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Secretory IgA Induces Tolerogenic Dendritic Cells through SIGNR1 Dampening Autoimmunity in Mice

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IgA plays ambivalent roles in the immune system. The balance between inhibitory and activating responses relies on the multimerization status of IgA and interaction with their cognate receptors. In mucosal sites, secretory IgA (SIgA) protects the host through immune-exclusion mechanisms, but its function in the bloodstream remains unknown. Using bone marrow–derived dendritic cells, we found that both human and mouse SIgA induce tolerogenic dendritic cells (DCs) following binding to specific ICAM-3 grabbing nonintegrin receptor 1. This interaction was dependent on Ca2+ and mannose residues. SIgA-primed DCs (SIgA-DCs) are resistant to TLR-dependent maturation. Although SIgA-DCs fail to induce efficient proliferation and Th1 differentiation of naive responder T cells, they generate the expansion of regulatory T cells through IL-10 production. SIgA-DCs are highly potent in inhibiting autoimmune responses in mouse models of type 1 diabetes and multiple sclerosis. This discovery may offer new insights about mucosal-derived DC immunoregulation through SIgA opening new therapeutic approaches to autoimmune diseases. The Journal of Immunology, 2013, 191: 2335–2343.

Immunglobulin A is the most abundant Ab isotype produced in the body and has a crucial role in the immune responses at mucosal surfaces, such as the gastrointestinal tract, the respiratory tract, and the vaginal tract. It exists in multiple molecular forms, and two subclasses have been described (1). Although monomeric IgA predominates in the circulation, dimeric IgA (dIgA) is dominant in exocrine secretions (2). IgA mediates its effector function through multiple mechanisms, including interactions with mucosal epithelial cells, binding to receptors, and high- and low-affinity Ag binding. IgA plays a major role in mucosal immunity, in which dIgA provides immune protection as a result of its ability to interact with the polymeric IgR (pIgR), a transporter expressed on the basolateral surface of epithelial cells (3). dIgA-pIgR are translocated to the apical surface of epithelial cells, thereby generating secretory IgA (SIgA) complexes that play multiple protective roles (4). SIgA promotes immune exclusion by entrapping dietary Ags and micro-organisms in the mucus, and it recognizes bacterial epitopes on commensal bacteria, thereby contributing to the maintenance of appropriate bacterial communities within specific intestinal segments (5–7). dIgA produced locally at submucosal sites by plasma cells can remove micro-organisms that have crossed the epithelial barrier by transporting them back into the lumen through the pIgR and by promoting their clearance via the myeloid IgA Fc receptor (CD89) expressed by dendritic cells (DCs), neutrophils, and other phagocytes, such as Kupffer cells (8–10), in humans.

In the human bloodstream, IgA can either inhibit or activate the immune system, depending on the type of interaction with CD89 (11, 12). Monomeric IgA inhibits the activation of heterologous receptors (such as other FcRs, cytokine receptors, chemotactic receptors, and TLRs), whereas IgA complexes induce several activating functions (11). These ambivalent functions of IgA in human cells are regulated by the CD89-associated FcγR-chain. Partial ITAM phosphorylation of the FcγR-chain results in its inhibitory configuration and SHP-1 phosphatase recruitment into inhibisome structures (13), whereas its full phosphorylation induces Syk kinase recruitment and cell activation (14).

IgA deficiency is the most common primary immunodeficiency worldwide, and patients with this selective Ig deficiency show an increased incidence of allergies and autoimmune disorders (8, 15). These clinical observations strongly suggest that IgA may exert major immunoregulatory functions. Despite the well-characterized inhibitory functions of the IgA monomer, it was suggested that SIgA prevents activation of the immune system. In mice, orally delivered SIgA induces mucosal and systemic responses associated with IL-10 and TGF-β expression in draining mesenteric
lymph nodes and the spleen (16). Although SIGAs are found in the human bloodstream of healthy individuals (17–19) and are increased in serum from patients with IgA-associated diseases (19–23), their systemic role in immunity remains unknown.

