, and liver. Counts of live bacteria are indicated per $10^8$ cells of tissue homogenate. Data are expressed as means ± SD ($n = 3$).

To examine whether SlgA Ab can deliver an associated bacterial Ag to PPs, the “natural” entry of such an Ag has to be avoided. S. flexneri represents an ideal candidate Ag because it does not infect the mouse by the intestinal route (30). SlgAC5-mediated translocation of S. flexneri represents thus an appropriate mouse model that further benefits from suitable controls, including IgGC20 mAb with the same anti-LPS specificity. The observation that DCs in the SED region bind and internalize SlgAC5-based immune complexes suggests that SlgA can serve as a delivery vehicle for large Ag in the physiological context. However, in face of the large excess of SlgA in intestinal secretions, the passage of immune complexes remains limited. Preferential uptake of SlgA-Ag complexes compared with free SlgA might be due to a conformational change(s) that unmasks the binding site to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor. For abundant commensal bacteria similarly coated with SlgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to the M cell receptor.

Discussion

The main function of the SlgA Ab is to avoid the attachment of Ag to mucosal surfaces, a mechanism referred to as immune exclusion. We have previously shown that after selective interaction with M cells, SlgA is targeted to DCs located in the SED region, resulting in limited mucosal and systemic immune responses against a non-self-associated protein Ag. In this work, we have first established that SlgA could direct a bacterial cargo to the SED region and then characterized the subtype of DCs involved in the sampling of the SlgA-Ag immune complex. Finally, we have demonstrated in the physiological context that SlgA restricts Ag mucosal dissemination to PPs and MLNs under noninflammatory conditions.

nor mononuclear cell infiltrates in either the PPs or adjacent intestinal villi (Fig. 5F).

In the absence of artifactual destruction of the epithelial mucosa, the mouse model is well suited to assess how far live S. flexneri associated with SlgA disseminate from the PPs. Following administration by intragastric gavage of immune complexes (or GFP:S. flexneri alone) to starved mice, PPs, MLNs, spleen, and liver were collected 16 h later and the presence of bacteria was assessed by culture on selective medium. Most bacteria were recovered in association with PPs and MLNs, reflecting limited spatial entry in the form of immune complexes (Fig. 5G). The level of uptake was low as compared with experimental shigellosis in rabbit (29), in accordance with the primary function of immune exclusion performed by SlgA, and the maintenance of epithelial integrity observed above. Occasionally, <10 S. flexneri were measurable in spleen and liver. As expected, we found no bacteria in all tissues tested after intragastric gavage with GFP:S. flexneri alone or in the form of IgGC20 Ab immune complexes (because the values for different tissues were equal to zero, the data are not depicted in Fig. 5G).

It is conceivable that early in life, immune complexes comprising maternal SlgA might contribute to educate the intestinal immune system of the breast-fed baby toward a tolerogenic or protective type of response, should the Ag be innocuous or harmful. In the case of pathogens, passive transfer of SlgA-based immune complexes could provide a “shield” during a period when the neonatal immune system is not mature enough to respond actively (34). In the context of mucosal vaccination, passive administration of SlgA-based immune complexes might deserve further evaluation, since this has the potential to direct small amounts of Ag to sampling and processing sites such as PPs in the absence of aggressive mucosal adjuvant. The stability of the SlgA molecule along with its delivery properties should favor the establishment of oral immunity through the contribution of effector
and/or regulatory pathways characteristic of the intestinal mucosal compartment.

