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Recognition of secretory IgA by DC-SIGN: Implications for immune surveillance in the intestine

Jan Baumann[a], Chae Gyu Park[b], Nicholas J. Mantis[a,*]

[a] Division of Infectious Diseases, Wadsworth Center, 120 New Scotland Avenue, New York State Department of Health, Albany, NY 12208, USA
[b] Laboratory of Cellular Physiology and Immunology, and Chris Browne Center for Immunology and Immune Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

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ABSTRACT

Secretory IgA (SIgA), the predominant class of antibody in intestinal secretions, serves as the first line of defense against enteric infections. SIgA has also been proposed to function in immune surveillance, given that both SIgA and SIgA-antigen complexes are actively transported by Peyer's patch M cells from the intestinal lumen to sub-epithelial dendritic cells (DCs). The goal of the present study was to identify the receptor(s) potentially utilized by mucosal DCs to recognize and internalize SIgA. We demonstrate that human colostral SIgA is recognized by purified recombinant human DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN) in a solid phase binding assay, as well as by DC-SIGN ectopically expressed on the surface of Chinese hamster ovary (CHO-S) cells. The interaction between SIgA and DC-SIGN was specific, given that it was Ca^{2+}-dependent and inhibited by mannan. Moreover, SIgA bound to, and was internalized by, endogenous DC-SIGN expressed on THP-1 cells following monocyte to macrophage-like cell differentiation by stimulation with phorbol ester and interleukin-4. These data identify DC-SIGN as a putative receptor for SIgA, and reveal a mechanism by which DCs could collaborate with M cells in immune surveillance at mucosal surfaces.

1. Introduction

As the largest continuous mucosal surface in the human body, the intestinal epithelium is constantly being exposed to potentially toxic environmental antigens, pathogenic food- and water-borne microorganisms, and commensal microflora [1]. To cope with the antigen barrage, the intestinal mucosa is endowed a local network of organized lymphoid follicles, commonly referred to as the mucosal immune system [2]. These organized lymphoid follicles, such as the Peyer's patches in the small intestine, contain germinal centers whose activity (*i.e.*, B cell differentiation and somatic cell hypermutation) is driven in response to antigens present in the intestinal lumen [3,4]. In the intestinal mucosa, the vast majority of plasma cells secrete dimeric IgA, which is then vectorally transported across the intestinal epithelium into the gut lumen by the polymeric immunoglobulin receptor (pIgR). A fragment of pIgR, known as secretory component (SC), remains covalently associated with IgA after the antibody is released on the luminal face of the epithelium, to form secretory IgA (SIgA) [3,5]. Once in the intestinal lumen, SIgA serves as an immunological barrier capable of preventing toxins and enteric pathogens from attaching to and penetrating the intestinal epithelium [5–7]. SIgA is heavily *N*- and *O*-glycosylated, a property integral of the antibody's function in intestinal secretions [8–11].

While the primary function of SIgA appears to be promoting exclusion of antigens and pathogens, there is evidence that a fraction of secreted antibody is actually transported "retrograde," back into the mucosa [12]. Specifically, we recently documented the selective adhesion and transepithelial transport of SIgA by Peyer's patch M cells [13]. M cells are specialized epithelial cells found exclusively within the epithelium that overlies organized mucosa-associated lymphoid tissues [2]. The primary function of M cells is the uptake and transepithelial transport of the intestinal epithelium is antigens, including viruses, bacteria, and parasites, from the lumen to an underlying network of B cells, T cells, macrophages, and dendritic cells (DCs) [2]. However, we and other have shown that M cells also mediate the transport of SIgA (but not IgG or IgM) and SIgA-antigen complexes from the intestinal lumen to the sub-epithelial compartment [13–15]. Following M cell transepithelial transport, SIgA and SIgA immune complexes associate primarily with DCs [14]. It has been postulated that retrograde transport of SIgA by M cells constitutes a mechanism by which sub-epithelial DCs can survey the antigenic status of the intestinal lumen [2,12].