In this article, we show that SIGA prevents activation of the immune system through the modulation of mouse bone marrow (BM)-derived DC (BMDC) function. SIGA-primed BMDCs (SIGA-DCs) are unable to mature and secrete proinflammatory cytokines after activation by various TLR agonists. Specific ICAM-3 grabbing nonintegrin receptor 1 (SIGNR1; the murine homolog of DC-SIGN) was identified as the SIGA receptor involved in the inhibition of DC maturation using sign1r−/− BMDCs. SIGA-DCs present a tolerogenic phenotype and produce large amounts of IL-10, inducing the expansion of IL-10–secreting regulatory T cells (Tregs). In vivo treatment with SIGA-DCs prevents the development of autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) and type 1 diabetes (TID). Therefore, these data suggest that SIGA has a hitherto unknown regulatory function in the bloodstream, opening new therapeutic avenues for the treatment of autoimmune and inflammatory diseases.

Materials and Methods

Mice and treatments

C57BL/6 mice (6–12 wk old) and lactating C57BL/6 mice (3–6 mo old) were purchased from Charles River Laboratory. OTII C57BL/6 mice and NOD mice were obtained from the U1016 facility, Cochin Institute. Foxp3-GFP+ mice (24) were backcrossed >10 times on the NOD background. BDC2.5 CD4.5+ Foxp3-GFP+ Ca2+/− NOD mice were generated by backcrossing to BDC2.5 CD4.5+ Foxp3-GFP+ Ca2+/+ NOD mice (25). In some experiments, NOD Ca2+/− recipient mice were injected i.p. with 0.25 mg/mouse anti-CD25 (PC1), anti–TGFB-β (2G7), or anti–IL-10R (1B1) mAbs or with the respective control isotype at the time of cell transfer. All animals were maintained in specific pathogen–free conditions in the U1016 facility, Cochin Institute. All experiments were approved by the local authorities (P2.AL.045.08).

dlGAs, SIGAs, and secretory component preparations

Human dlGAs and secretory component (SC) were purified as described (26). Human SIGAs were purchased from Euromedex and B Cell Design. SIGAs from Euromedex were purified from colostrum (polyclonal SIGA), and SIGAs from B Cell Design were obtained from hybridoma culture supernatant (monoclonal SIGA, anti–β-lactoglobulin) associated with purified SC. SIGA from both origins were used indirectly and harbored the same regulatory activity. Mouse SIGA was isolated from C57BL/6 mouse milk, according to the method described by Parr et al. (27), with a few modifications. Milk samples were collected from lactating mice between days 5 and 8 postpartum. Pups were removed ≥12 h before the procedure. Females were anesthetized with an i.m. injection of ketamine-xylazine and were given 1 IU oxytocin (Novartis) immediately before milking. The milk was collected with a 2-ml syringe and centrifuged at 2000 × g for 10 min at 4˚C to remove the fat layer. After precipitation with a half-saturated ammonium sulfate solution, SIGA was separated using diethylaminoethyl-cellulose column chromatography (Pall) equilibrated in 50 mM NaCl, 10 mM phosphate buffer (pH 7.5). Elution of SIgA was carried out using a linear gradient of NaCl from 0 to 1 M. The total protein concentration of the sample was measured using a NanoDrop spectrophotometer. Purity was assessed by SDS-PAGE analysis. Before use, purified SIGA was dialyzed against PBS (pH 7.4) and passed through a 0.2-µm filter.

Preparation of BMDCs

BMDCs were prepared from progenitor cells isolated from the femurs and tibias of female mice between 6 and 8 wk of age, as previously described (28). Briefly, BM cells were plated on six-well low-cluster plates in RPMI 1640 medium containing 10% FCS and penicillin/streptomycin and supplemented with 10 ng/ml murine GM-CSF (R&D Systems) for 8 d. Half medium changes took place on day 4 of culture. SIGA (100 µg/ml) was added on day 4 in all experiments. In some experiments, LPS (100 ng/ml), CpG (ODN1826) (1 ng/ml), zymosan (40 particles/cell), or polynosinic-polycytidylic acid [poly (IC)] (50 µg/ml) (all from InvivoGen) were added 12 h before DC analysis on day 8.