In contrast to the unique DC population derived in vitro from mouse bone marrow cells, DCs in PPs consist of at least three major subtypes (35). By choosing to work with freshly isolated PP DCs that most likely resemble DCs in situ, we found selective association of SlgA as a function of the subtypes under analysis. Internalization was observed for myeloid CD11b+/DCs, while surface binding was detected for the novel CD19+ DCs. DCs isolated from MLNs showed SlgA internalization, whereas DCs from other tissues including BLNs, ILNs, and spleen did not interact with SlgA, indicating that the tissue origin represents an essential feature to be considered for analysis of DC function. Myeloid DCs isolated from murine PPs produced high levels of IL-10 and induced naïve T cells to differentiate into cells that produced much higher levels of IL-10 than T cells primed with any other DC subset (36). PP DC-mediated induction of IL-10-producing CD4+ T cells (37, 38), and expression of TGF-β in the lung and gastrointestinal mucosa, play a particularly important role in maintaining local tolerance and homeostasis (37, 39, 40). Similarly, oral delivery of exogenous recombinant SlgA and SlgA-based immune complexes contribute to orchestrate noninflammatory responses (this study) by triggering secretion of IL-10 and TGF-β by mucosal CD4+ T cells (40). Because these cytokines are also involved in IgA class switch, the presence of natural (32) or preexisting SlgA after a recall challenge might permit to keep inducing mucosal Ab production against a variety of microbial Ags, while minimizing any proinflammatory, deleterious effects on the integrity of the mucosal barrier.

The current study demonstrates that immune complexes formed by SlgA and S. flexneri were found in association with DCs in the SED region of PPs. The fact that either Ag-bound or free SlgA are captured by DCs suggests that a specific receptor for this Ab isotype exists on these cells. We checked that established receptors for IgA appear not to be involved in SlgA binding and uptake. The possible capture of SlgA by DC-specific DC receptors such as Langerin and DC-SIGN and C-type lectin R1 (41) needs further investigation using DCs isolated from PPs. Although the nature of the receptor remains to be elucidated, binding of SlgA does not lead to activation of DCs as reflected by the weak modulation of surface costimulatory molecules CD80/CD86 (10). Limited responsiveness of DCs to the SlgA-Ag immune complexes can possibly be due to the high degree of glycosylation of SlgA that would result in poor processing by DCs (42). Along the same line, SlgA can “sequester” the associated Ag, which leads to insufficient levels of immunostimulatory Ag to efficiently prime naïve T cells (43). Differential uptake, routing, and processing of the Ag (44 –46) by myeloid CD11c+CD11b+ DCs that are potent inducers of IL-10-secreting T cells (47) and IgA production from naïve B cells (48) might as well contribute to modulate local responses. Because DCs constitutively traffic from the intestinal epithelium and PPs to the MLNs (28, 49), this represents a mechanism whereby soluble immune complexes can be taken to the MLNs to promote T cell responses (50).

Our experiments show that the function of SlgA is not only immune exclusion. Although active in facilitating sampling of luminal bacteria, SlgA restricts penetration at the level of PPs and MLNs, as this has been observed for nonpathogenic commensals (51, 52). In association with SlgA, steady-state Ag acquisition from the luminal content by underlying DCs may favor tolerance induction under homeostatic conditions or induce defense mechanisms under reduced inflammatory conditions in response to mucosal pathogens. From a clinical point of view, the absence of IgA is associated with the development of allergy, autoimmune diseases, inflammatory bowel diseases, and recurrent infections (53–55). In individuals with IgA deficiency, SlgM could compensate for SlgA in terms of immune exclusion, particularly in the gut (2). However, SlgM cannot deliver Ags to PPs because it cannot bind to M cells (8). Should the mother’s SlgA have the capacity to deliver novel Ags such as those associated with foods and the commensal microbiota without damageable consequences, it would be relevant to evaluate the incidence of allergies or autoimmune disorders in infants born from IgA-deficient parents.

The Fc portion of IgA Abs does not confer to the molecule proinflammatory properties or effector functions on target cells (56–58), as this is known for other isotype Abs including IgG, IgM, and IgE. Unique transport features associated with SlgA might permit one to extend this notion to the mucosal compartment and underscores the multitask role of SlgA in protecting against foreign substances and microbes, in regulating the commensal microbiota, while at the same time not subjecting the mucosa to undue inflammation.

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Disclosures

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References


