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Antibodies Enhance Interaction of *Vibrio cholerae* with Intestinal M-Like Cells▽†

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Intestinal M cells bear a receptor for secretory immunoglobulin A (IgA) (sIgA) facing the lumen of the epithelial surfaces. Cells bearing this receptor are also found throughout an experimental monolayer consisting of polarized Caco-2 cells, a colon adenocarcinoma cell line. The presence of antibodies (mainly sIgA) in the lumen of the small intestine led us to explore the participation of the sIgA receptor and antibodies in the interaction of Caco-2-associated M-like cells with the mucosal pathogen *Vibrio cholerae*. Here, we demonstrate that sIgA antibodies isolated from pooled healthy human colostrums, as well as IgG from pooled healthy human serum, can recognize *V. cholerae*. Furthermore, opsonization enhances M-like-cell transcytosis of *V. cholerae* strains. We also show that the cholera toxin (CT) receptor ganglioside GM_1 colocalizes with the sIgA receptor in cells of the epithelial monolayer. Both sIgA and IgG antibodies compete for the attachment of soluble CT subunit B to immobilized GM_1. Our results indicate that in this in vitro model system of intestinal epithelia, human sIgA and IgG contribute to the uptake of *V. cholerae* by M-like cells, probably through an interaction with GM_1. Our results support previous findings of others showing that sIgA can act as an endogenous adjuvant and that sIgA is important for the antigen-sampling function of M cells.

M cells are considered to be a gateway to the mucosal immune system; they are interspersed mainly in the epithelial layer over the Peyer's patches of the small intestine, but recently, M cells were also detected through the villous epithelia of murine small intestines (7) and in the mucosal airway tissue (11). These cells are distinguished by their ability to internalize and transport lumenal elements including microorganisms, viruses, and particles. Morphologically, M cells lack the organized brush border and have a basolateral pocket where lymphocytes and dendritic cells reside (5, 14, 15, 21). Markers for human M cells are rare, but in tissue sections of the small intestine, M cells facing the lumen display a specific receptor for secretory immunoglobulin A (IgA) (sIgA) that is present in human, rabbit, and mouse M cells and is absent in adjacent enterocytic cells (8, 12). However, both the molecular nature and function of this receptor remain to be elucidated (12).

We demonstrated that interspersed within a polarized monolayer of Caco-2 enterocytes are cells that express the receptor for sIgA (1). We also demonstrated that another human M-cell-specific marker, the carbohydrate antigen known as sialyl-Lewis A, colocalizes with the sIgA receptor in the Caco-2 monolayer system (1). We thus concluded that Caco-2 monolayers harbor cells with features of M cells. Supporting this conclusion was our observation that the mucosal pathogen *Vibrio cholerae* could be taken up and transcytosed across the Caco-2 monolayer and was found in cells bearing M-cell markers (1).

In this study, we exploited this in vitro cell system to investigate the identity and function of the receptor for sIgA in context of the interaction between M-like cells and *Vibrio cholerae*.

An innate immune function for sIgA has recently been deduced from its structure (20). Glycans located in the constant region of the molecule may play a role in recognizing microbial polysaccharides. This finding is relevant to understanding mucosal immunity, because sIgA antibodies would thus have the potential for specific recognition of the triggering antigen as well as more nonspecific recognition of a variety of microorganisms. Indeed, innate recognition of microbes by glycans in sIgA could compensate for the restricted repertoire of sIgA (24). In fact, a significant number of the anaerobic bacteria isolated from human feces are coated with sIgA (26), sIgA-coated microorganisms have been observed within biofilms present in the small intestine of different species (17), and sIgA aids in biofilm formation in *Escherichia coli* (2). It has been assumed that the main role of lumenal sIgA opsonization of microorganisms is immune exclusion, which may block colonization and aid in elimination (10). Due to the presence of the receptor for sIgA on M cells, we sought to determine the effect of opsonization on the internalization of microorganisms through this mucosal immune gateway.

A number of lines of evidence suggest that M cells and sIgA collaborate to induce mucosal immune responses. First, M cells are closely associated with sIgA in tissue sections from the rabbit small intestine (8). Second, M cells in orally fed mice internalize IgA-opsonized colloidal gold particles, beads, and immune complexes more efficiently than nonopsonized elements or those coated with a nonrelevant protein such as bovine serum albumin (BSA) (18, 23, 27). Third, beads opsonized with sIgA that were orally administered to rats were found to be more significantly transported both through M cells and

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to the mesenteric lymphoid fluids than beads opsonized with a nonrelevant protein, i.e., bovine growth hormone (23). Fourth, IgA-coated liposomes containing ferritin induce a better mucosal immune response against ferritin in rectally immunized mice than do noncoated liposomes (29). Fifth, sIgA, a recombinant bacterial epitope expressed within the secretory component and orally administered to mice, was able to induce specific systemic and mucosal antibodies against the bacterial epitope (3). Finally, it was also demonstrated that after transport through M cells, sIgA colocalizes with CD4 lymphocytes and is internalized by subjacent dendritic cells in mouse Peyer's patch tissue (19). However, the contribution of the opsonization by sIgA to the internalization and trafficking of a pathogenic bacterium such as *V. cholerae* through M cells is unknown.