Flow cytometry

Cell suspensions were prepared from cell culture or tissue and were stained at 4˚C in PBS containing 2% FBS and 1% EDTA after blocking FcγR with 2.4G2 mAb. Surface staining was performed with Abs from BD Pharmingen or eBioscience. For IFN-γ and IL-10 intracellular staining, single-cell suspensions were stimulated with PMA (100 ng/ml) and ionomycin (100 ng/ml) for 5 h at 37˚C in the presence of 1 µg/ml brefeldin A (all from Sigma). Tregs were detected using the anti-mouse/rat Foxp3 staining set (IFK-16s; eBioscience). Stained cells were analyzed on a Fortessa flow cytometer (BD Biosciences). For SIGA-binding experiments, unstained BMDCs were incubated with SIGA (100 µg/ml) for 30 min at 4˚C in HBSS and then stained with anti–IgA-FTTC and the other markers, as described above.

Phagocytosis assay

Phagocytosis assay was performed using the Escherichia coli BioParticles Phagocytosis Kit for flow cytometry, as recommended by the manufacturer (Invitrogen).

Determination of cytokine production

A total of 5 × 105 DCs was cultured in 96-well flat-bottom plates with 100 µl complete RPMI medium containing 10% FCS for 48 h in the absence or presence of LPS. Cytokine levels (IL-10 and IL-12p70) in the supernatant were assayed using an ELISA kit (eBioscience).

Isolation and transfer of BDC2.5 cells

BDC2.5 cells were obtained from BDC2.5 Ca2+/− NOD mice before they developed diabetes (5–7 wk of age). Splenocyte suspensions were prepared, and RBCs and B cells were removed by hypotonic lysis and by sheep anti-mouse IgG beads (Dynal), respectively. CD62L+ splenocytes were positively selected with biotinylated anti-CD62L mAb and Streptavidin MicroBeads (Miltenyi Biotec). BDC2.5 CD62L−CD4+ T cells were transferred at the dose of 1.5 × 107 cells to 6–7-wk-old NOD Ca2+/− recipient mice. All recipient mice were used for BDC2.5 T cell transfer at 6–7 wk of age. For diabetes diagnosis, mice were tested daily from day 5 until disease onset, using Glukotest and Haemo-Glukotest kits (Boehringer).

In vitro culture of DCs and T cells

For in vitro stimulation, CD62L+ BDC2.5 CD4+ T cells (5 × 104) or OTII CD4+ T cells were incubated with LPS-activated BMDCs (5 × 103) loaded with the specific peptide for each T cell clone in the presence of recombinant human IL-2 (50 U/ml) and 1 ng/ml rTGFB-β (when indicated). In some experiments, T cells were stained with CFSE before culture.

EAE induction

For EAE induction, mice were injected s.c. with 200 µg myelin oligodendrocyte glycoprotein (MOG)35–55 peptide in 100 µl PBS emulsified with 100 µl CFA and further enriched with 5 mg/ml Mycobacterium tuberculosis (H37Ra). In addition, 500 ng pertussis toxin was injected i.p., on days 0 and 2. Clinical symptoms were monitored daily after immunization. The clinical score was graded as follows: 0, no disease; 1, tail limpness; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; 5, quadriplegia; and 6, death.

In vivo DC treatment in autoimmune diabetes and EAE

For in vivo DC treatment, BMDCs (SIGA-DCs or DCs) were incubated with 100 µg/ml the relevant peptide (1040-51 peptide recognized by BDC2.5 T cells for diabetes or MOG35-55 peptide for EAE) in complete medium for 4 h at 37˚C before disease induction. After intensive washing, 1040-51 peptide–pulsed DCs (5 × 107) were injected i.v. into NOD mice with BDC2.5 T cells, and MOG35-55 peptide–pulsed DCs (5 × 107) were injected i.v. into C57BL/6 mice on day −7 before EAE induction (day 0).

