SIgA–*Shigella* Immune Complexes Interact with Dectin-1 and SIGNR3 to Differentially Regulate Mouse Peyer's Patch and Mesenteric Lymph Node Dendritic Cell's Responsiveness

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Abstract

In addition to contributing to immune exclusion at mucosal surfaces, secretory IgA (SIgA) made of polymeric IgA and secretory component is able to selectively reenter via microfold cells into Peyer's patches (PPs) present along the intestine and to associate with dendritic cells (DCs) of the $CD11c^+CD11b^+MHCII^+F4/80^-$ $CD8^-$ phenotype in the subepithelial dome region and the draining mesenteric lymph nodes (MLNs). However, the nature of the receptor(s) for SIgA on murine PP and MLN DCs is unknown. We find that glycosylated secretory component moiety and polymeric IgA are both involved in the specific interaction with these cells. Using blocking antibodies and competition experiments, we identify Dectin-1 and specific intercellular adhesion molecule-3 grabbing non-integrin receptor 3 (SIGNR3) as receptors for SIgA. While SIgA-commensal immune complexes (ICs) contribute to local homeostasis upon interaction with mucosal DCs, the picture is less clear for pathogenic agents. We find that in comparison with incubation of *Shigella flexneri* alone, association of the enteropathogen with SIgA prompts freshly isolated DCs from PPs and MLNs to invert the production of pro- *versus* non-inflammatory cytokines/chemokines. The sum of the data suggests that in contrast to IgG-based ICs boosting immune reactivity of antigen-presenting cells, SIgA produced during an ongoing immune response can, in addition to its known function of immune exclusion, modulate mucosal DC conditioning via specific interaction with Dectin-1 and SIGNR3.

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Introduction

IgA is the antibody that dominates the mucosal humoral immunity [1]. Following binding to epithelial polymeric Ig receptor and subsequent transcytosis, the antibody is released in the mucosal lumen in the form of a complex with secretory component, resulting in the formation of secretory IgA (SIgA) [2]. SIgA is endowed with the capacity to exert protective and regulatory functions at mucosal surfaces [3]. Interaction between SIgA and microorganisms in the intestine ensures protection against pathogens [4–7] or symbiosis with commensals [8,9], a dual function crucial to guarantee the integrity of the gut epithelium and associated local homeostasis [10].

An intriguing feature of luminal SIgA is its capacity to cross the intestinal barrier via sampling microfold (M) cells overlying Peyer's patches (PPs) [11] and to subsequently associate with underlying dendritic cells (DCs) in the subepithelial dome region, either in the form of free antibody or in complex with antigens [12–14]. Focusing on $CD11c^+CD11b^+$ $MHCII^+F4/80^-CD8^-$ DCs freshly isolated from mouse PPs and mesenteric lymph nodes (MLNs), that is, their natural environment, we have shown *ex vivo* that SIgA associated with a commensal bacterium participates in educating such DCs to trigger the onset of functional regulatory T cells [9]. In contrast, the functional consequence on PP and MLN DC's conditioning by SIgA combined to an enteropathogen is unknown.

Another missing piece of information is the nature of the receptor(s) involved in the recognition of SIgA by murine PP and MLN DCs. Scarce data exist as to the possible identity of such receptor(s) in the mouse. They include bone-marrow-derived DCs specific intercellular

adhesion molecule-3 grabbing non-integrin receptor 1 (SIGNR1; CD209b) [15], a close mouse homolog of human CD209/DC-SIGN, and Dectin-1 present on M cells overlying PPs [16]. Moreover, human THP-1 monocytic cell line recognizes SIgA through DC-SIGN [17], similar to Chinese hamster ovary cells ectopically expressing the receptor [16,17].