In this work, we wanted to test whether sIgA isolated from pooled healthy human colostrums was able to recognize *V. cholerae* and, if so, to determine whether it can play a role in the uptake and transcytosis of *V. cholerae*, perhaps through the receptor for sIgA present on M-like cells.

MATERIALS AND METHODS

Bacteria, cell line, culture conditions, and reagents. The bacterial strains used in this work were *Vibrio cholerae* LPB1, a transcytosis-proficient laboratory collection strain derived from O395, and the cholera toxin (CT) mutant *V. cholerae* O395NT with a deletion/insertion of a kanamycin cassette at the *ctxAB* locus, which was kindly provided by J. J. Mekalanos (13). LPB1 was spontaneously derived from O395; strains phenotypically similar to LPB1 (exhibiting an enhanced ability to transcytose through M-like cells) can be isolated as resistant colonies after mytomicin C treatment of O395 by a mechanism that is currently under extensive research in our laboratory (our unpublished data). Bacterial strains were kept at −80°C in Luria broth containing 20% glycerol and were cultured in Luria broth at 37°C. The eukaryotic cell line used in this work was Caco-2, obtained from the ATCC (HTB-37) and derived from a colon adenocarcinoma at passages 1 to 25 since it was received from the ATCC. Cells were cultured in 125-ml cell culture flasks replaced with fresh medium three times a week, and new passages were prepared once a week following trypsin treatment. The Caco-2 cells were seeded at a density of 1×10^5 cells/ml in 10 ml of fresh Dulbecco's modified essential medium (DMEM) (complete DMEM) containing 0.1 mM modified essential medium nonessential amino acids, 2 mM glutamine, high glucose, 25 mM HEPES buffer, and 10% fetal bovine serum from GIBCO at 37°C with 5% CO_2. Pure soluble CT subunit B (CTB), human sIgA isolated from colostrum, human serum from pooled sera of healthy males (AB^+ blood group), polyclonal rabbit antibody against CT, and the secondary conjugated antibodies anti-rabbit IgG, anti-rabbit IgG-tetramethyl rhodamine isocyanate (TRITC), anti-rabbit IgG-fluorescein isothiocyanate (FITC), and anti-rabbit IgG-alkaline phosphatase were obtained from Sigma Aldrich; polyclonal rabbit antibody against *V. cholerae* was obtained from LEE Laboratories; and the anti-GM_1 polyclonal rabbit antibody was obtained from Calbiochem. A Pro-Long kit and 4′,6′-diamino-2-phenylindole, dilactate (DAPI), anti-rabbit-IgG Alexa 350, and anti-human IgG Alexa 555 were obtained from Molecular Probes. GM_1 was obtained from Matreya LLC.

Generation of M-like cells: transcytosis assays. Polarized epithelial monolayers of Caco-2 cells containing M-like cells were obtained as previously described (1), except that complete DMEM was exchanged for DMEM without fetal serum 1 h before the infection experiments.

The receptor for sIgA was detected by incubating the Caco-2 monolayer with human sIgA isolated from colostrum (200 μg/ml) followed by anti-human IgA antibody conjugated with FITC (1:4). Opsonization of bacteria prior to infection was done by incubating bacteria with 1 mg/ml sIgA in DMEM (without fetal serum) for 5 min at 37°C; throughout the infection, sIgA remained at a final concentration of 0.2 mg/ml. For IgG, human serum was diluted 1:8 in DMEM to opsonize *V. cholerae* and then remained at a 1:50 concentration in DMEM without fetal serum during the transcytosis assay. The effect of CTB on the transcytosis of sIgA-opsonized *V. cholerae* was determined by incubating the Caco-2 cells for 10 min with 400 μg of CTB before and throughout the transcytosis assay.