Statistical analyses

Data are mean ± SEM of n determinations. When analyzing experiments performed at multiple time points, ANOVA for repeated measurement statistics was used to calculate the overall significance and average difference between the two groups, followed by a standard t test to calculate differences at the individual time points. Incidences between groups were compared with the log-rank test. A p value < 0.05 was considered significant. All data were analyzed using GraphPad Prism v5 software.
Results
S IgA induces tolerogenic DCs

Because myeloid cells express several molecules that can bind IgA (9), and DCs are key players in immune regulation, we investigated the role of SIgA in DC function. In vitro cultures of mouse BM precursors with GM-CSF induce the differentiation of BMDCs, which mimic conventional CD11c<sup>+</sup> CD11b<sup>+</sup> DCs both phenotypically and functionally (28, 29). BMDCs, treated or not with SIgA at day 4, were characterized at day 8 for their phenotype, morphology, and ability to undergo maturation. BMDCs were treated with human (h) SIgA (hSIgA) purified from colostrum (hSIgA-DCs).

hSIgA was added at physiological concentrations observed in blood (100 μg/ml), and we observed that hSIgA-DCs were phenotypically similar to conventional BMDCs with regard to their high expression of CD11c and CD11b markers, confirming that the addition of hSIgA to the culture did not affect DC differentiation (Fig. 1A). Morphologically, both DC preparations appeared relatively homogeneous with classical cytological DC features. Cytological characteristics of DCs included the presence of large nuclei with open chromatin and rare nucleoli, slightly basophilic cytoplasm, minor vacuolation, and extensive membrane protrusions consistent with the presence of dendritic processes (Fig. 1B). Phagocytosis by each type of BMDCs was evaluated using fluorescent E. coli.
particles. No significant difference was observed between DCs and SlgA-DCs (Fig. 1C). To further evaluate the ability of hSlgA-DCs to respond to an inflammatory stimulus, DCs and hSlgA-DCs were cultured in the presence of LPS, a well-known TLR4 agonist. As shown in Fig. 1D and 1E, we observed a significant reduction in the upregulation of costimulatory molecules (CD86, CD80, CD40) at the surface of hSlgA-DCs. However, hSlgA-DCs harbored similar expressions of PD-L1 and PD-L2 molecules compared with untreated DCs. Thus, although DCs were fully matured in the presence of LPS, hSlgA dampened such maturation. We next evaluated the cytokine profile of both DC types before and after LPS stimulation (Fig. 1F). Although IL-10 and IL-12 were not produced by DCs in the absence of stimulation, both cytokines were induced by the addition of LPS, as expected, because these cytokines are involved in an autocrine loop controlling DC responsiveness to TLR agonists (30). In contrast, hSlgA-DCs failed to produce IL-12 in the presence of LPS, whereas they produced large amounts of IL-10 even in the absence of LPS. Therefore, we investigated whether the lack of response of hSlgA-DCs was restricted to LPS by comparing hSlgA-DCs responses after triggering with other TLR agonists, including zymosan (TLR2 and TLR6 agonist) and CpG1826 (TLR9 agonist). Importantly, hSlgA-DCs were unable to upregulate the costimulatory molecule (CD86) in response to these TLR agonists (Fig. 1G), contrasting with the expected maturation of BMDCs. A similar impairment was observed for other costimulatory molecules (CD80, CD40; data not shown). We evaluated the ability of hSlgA-DCs to secrete IL-12p70 in response to TLR stimulation. In contrast to DCs, hSlgA-DCs were not able to secrete IL-12p70 after LPS, zymosan, or CpG1826 stimulation (Fig. 1H). Altogether these data suggest that TLR-dependent DC maturation and proinflammatory cytokine secretion are markedly inhibited by hSlgA treatment.