While both mouse- and human-derived DCs are capable of binding and internalizing SIgA, the specific IgA receptor(s) on DCs involved in immunoglobulin A recognition have not been identi-

* Corresponding author. Tel.: +1 518 473 7487; fax: +1 518 402 4773.
E-mail address: nmantis@wadsworth.org (N.J. Mantis).

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fied. Heystek and colleagues demonstrated that the interaction of SIgA with human monocyte-derived DCs (MoDCs) was not diminished by the addition of anti-CD89 antibody, thus indicating that the interaction is not mediated by the one known human FcαR [16]. In contrast, the binding was abrogated by the addition of mannose or fucose, suggesting that the *N*- and/or *O*-linked oligosaccharides on SIgA are being recognized by a member(s) of the C-type lectin family of receptors known to be expressed on DCs. This family includes the mannose receptor (MR) and DC-specific ICAM-3 grabbing non-integrin (DC-SIGN) [17,18]. Because antibodies against the MR only marginally reduced the interaction of SIgA with MoDCs [16], we sought to identify a role for DC-SIGN in this interaction. In this study, we report that DC-SIGN, in recombinant form or expressed on the surfaces of CHO-S cells, selectively and specifically binds to human SIgA. Moreover, we present evidence that SIgA is endocytosed following association with DC-SIGN on the cell surface. Based on these results we propose that DC-SIGN may serve as the receptor on mucosal DCs involved in the recognition and internalization of SIgA, and possibly SIgA-antigen complexes.

2. Materials and methods

2.1. Chemicals, reagents, buffers and antibodies

Purified human colostral IgA, bovine serum albumin (BSA), ovalbumin (OVA), mannan from *Saccharomyces cerevisiae*, and streptavidin conjugated to horseradish peroxidase (SA-HRP), were purchased from Sigma–Aldrich (St. Louis, MO). Purified human plasma Igs (IgA, IgM, and IgG) and myeloma proteins (IgA1 and IgA2) were obtained from EMD Biosciences (San Diego, CA). Human SC was obtained from Nordic Immunology (Tilburg, The Netherlands). The lectin RCA-II was obtained from Vector Labs (Burlingame, CA). Recombinant mouse macrophage mannose receptor (CD206), recombinant human DC-SIGN-Fc chimera [19], and anti-human DC-SIGN mouse monoclonal antibody conjugated to phycoerythrin (PE) were obtained from R&D Systems (Minneapolis, MN). EZ-Link sulfo-NHS-LC-biotin, biotin-LC-hydrazide and fluorescein isothiocyanate (FITC) were purchased from Thermo Fisher Scientific (Waltham, MA). Biotinylation was performed in phosphate buffered saline (PBS; pH 7.4), whereas FITC-conjugation was performed in bicarbonate buffer (0.1 M carbonate, 0.1 M bicarbonate pH 9.0). Tween-20 was obtained from BioRad (Torrance, CA), and paraformaldehyde (16%) was purchased from Electron Microscopy Sciences (Fort Washington, PA). Dialysis was performed with a Slide-a-Lyzer (10,000 molecular weight cut-off) purchased from Thermo Fisher Scientific. The following buffers were prepared by the media facility at the Wadsworth Center: Dulbecco's PBS (DPBS), calcium magnesium-free (CMF)-DPBS supplemented with EDTA (1–5 mM), Hanks' balanced salt solution (HBSS), CMF-HBSS, and enhanced HBBS (E-HBSS) supplemented with 10 mM $CaCl_2$.