GM_1-ELISA. To determine the ability of the antibodies to opsonize *V. cholerae*, the classical GM_1–enzyme-linked immunosorbent assay (ELISA) method to measure the amount of CT in solution was modified using whole, previously washed *V. cholerae* as described previously (1). sIgA isolated from colostrums (Sigma Aldrich) was used at a 1.6-mg/ml concentration, and the human serum was diluted 1:8 to opsonize *V. cholerae* in DMEM; bacteria were incubated for 10 min at 37°C and washed in DMEM before the GM_1-ELISA was performed as previously described (1). In the GM_1-ELISA competition assay between CTB and the antibodies, pure CTB was added at 500 ng/ml in the undiluted initial well in DMEM (50 μl), and serial twofold dilutions were the prepared in a total volume of 50 μl; the antibodies were added in a fixed concentration of 200 μg/ml sIgA and a 1:8 dilution of the serum containing IgG to a total volume of 100 μl in each well, and the incubation was done for 2 h at 37°C in the cell culture incubator with 5% CO_2. Following by a brief wash with phosphate-buffered saline (PBS)–0.05% Tween, the plate was incubated 2 h with PBS–Tween–2% BSA at 4°C. Each well was the incubated overnight at 4°C with 100 μl of rabbit anti-CT antibody (1:50) in PBS–Tween–1% BSA. Following three washes of 5 min each, one with PBS-Tween, the wells were incubated for 2 h at 4°C with 100 μl of secondary anti-rabbit IgG-alkaline phosphatase antibody, anti-human IgA-alkaline phosphatase antibody, or anti-human IgG-alkaline phosphatase antibody diluted 1:5,000 in PBS–Tween–1% BSA. Each well was again washed three times with PBS-Tween, and alkaline phosphatase activity was determined using the substrate *p*-nitro-phenyl-phosphate (0.4%) in 10 mM Tris buffer, pH 8.0. The optical density at 405 nm was determined using an EL309 ELISA microplate autoreader (BIO-TEK Instruments). Negative control wells had DMEM alone.

Laser confocal microscopy. To visualize the colocalization of the receptor for sIgA and GM_1 ganglioside, the Caco-2 monolayer was treated with sIgA (0.2 mg/ml) for 1 h followed by fixation with 1% formaldehyde in DPBS for 5 min. After a brief wash with Dulbecco's PBS (DPBS), anti-GM_1 antibody was added (1:25) and incubated for 1 h at 37°C. After three washes with DPBS (GIBCO), anti rabbit-TRITC (1:4) and anti-human IgA-FITC (1:4) were added and incubated overnight at 4°C. After three washes with DPBS, the membrane was incubated for 24 h in DPBS containing 500 nM DAPI. The membrane containing the epithelial cells was cut from the transwell with a scalpel and mounted between a microscope slide and a coverslip submerged in Pro-Long (Molecular Probes). Samples were observed using the PlanApo 63× objective under a Zeiss LSM 510 laser confocal microscope using the argon, HeNe, and Enterprise lasers and LS10 META software. A similar procedure was followed to detect sIgA-opsonized *V. cholerae* or IgG-opsonized *V. cholerae* attached to the epithelial monolayer of Caco-2 cells. To detect sIgA-opsonized bacteria trafficking through M-like cells, fixed Caco-2 cells were permeabilized for 20 min in DPBS containing 0.2% Triton X-100. After washing with DPBS, fixed and permeabilized cells were treated with antibodies and DAPI.

RESULTS AND DISCUSSION

Antibodies opsonize *V. cholerae* and interact with M-like cells. In order to determine whether sIgA isolated from pooled healthy human colostra or IgG from pooled healthy human sera could recognize *V. cholerae*, we used a whole-microbe binding ELISA as previously described (1). *V. cholerae* strain LPB1 opsonized with sIgA was preferentially recognized by anti-IgA antibody, and IgG-opsonized *V. cholerae* strain LPB1 was better recognized by anti-IgG antibody (Fig. 1A). An anti-*V. cholerae* antibody detected equivalent microbe binding to immobilized GM_1 under three different conditions used in this assay (Fig. 1A). Hence, both sIgA and IgG recognize *V. cholerae*, although the precise molecular basis for this interaction is not clear, because these antibodies are derived from healthy individuals not previously exposed to cholera. They may recognize bacteria through nonspecific polysaccharide moieties present in the constant portion of the antibody molecules as proposed previously (20). It is also possible, however, that natural antibodies produced against normal flora may cross-react with polysaccharide epitopes on the surface of *V. cholerae*, and in this case, the antibodies would be recognizing *V. cholerae* through the Fab portion.