There is evolutionary conservation of the regulatory function of SlgA between mice and humans

The SlgA structure is highly conserved throughout evolution. This is illustrated by the ability of the J-chain to polymerize IgA originating from different mammals (31). We assessed whether mouse (m)SlgA was able to induce TLR-unresponsive DCs after ligand stimulation, as shown above with hSlgA. As for hSlgA, mSlgA was purified from colostrum and added to BMDC cultures. We first observed that both human and mouse SlgA are able to bind mouse BMDCs (Fig. 2A). Accordingly, SlgA from both human and mouse rendered BMDCs unresponsive to LPS stimulation, as shown by the weak expression of costimulatory molecules CD80 and CD86 (Fig. 2B, 2C) and by the blocking of IL-12p70 secretion in response to TLR agonists (Fig. 2D), contrasting with the expected maturation of BMDCs. A similar impairment was observed for other costimulatory molecules (CD80, CD40; data not shown). We evaluated the ability of hSlgA-DCs to secrete IL-12p70 in response to TLR stimulation. In contrast to DCs, hSlgA-DCs were not able to secrete IL-12p70 after LPS, zymosan, or CpG1826 stimulation (Fig. 1H). Altogether these data suggest that TLR-dependent DC maturation and proinflammatory cytokine secretion are markedly inhibited by hSlgA treatment.

**FIGURE 2.** The regulatory function of SlgA is conserved between mice and humans. (A) BMDCs were incubated in the presence of human (h) or murine (m) SlgA or dIgA or IgG control and then stained with the corresponding secondary Ab to assess Ig binding. Data are mean ± SEM of three independent experiments, with two independent mice/group for each experiment. *p < 0.05, SlgA versus IgG. (B and C) BMDCs were cultured in the presence of human (h) SlgA or murine (m) SlgA, and expression of CD80 and CD86 was evaluated by flow cytometry 12 h after LPS activation. Data are mean ± SEM of three independent experiments, with two independent mice/group for each experiment. (D) IL-12p70 and IL-10 production was assessed in cultures of DCs, hSlgA-DCs, and mSlgA-DCs 12 h after LPS activation (left panel) or without LPS (right panel). Data are mean ± SEM of three independent experiments, with two independent mice/group for each experiment. (E and F) DCs were cultured with 100 µg/ml or with increasing doses of human SlgA, dIgA, or SC and stimulated with LPS for 12 h. IL-10 production was measured by ELISA, and the percentage of inhibition of CD86 expression was calculated relative to the untreated group (no SlgA). Data are mean ± SEM of two independent experiments, with two independent mice/experiment. *p < 0.05, **p < 0.01.
(Fig. 2D, left panel) following stimulation. Furthermore, as previously shown with hSIgA, mSIgA induced the production of IL-10 by BMDCs in the absence of LPS-stimulation (Fig. 2D, right panel). These data suggest that SIgA can regulate the maturation of DCs and that this phenomenon is conserved in mammals.

Because SIgA is made of dIgA linked to SC, the extracellular domain of pIgR, we next addressed whether isolated molecular components of hSIgA could mediate DC modulation. Therefore, BMDCs were incubated with purified human SC, dIgA, or SIgA. Only SIgA induced IL-10 secretion and prevented CD86 upregulation by BMDCs after LPS stimulation (Fig. 2E, 2F). Furthermore, this inhibitory effect was dose dependent (Fig. 2F). Therefore, association of the SC with dIgA is required for DC interaction and to induce tolerogenic DCs.

**SIgA-mediated tolerogenic effect on DCs is dependent on SIGNR1**

Because mice do not express the CD89 homolog (8, 9), we searched for other potential SIgA receptor candidates expressed on myeloid cells. Because complement receptor 3 (Mac-1, CD11b/CD18) was shown to play a crucial role in the binding of SIgA, but not of serum IgA, to human myeloid cells (32), we first examined...