While much of the literature deals with the identification of microorganism-derived stimuli that lead to maturation of mucosal DCs, it is similarly important to decipher how such DCs return to steady state to ensure gut-prone homeostasis. We hypothesized that association of oral noxious antigens with SIgA present during an ongoing immune response may serve as a mechanism to regulate DC responsiveness via specific receptors. To verify this assumption, we examined the interaction of murine SIgA with DCs isolated from PPs and MLNs, that is, the tissues relevant to the study. Dectin-1 and specific intercellular adhesion molecule-3 grabbing non-integrin receptor 3 (SIGNR3) were identified as receptors for the antibody. Using *Shigella flexneri* (Sf) as an inflammatory inducer, the role of bound SIgA in modulating the conditioning of PP and MLN DCs was evaluated. Differential sensing of Sf and Sf-SIgA by DCs underscored SIgA-mediated regulation in favor of a non-inflammatory type of response, as reflected by decreased secretion of IL-12p70, keratinocyte-derived cytokine (KC), and IL-6 and by increased production of TGF-β. Blocking of either Dectin-1 and/or SIGNR3 revealed that context-dependent signals emanating from Sf-SIgA can differentially shape the ensuing production of cytokines/chemokines by mucosal DCs. This suggests that SIgA, upon targeting of local DCs, is involved in the control of pathogen sensing following recruitment by Dectin-1 and SIGNR3.

Results

Characterization of the interaction between SIgA and mucosal DCs

Although it has been demonstrated that both commensal and pathogenic bacteria in complex with SIgA, and free SIgA, are targeted to underlying DCs in the subepithelial dome region [12,13,18], the receptor(s) involved in this interaction has/have not been identified to date. In view of the recent demonstration that the $CD11c^{+}CD11b^{+}MHCII^{+}F4/80^{-}CD8^{-}$ DC subtype present in PPs and MLNs binds preferentially with SIgA [9,12], these cells were selected (Fig. 1a) to question *ex vivo* the identity of the putative SIgA receptor(s) on this particular mucosal subtype.

The interaction between PP and MLN DCs and fluorescently (Cy5) labeled SIgA (SIgA-Cy5) as a function of the abundance of the antibody and the time of incubation was examined first. We found both a quantity- and time-dependent interaction of SIgA with DCs isolated from either mucosal tissue, as reflected by increasing mean fluorescence intensity (Fig. 1b). Optimal binding occurred at 37 °C, was reduced by a factor of 2 at room temperature (RT), and abolished when the experiment was performed at 4 °C (J. Mikulic, unpublished data). To gain insight into the nature of the molecular partner of SIgA involved, we evaluated the specific interaction of polymeric IgA (pIgA) and free murine secretory component separately. In titration experiments, the two proteins associated with PP and MLN DCs with a similar pattern dependent on time and amount (Fig. 1c). Together, the use of purified murine secretory component and pIgA allowed us to unravel that both contribute to the recognition of SIgA by DCs prepared from these two mucosal tissues.

Competition assays using preincubation of DCs with a 25-fold molar excess of unlabeled SIgA, pIgA, free murine secretory component, or IgG were performed next. All components of SIgA led to a statistically significant reduction of SIgA-Cy5 binding capacity, as compared to control with no competitors (Fig. 1d); no effect of IgG was detected. This confirms that structural features within either pIgA or murine secretory component are required for interaction with DCs. As the highest reduction in the interaction was observed upon competition with SIgA itself, this indirectly suggests that the anchoring sites for pIgA and murine secretory component most likely do not fully overlap. The fact that murine secretory component competes slightly better than pIgA argues in favor of the hypothesis that the presence of secretory component in the secretory antibody is of primary importance in the association with PP and MLN DCs (Fig. 1d).

To gain further insight into the molecular features of murine secretory component intervening in DC binding, we speculated that the numerous glycosylation sites present on murine secretory component could well play a role in the process. Enzymatically deglycosylated murine secretory component (see Experimental Procedures) was reassociated with pIgA to yield degSIgA, and the interaction with mucosal DCs was compared to "unmodified" SIgA. Statistically significant lower MFI was observed when comparing the binding of degSIgA and SIgA (Fig. 1e). The results assign to the carbohydrate moieties carried by secretory component in the SIgA molecule, an essential role in the interaction of the antibody with PP and MLN DCs. Because complete deglycosylation of pIgA requires that the protein is irreversibly denatured, similar experiments on the role of glycans could not be carried out.

SIgA binds to mucosal DCs via Dectin-1 and SIGNR3 receptors

Receptors for SIgA on mouse and human hematopoietic cells have been identified [15–17], but not

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