To determine whether the sIgA receptor on M-like cells

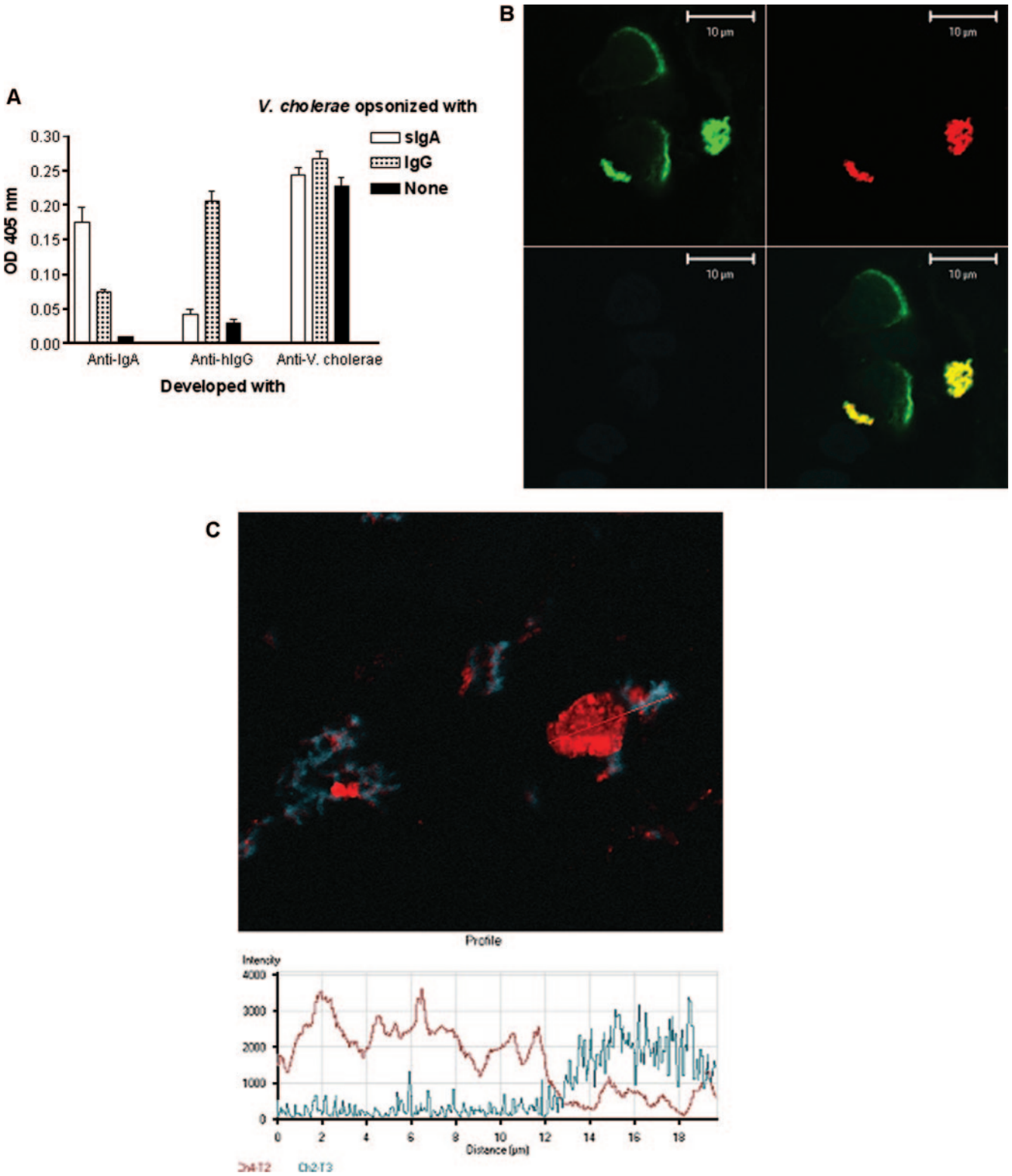

FIG. 1. Secretory IgA and IgG opsonize *V. cholerae* and interact with M-like cells. (A) GM_1-ELISA data with whole *V. cholerae* strain LPB1 opsonized by sIgA or IgG or without previous opsonization (None) and detected by the respective secondary antibody-alkaline phosphatase conjugate. The optical density (OD) at 405 nm measures the positive signal detected under each condition. (B) Laser confocal *x-y* views showing clumps of sIgA-opsonized *V. cholerae* (sIgA is the green fluorophore and the microbe is red fluorophore in the upper panels) and, in the merge at the bottom right panel, the yellow fluorescence of opsonized bacteria attached to the surface of M-like cells positive for the receptor for sIgA (green fluorescence). (C) *x-y* view of IgG-opsonized *V. cholerae* attached to the surface of the epithelial monolayer of Caco-2 cells. The image displays an IgG-positive cell (red fluorescence) and several clumps of *V. cholerae* (detected with a blue fluorophore); at the bottom, a graphic shows the profile of fluorophore intensities through the red line depicted.