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**FIGURE 3.** SIgA modulates DC function through SIGNR1. (A) BMDCs from WT, CD11b−/−, and FcRγ−/− C57BL/6 mice were cultured in the presence or absence of human SIgA. At day 8 of culture, cells were activated by the addition of LPS (100 ng/ml) for 12 h. Surface expression of CD86 was analyzed by flow cytometry. Data are mean ± SEM of four independent experiments, with two independent mice/experiment. (B) SIGNR1 expression was evaluated by flow cytometry on BMDCs from WT or signr1−/− C57BL/6 mice. Data are representative of two independent experiments. (C and E) BMDCs from WT or signr1−/− mice were cultured in the presence of hSIgA (solid line) or human IgG (dashed line) and then stained with the corresponding secondary Ab to assess Ig binding. In some experiments (E), anti-SIGNR1 mAb (100 µg/ml), mannan (1 mg/ml), or EDTA (5 mM) was added with SIgA. Data are mean ± SEM from three independent experiments, with two independent mice/group for each experiment. *p < 0.05, SIgA versus IgG. (D) BMDCs derived from WT or signr1−/− mice were cultured in the presence or absence of human SIgA, and CD86 expression was assessed by flow cytometry 12 h after activation with LPS (100 ng/ml) or poly (I:C) (10 µg/ml). Dotted lines represent the basal expression obtained after staining with isotype-matched control Ab. Data are mean ± SEM from three independent experiments, with two independent mice/group for each experiment. *p < 0.05.
whether BMDCs from CD11b<sup>−/−</sup> mice were affected by hSlgA. No difference was observed between LPS-treated wild-type (WT) and CD11b<sup>−/−</sup> BMDCs (Fig. 3A). Similarly, deficiency in the FcRγ-chain did not prevent the hSlgA inhibitory function on BMDCs, thereby excluding the potential role of an as-yet unidentified FcRγ-associated SlgA receptor (Fig. 3A). Therefore, we investigated whether the mouse homolog of DC-SIGN, a recently described receptor for hSlgA on the cell surface of DCs (33), was mediating the hSlgA inhibitory function. As expected, WT BMDCs expressed SIGNR1 (Fig. 3B). Importantly, hSlgA completely failed to bind (Fig. 3C) and to inhibit CD86 upregulation on signr1<sup>−/−</sup> DCs after LPS or poly (I:C) stimulation, whereas it efficiently impaired WT DC maturation (Fig. 3D). This observation is in agreement with the blockade of SlgA binding on BMDCs by anti-SIGNR1 mAb (Fig. 3E). Finally, we observed that SlgA binding to BMDCs was dependent on mannose and Ca<sup>2+</sup> because this binding was abrogated by the addition of mannan and EDTA, respectively (Fig. 3E). These data reveal that the hSlgA-inhibitory effect on DCs is mediated by SIGNR1.

**SIgA-DCs induce Treg expansion**

There is a general consensus that, although mature DCs induce immunity, immature DCs induce tolerance (34). The molecular mechanism implicated seems to be dependent on both costimulatory molecules and environmental cytokines, which modulate immune functions. First, we analyzed the ability of hSIgA-DCs to induce Ag-specific CD4<sup>+</sup> T cell proliferation using the OVA-specific OTII system (Fig. 4A). Although LPS-stimulated BMDCs induced the proliferation of OTII CD4<sup>+</sup> T cells in an Ag dose-dependent manner, hSIgA-DCs failed to induce such proliferation. Of note, hSIgA-DCs also induced a weaker proliferation of OTI CD8<sup>+</sup> T cells compared with untreated DCs (Supplemental Fig. 1).
Previous studies described that semimature tolerogenic DCs can induce Foxp3+CD25+CD4+ Treg expansion (34). Thus, we assessed whether hSIgA-DCs could induce Tregs using OTII CD4+ T cells (Fig. 4B). Incubation of T cells with hSIgA-DCs promoted a relative enrichment in Tregs compared with control DCs. This was observed in the absence, as well as after the addition, of exogenous TGF-β. The difference in Treg expansion between the two types of DCs was also observed, even when using high doses of exogenous TGF-β (Fig. 4C). Using CFSE-labeled OTII T cells, we further confirmed that, in the presence of TGF-β, hSIgA-DCs induced the proliferation of Tregs, limiting the expansion of conventional T cells (Fig. 4D). We further investigated whether Tregs could be induced by hSIgA-DCs in an autoimmune-prone mouse background using naive (CD62L+) diabetogenic CD4+ T cells isolated from BDC2.5 Cα2/2 NOD mice. These T cells were cultured in vitro with hSIgA-DCs loaded with the relevant mimotope peptide 1040-31, and Foxp3+ Treg expansion was evaluated (Fig. 4E). As observed with OTII cells, hSIgA-DCs promoted 2-fold expansion of Tregs compared with control DCs. This Treg expansion was dependent on IL-10 and TGF-β, as demonstrated by the addition of blocking mAbs to the culture.