2.2. Solid phase binding assays

Lyophilized recombinant human DC-SIGN and recombinant mouse MR were dissolved in PBS (pH 7.4) to a concentration of 8 nM and then used to coat wells (100 μL per well) of Nunc MaxiSorb™ 96 well plates (Thermo Fisher Scientific). The plates were incubated in a humidified chamber at 4 °C for 24 h, and then washed with PBS-T (0.05%) and blocked with Ig-free BSA (2%, w/v in PBS). Biotinylated Ig or control proteins were diluted into E-HBSS or CMF-HBSS to a final concentration of 2 nM and then applied to the plates and incubated for 30 min incubation period at 37 °C. The plates were developed with SA-HRP (1.0 μg/mL) and one-component tetramethylbenzidine (TMB) colorimetric substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Plates were read at 450 nm on a SpectraMax 250 microtiter plate reader (Molecular Devices, Sunnyvale, CA) with accompanying Softmax software.

2.3. Cell culture

CHO-S cells were obtained from Invitrogen (Carlsbad, CA). CHO-S cell lines stably transfected with DC-SIGN or another C-type lectin were obtained from the laboratory of Dr. Ralph M. Steinman (The Rockefeller University, New York, NY). CHO-S cells were maintained in DMEM with high glucose (4500 mg/L) supplemented with fetal bovine serum (10%, v/v) and non-essential amino acids (Invitrogen). When necessary, G418 (Sigma–Aldrich) was added to the medium to a final concentration of 0.5–1.5 mg/mL. All cell culture media were prepared by the Wadsworth Center media facility. Cells were routinely maintained in a humidified incubator at 37 °C with 5% CO_2.

The human monocytic leukemia cell line THP-1 was obtained from ATCC (Manassas, VA). The cells were grown in RPMI 1640 supplemented with 10% FBS. Differentiation was induced by treatment with phorbol 12-myristate 13-acetate (PMA; EMD Biosciences) and recombinant human interleukin 4 (IL-4; EMD Biosciences), as previously described [20]. Cells were seeded at $\sim 5 \times 10^5$ cells/mL in a T_{75} cm^2 tissue culture flask and then treated with PMA (10 ng/mL) for 24 h. The culture medium was then further supplemented with IL-4 (200 ng/mL) for an additional 72 h before the cells were collected for analysis by flow cytometry or microscopy. Control cells were treated in parallel with 0.1% BSA instead of PMA and IL-4.

2.4. Flow cytometry

CHO-S cells and C-type lectin-transfected derivatives were detached from the surfaces of cell culture flasks by treatment with CMF-DPBS containing 5 mM EDTA for ~30 min on ice, collected by centrifugation, washed with serum-free DMEM, and then adjusted to $\sim 1–5 \times 10^6$ cells/mL in E-HBSS or CMF-HBSS. The cells were then treated with FITC-labeled ligand (*e.g.*, SIgA, IgA1, IgA2, IgG, OVA) for 30 min at 4 °C or 37 °C. For immunolabeling, the cells were suspended in HBSS and then incubated with fluorophore-conjugated antibodies, at concentrations recommended by the manufacturer, for 60 min at 37 °C. The cells were washed three times to remove unbound ligands or antibodies, and fixed with 1% paraformaldehyde for 10 min at room temperature. Following fixation, the cells were suspended in PBS containing 1 mM NaN_3 and 2% goat serum (Invitrogen), and were then subjected to flow cytometry using a FACSCalibur (BD Biosciences, Franklin Lakes, NJ). A minimum of 10,000 cells were analyzed per sample.

2.5. Fluorescence microscopy

CHO-S cells and C-type lectin-transfected derivatives were detached from cell culture flasks by treatment with trypsin, collected by centrifugation, washed with serum-free DMEM, and then adjusted to $\sim 1–5 \times 10^6$ cells/mL. The cells were then seeded onto sterile, poly-L-lysine coated glass cover slips placed in 6 well cell culture plates (Costar) and incubated overnight at 37 °C with 5% CO_2. The following morning, the cells were treated with FITC-coupled ligands for 30 min at 4 °C. To assess ligand endocytosis, the cells were then transferred to 37 °C for a additional 30 min incubation. The cells were fixed with 4% PFA, and then mounted on microscope slides using VectaShield (Vector Labs). The cells were visualized using a Zeiss Axioskop 2 fluorescence microscope or a Leica TCS SP5 confocal laser scanning microscope.