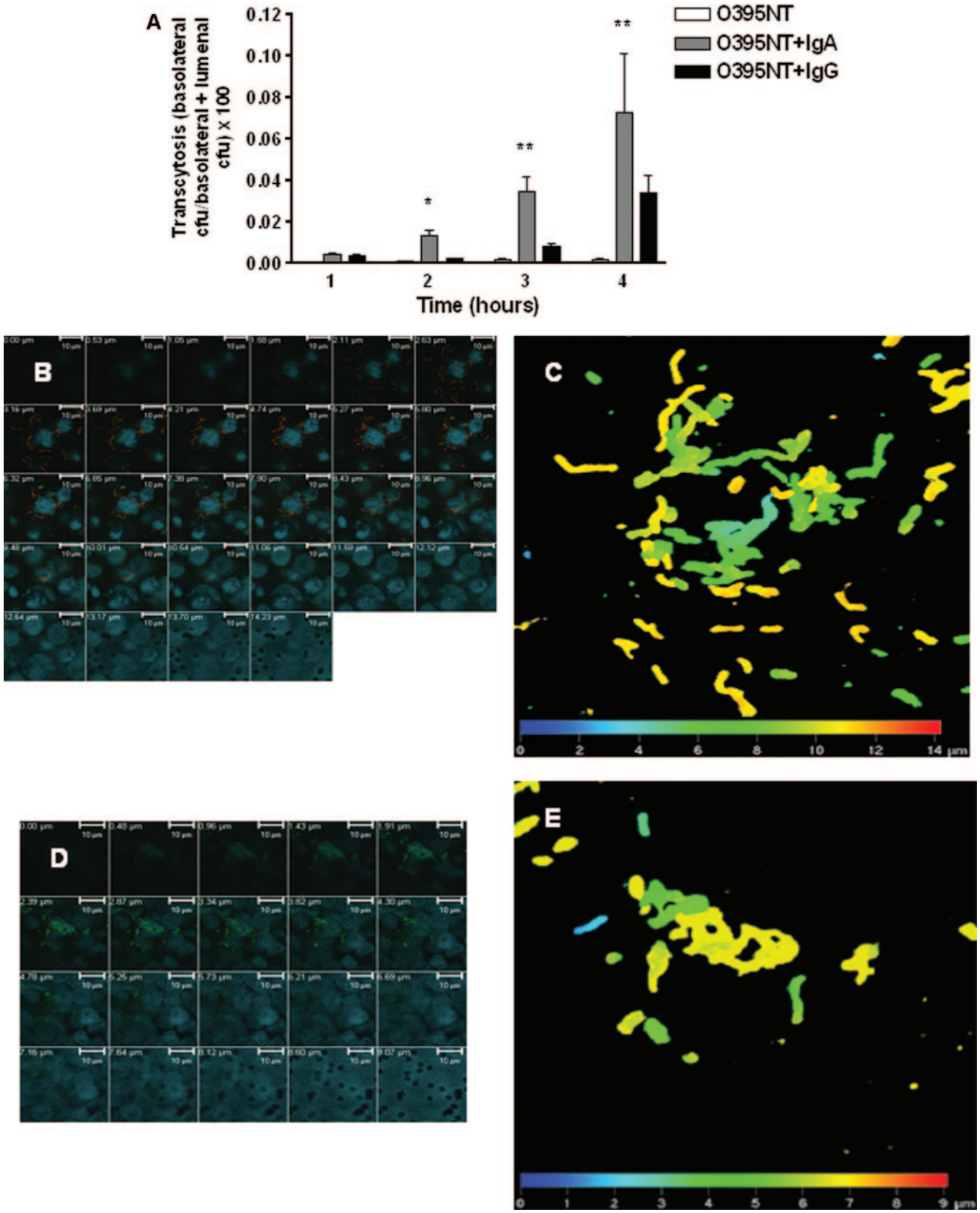

FIG. 2. Secretory IgA and IgG enhance the transcytosis of a CT-deficient mutant of *V. cholerae* O395NT through M-like cells. (A) Transcytosis of the O395NT mutant strain measured over time through the epithelial monolayer of Caco-2 cells that is enhanced by sIgA or IgG opsonization. Shown are the averages ± standard errors of three independent experiments. *, $P < 0.05$; **, $P < 0.01$ (analysis by Student's *t* test). (B and D) *z*

could play a role in the transcytosis of *V. cholerae*, we first studied the ability of *V. cholerae* strain LPB1 to interact with sIgA receptor-positive cells on the surface of Caco-2 cells. As shown by laser confocal microscopy in the *x-y* view in Fig. 1B, sIgA-opsonized *V. cholerae* (red) and sIgA (green) associate with the surface of cells expressing the sIgA receptor (2 out 10 cells are positive for the sIgA receptor) (Fig. 1B, upper left panel). In the merged view at the bottom right of Fig. 1B, it is evident that *V. cholerae* is opsonized by sIgA (yellow fluorescence). We also detected sIgA-opsonized *V. cholerae* trafficking through the cell by laser confocal microscopy of the epithelial monolayer, which was fixed and permeabilized 1 h after the addition of *V. cholerae* (see the movie in the supplemental material). IgG also recognizes some cells in the Caco-2 monolayer (1 out of 30 cells is recognized by IgG) (Fig. 1C, red) and the *V. cholerae* microbe (Fig. 1C, blue). To confirm that IgG is opsonizing the bacteria, a profile analysis through the red line is shown in Fig. 1C, where the blue channel (bacteria) shows red fluorescence (IgG) concomitantly.

The human neonatal Fc receptor expressed in enterocytes is able to transport immune complexes with IgG from the lumen to the basolateral side of the small intestine (28). Whether this neonatal Fc receptor could also recognize sIgA cannot be predicted, because M cells were not analyzed directly, and sIgA was not tested (28). Two previously published observations suggest that IgG might associate with M cells in vivo. First, IgG-opsonized colloidal gold particles bind to murine M cells with an efficiency similar to that of IgA-opsonized colloidal gold particles and better than that of BSA-opsonized colloidal gold particles, and exogenously added IgG competes with the binding of IgA-opsonized particles (27). Thus, it is not unexpected that IgG might bind to cells in this monolayer, which includes M-like cells (1).