However, TGF-β production by hSIgA-DCs was not detected (data not shown). Therefore, it is likely that Tregs are the source of this cytokine, which promotes their expansion, as previously reported (35). Together, these data demonstrate that hSIgA-DCs induce the expansion of Tregs in an Ag-specific manner.

**SIgA-DCs prevent the development of autoimmune diseases**

To assess the regulatory role of SIgA-DCs in vivo, a model of EAE induced in C57BL/6 mice was used. DCs or hSIgA-DCs were loaded with MOG35–55 peptide and injected 7 d before peptide immunization and EAE induction. Mice were scored for clinical symptoms of EAE (n = 15 mice/group). Incidence of diabetes after transfer into NOD Ca−/− mice of BDC2.5 T cells precultured with DCs or SIgA-DCs (G) or after cotransfer of BDC2.5 T cells and DCs or SIgA-DCs (D, E), with or without treatment with anti–IL-10R, anti–TGF-β, and anti-CD25 mAbs. Mice were injected with 5 × 10^6 T cells and/or 5 × 10^5 DCs or SIgA-DCs/mouse (n = 10 mice/group). (F) Treg frequency among transferred T cell populations was analyzed in the pancreatic lymph nodes, mesenteric lymph nodes, and the spleen 7 d after cotransfer of GFP−/− BDC2.5 T cells (from Foxp3-GFP−/− mice) and DCs or SIgA-DCs into NOD Ca−/− mice. (G) Intracellular staining for IFN-γ and IL-10 in the T cell population 7 d after cotransfer of BDC2.5 T cells and DCs or SIgA-DCs into NOD Ca−/− mice. Data are mean ± SEM of four independent experiments, with two pooled mice/group. *p < 0.05, DCs versus SIgA-DCs.
treated DCs had no influence on diabetes onset. Importantly, diabetes protection conferred by the transfer of hSIgA-DCs was abrogated by the injection of blocking anti–IL-10R, anti–TGF-β, or depleting anti-CD25 mAbs, revealing the requirement of these suppressive cytokines and Tregs in disease prevention (Supplemental Fig. 2). To investigate further whether a cell-conversion mechanism was involved in the induction of Tregs by hSIgA-DCs in NOD mice, naïve GFP+CD8+ T cells cotransferred with loaded hSIgA-DCs in NOD mice, and in vivo conversion of Tregs was analyzed in the pancreatic and mesenteric lymph nodes and spleen (Fig. 5F). Treg induction was significantly higher than that observed after cotransfer of T cells with untreated DCs. Finally, intracellular staining for IFN-γ and IL-10 of BDC2.5 T cells cotransferred with loaded hSIgA-DCs in NOD mice showed that >50% of T cells in the pancreatic lymph nodes were IFNγ−/− IL-10+ compared with only 3.5% when BDC2.5 T cells were cotransferred with untreated DCs (Fig. 5G). Altogether, our data confirm the tolerogenic potential of SIgA-DCs in vivo and their capacity to induce Tregs, preventing autoimmune diseases in both C57BL/6 and NOD mice.

Discussion

Our data show that SIgA strongly impacts the immune system through interaction with DCs. SIgA-DCs present a tolerogenic phenotype and produce large amounts of IL-10, inducing the expansion of IL-10–secreting Tregs. The functional relevance of these findings is supported by the fact that SIgA-DC treatment prevents the development of autoimmune diseases, such as EAE and T1D. Thus, circulating SIgA in bloodstream constitutes a natural anti-inflammatory-stimulatory systemic key factor through interaction with DCs.

SIgA has a powerful anti-inflammatory effect due to its ability to interact with DCs through SIGNR1 receptor. SIGNR1 is a mouse homolog of DC-SIGN, a C-type lectin receptor that was recently described as a receptor for human SIgA on the cell surface of DCs (33). Our data suggest that SIGNR1 expressed by DCs serves as a SIgA-specific receptor, because dIgA and SC are not able to modulate the DC tolerogenic profile. Thus, we suggest that the structural domains involved in SIgA–SIGNR1 interactions are generated upon dIgA trancytosis on epithelial cells when SC (a major fraction of the extracellular motif of pIgR) becomes covalently attached to IgA (31). Moreover, similar to DC-SIGN (33), SIgA–SIGNR1 interactions are dependent on sugars, notably mannose residues, and are Ca2+ dependent. Thus, this receptor does not behave like the human myeloid FcαRI CD89, which binds IgA in the CH2/CH3 boundaries independently of SC association (9). Interestingly, previous studies using DCs loaded with anti–DC-SIGN fusion proteins in the presence of other TLR or cytokine-derived signals induced IL-10 secretion by DCs, further confirming the DC tolerogenic activity dependent on DC-SIGN triggering (37, 38). Moreover, in human mucosal tissues, such as the rectum, uterus, and cervix, DC-SIGN is expressed abundantly by DCs present in the lamina propria and Peyer’s patches, further substantiating the importance of the localization of DC-SIGN+ DCs as a first-line of defense against viruses and pathogens. Our data support the notion that delivery of SIgA in the circulation alone, in the absence of Ags, may initiate anti-inflammatory responses through DC-SIGN+ DCs inducing tolerance, whereas delivery of SIgA-based immune complexes following retrotranscytosis by epithelial cells (39, 40) may induce activating immune responses.

By binding to ICAM-3, DC-SIGN receptor favors the generation of Ag-specific suppressive CD4+ T cells that produce IL-10 (41), a cytokine that intervenes in both intestinal homeostasis and the production of local IgA. Our data show that SIgA-DCs express a low level of costimulatory molecules and produce IL-10, rather than IL-12, in response to external stimuli (i.e., TLR agonists). Therefore, these cells are not able to trigger deleterious Ag-specific T cell responses. Instead, they generate efficient immunoregulation. Together, our data indicate that SIgA induces a semi-mature phenotype of DCs resembling the tolerogenic DCs described previously (34, 42). Induction of tolerogenic DCs by SIgA occurs at physiological concentrations of SIgA and unveils a hitherto unknown role for this molecule in the bloodstream. The prevention of two autoimmune diseases, induced either by polyclonal (EAE) (43) or monoclonal T cells (T1D) (44), strengthened the regulatory role of SIgA. Similarly, it was shown recently that the lack of SIgA in plgR-deficient mice enhances systemic hyperreactivity during intradermal OVA challenge (45). Interestingly, a rapid compensatory response was shown to operate in these animals to avoid detrimental effects, further suggesting that SIgA could constitute a first barrier controlling T cell responses.

In summary, to our knowledge, this study identified for the first time that SIgA plays ambivalent roles in the immune system. SIgA can simultaneously mediate protection at mucosal surfaces through Ab-mediated functions (26, 46) and exert a complementary inhibitory tolerogenic function at systemic levels through a SIGNR1+ DC-dependent pathway when delivered alone in the submucosa. This study provides evidence for the concept of establishing a method to produce potent tolerogenic DCs able to prevent inflammatory responses, opening new therapeutic avenues for inflammatory and autoimmune diseases.

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Disclosures

The authors have no financial conflicts of interest.
Figure S1: SlgA-DC are weak inducers of CD8⁺ T cell expansion.
LPS-stimulated DC or hSlgA-DC were co-cultured for 5 days with naive monoclonal OTI CD8⁺ T cells (1 DC/10 T cells) in the presence of increasing concentrations of OVA₂₅₇₋₂₆₄ peptide, and T cell proliferation was measured by H³-thymidine incorporation. Data are mean values +/- SEM from three independent experiments with two independent mice per group for each experiment. *: p < 0.05.
Figure S2: Effect of blocking mAbs against IL-10, TGFβ and Treg cells on BDC T cell-induced diabetes development.
Incidence of diabetes after transfer into NOD Cα−/− mice of BDC2.5 T cells with or without treatment with anti-IL-10R, -TGFβ and -CD25 mAbs. Mice were injected with 5 x 10^4 T cells *: p < 0.05, n=6 mice per group.