Antibodies enhance transcytosis of a cholera toxin-deficient mutant of *V. cholerae* O395NT through M-like cells. Our results suggest that the sIgA receptor on M-like cells can interact with *V. cholerae*. Previously, we demonstrated that the transcytosis of *V. cholerae* through M-like cells correlates with the ability to bind to immobilized GM_1, and we hypothesized that this was due to bacterial cell-associated CT expression (1). CT mutant strain O395NT is taken up and transcytosed poorly in this model system (Fig. 2A) (1). We hypothesized that the interaction between antibodies and *V. cholerae* might allow for higher levels of transcytosis through the interaction with M-cell receptors that can recognize antibodies, as suggested above to be the case.

To test this hypothesis, we opsonized the transcytosis-defective strain O395NT with either human colostrum-derived sIgA or human serum containing IgG and quantified the transcytosis of bacteria through the epithelial monolayer. Prior opsonization with either class of antibody enhanced transcytosis of the toxin mutant O395NT (Fig. 2A). Transcytosis through M-like cells by O395NT at 4 h was 45- and 21-fold higher for the sIgA-opsonized or the IgG-opsonized O395NT mutant, respectively, than for the nonopsonized O395NT mutant (Fig. 2A). These experiments consistently showed that antibodies, most notably sIgA, enhance the transcytosis of strain O395NT (Table 1). The predominant antibody isotype in the lumen of the small intestine is IgA, and due to its association with the secretory component, the sIgA isotype is more resistant to proteolytic degradation than IgG (25). Therefore, in healthy individuals, sIgA will predominate over IgG in the gut lumen. Our results could explain why some of sIgA-immunodeficient patients remain healthy; IgG (and perhaps IgM) may replace sIgA in this immune adjuvant function. Supporting this suggestion, increased numbers of intestinal IgG- and/or IgM-producing plasma cells arise in healthy sIgA-deficient patients (4, 16).

TABLE 1. Antibodies enhance transit of the transcytosis-deficient strain O395NT through M-like cells[a]

Time (h)	No. of CFU detected in the basolateral compartment (avg ± SE)		
	O395NT	IgG-O395NT	sIgA-O395NT
1	50 ± 50	267 ± 148	543 ± 346
2	100 ± 52	617 ± 239[b]	3,733 ± 1,787[b]
3	120 ± 73	1,950 ± 988[b]	4,833 ± 2,303[b]
4	420 ± 121	2,800 ± 986[b]	29,680 ± 12,370[b,c]

[a] The averages from two different experiments ± standard errors for at least five wells under each condition are shown.
[b] $P < 0.05$ compared to O395NT using Student's *t* test analysis.
[c] $P < 0.05$ compared to IgG-O395NT using Student's *t* test analysis.

We corroborated the quantitative effect of sIgA on the transcytosis of the O395NT mutant through M-like cells using laser confocal microscopy and depth analysis based on *z* sections from the confocal images. Analysis of opsonized versus nonopsonized O395NT mutant trafficking through a fixed and permeabilized epithelial monolayer of Caco-2 cells is presented in Fig. 2B to E; signals go from yellow to green to blue as their depth from within the monolayer increases. sIgA-opsonized O395NT reached deeper planes throughout the membrane (as signified by more blue- and green-coded bacteria) (Fig. 2C) than nonopsonized O395NT (more yellow-coded bacteria) (Fig. 2E). In addition, the overall number of opsonized bacteria found within the monolayer is greater than that of the nonopsonized bacteria (compare the events in Fig. 2C and E). As a broad variety of microorganisms, viruses, and proteins can be recognized by human sIgA from colostrum (4), transcytosis through M cells by a variety of microorganisms and antigens would be enhanced by an sIgA-dependent mechanism, and this improved antigen sampling may be of physiological significance in triggering immune responses. Indeed, transcytosis-proficient *V. cholerae* strain LPB1 is also taken up better after sIgA opsonization (Fig. 3E).

sections of laser scanning microscopy through the infected Caco-2 monolayer using sIgA-opsonized O395NT or nonopsonized O395NT, respectively. (C and E) Depth analysis of the respective *z* sections showing the depth reached through the monolayer by sIgA-opsonized O395NT and nonopsonized O395NT, respectively. Blue indicates a deeper level closer to the filter (basolateral side) of the epithelial monolayer, and yellow shows bacteria closer to the surface or shallower within the epithelial monolayer.

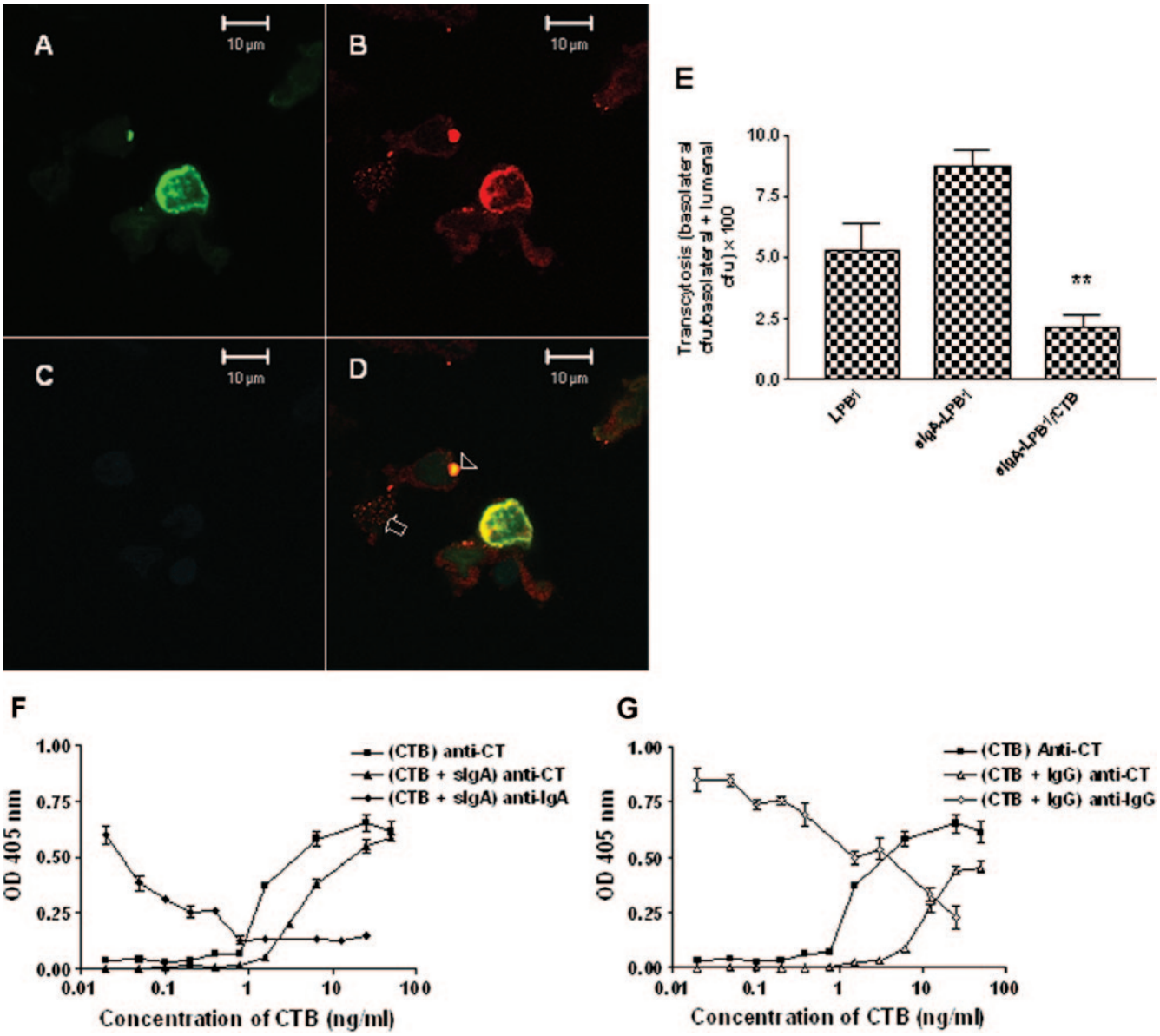

FIG. 3. GM_1 ganglioside participation in the antibody-assisted transcytosis of *V. cholerae*. Shown in A through D is an *x-y* view of the surface of a fixed epithelial monolayer of Caco-2 cells containing one strong positive cell for both (A) the receptor for sIgA (green channel) and (B) GM_1 (red channel). (C) Some nuclei are shown (DAPI staining is blue). (D) Merged image of panels A to C. In panel D, the arrowhead shows the focal stain of a cell positive for both sIgA and GM_1, and the arrow shows a GM_1-positive stained cell with a dotted pattern. (E) Transcytosis of *V. cholerae* strain LPB1 through the epithelial monolayer of Caco-2 cells after 2 h. sIgA-LPB1 indicates the transcytosis reached by sIgA-opsonized LPB1, and sIgA-LPB1/CTB is the transcytosis of sIgA-opsonized LPB1 in the presence of exogenously added CTB (400 μg). Shown are the averages ± standard errors of two different experiments. **, $P < 0.01$ (analysis by Student's *t* test, different from the sIgA-LPB1 condition). (F and G) Competition assay for CTB attachment to GM_1-coated ELISA wells by sIgA and IgG, respectively. In the presence of the antibodies, the CTB binding curve has a shift to the right (triangles) compared with the curve of CTB alone (squares). Also shown is the direct attachment of sIgA or IgG antibody in the presence of CTB to immobilized GM_1 in the curve with the diamonds in F and G, respectively.

GM_1 ganglioside participation in antibody-assisted transcytosis of *V. cholerae*. We recently provided evidence that GM_1 ganglioside participates in the transcytosis of *V. cholerae* through M-like cells (1). To test whether GM_1 may play a role in sIgA-assisted bacterial transcytosis as well, we analyzed the localization of the sIgA receptor in relation to GM_1 on the surface of the Caco-2 monolayer. Using laser confocal microscopy, we detected the colocalization of the GM_1 molecule with the sIgA receptor on the surface of some cells within the Caco-2 epithelial monolayer (Fig. 3A to D). Figure 3 shows a cell that is positive for the sIgA receptor (Fig. 3A, green) and GM_1 (Fig. 3B, red), with colocalization demonstrated in the merged image for both fluorophores in Fig. 3D. Another cell is positive for GM_1 in a dotted pattern (arrow in Fig. 3D), while another cell expressed the receptor for sIgA in concert with the GM_1 molecule (Fig. 3D, arrowhead). The colocalization of the

sIgA receptor and GM_1 on the surface of certain cells (approximately 7% in the field shown in Fig. 3D) suggests that the receptor for sIgA and GM_1 may share the lipid raft microdomains where GM_1 is anchored. CT is able to cross-link GM_1 with two proteins from the detergent-resistant fractions of membranes isolated from different cell lines, including Caco-2 (22). It was proposed that these proteins have roles in endocytosis and retrograde traffic of CT (22). Taken together with our results, we speculate that one of these two GM_1-associated proteins may be the receptor for sIgA. Previous attempts to determine the nature of the sIgA receptor in M cells failed, perhaps because the sIgA used was not associated with an antigen (12).

Whether the recognition of GM_1 over the epithelial monolayer is playing a direct role in the transcytosis of the opsonized microbe was investigated by performing the transcytosis assay in the presence of exogenously added CTB, the GM_1 binding moiety of cholera toxin. If GM_1 is playing a role in the transcytosis of opsonized *V. cholerae*, we hypothesized that adding CTB would diminish transcytosis. As predicted, the transcytosis of sIgA-opsonized *V. cholerae* strain LPB1 was reduced after 2 h by the addition of exogenous CTB compared to the transcytosis of the opsonized strain LPB1 in the absence of CTB (Fig. 3E). This result suggests that ganglioside GM_1 may be involved in the interaction of sIgA-opsonized *V. cholerae* with M-like cells. In a previous study, we determined that the transcytosis of nonopsonized *V. cholerae* was not inhibited by exogenously added CTB (1). Our present result suggests that the interaction of sIgA-opsonized *V. cholerae* with membrane-associated GM_1 is more sensitive to exogenous CTB than is the interaction of nonopsonized *V. cholerae* with M-like cells and GM_1.

One hypothesis that explains our findings is that GM_1 may be working as coreceptor for antibodies. If this were the case, we would expect that sIgA or IgG might compete with CTB for GM_1 binding. To test this, we performed a GM_1-based ELISA in which CTB was serially diluted, and a fixed amount of either sIgA (Fig. 3F) or IgG (Fig. 3G) was added to each well concomitantly. In the presence of antibodies (triangles in Fig. 3F and G), a higher CTB concentration was required to achieve the same absorbance in the assay compared with that observed in the absence of antibodies (closed squares in Fig. 3F and G, respectively). The antibodies are not washing the CTB out because either sIgA or IgG was directly bound to the wells throughout the assay, as was detected by antibodies against sIgA or IgG (diamonds in Fig. 3F and G, respectively). In conclusion, both sIgA and IgG compete with CTB for the association with GM_1 ganglioside. These titration curves fit a nonlinear regression curve (one-site binding hyperbola) analysis with the following dissociation constant values: only CTB, 2.136 ± 0.44 ng/ml; with sIgA, 7.548 ± 1.167 ng/ml; and with IgG, 26.390 ± 6.639 ng/ml. These values indicate that IgG has more affinity for immobilized GM_1 than sIgA.

An endogenous adjuvant property of sIgA has been hypothesized previously (6), and based on our results, we propose that the well-recognized mucosal adjuvant CTB is in fact mimicking an endogenous pathway controlled by the interaction between sIgA and GM_1. The ability of antibodies to recognize GM_1 could be relevant for the function of other immune cells, such as dendritic cells, where the interaction of GM_1 with CTB induces the translocation of NFκB from the cytoplasm to the nucleus (9). The physiologic relevance of our findings for systemic immunity requires further experimentation.

We conclude that in this in vitro model system of an intestinal epithelium, human sIgA contributes to the uptake of *V. cholerae* by M-like cells, and we hypothesize that human sIgA is working as an endogenous adjuvant, an important and novel immune function for these secretion-abundant antibodies.

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