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Original Research Article

An IgA mimicry of IgG that binds polymeric immunoglobulin receptor for mucosa transcytosis

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ABSTRACT

Most pathogens establish infection through mucosa, where secretory immunoglobulin A (sIgA) plays an 'immune exclusion' role in humoral defense. Extravasation of intravenously (i.v.) administrated therapeutic immunoglobulin G (IgG) mainly relies on convection and/or neonatal Fc receptor-mediated transcytosis from circulation into interstitial space. Active transport of interstitial IgG further across epithelium into mucosa, like sIgA, is a much desired feature for the next generation of therapeutic antibodies, especially for anti-infection purposes. For the first time, we report the engineering of an IgA mimicry of IgG, with its Fc portion in fusion with the 18-aa tail piece (tp) of sIgA and the J chain, possessing sIgA's full binding activity towards polymeric immunoglobulin receptor that mediates mucosa transcytosis. In a diphtheria toxin receptor (DTR) knockin mouse model, i.v. injected anti-diphtheria toxin (DT) IgG(tp)J protected DTR+ cells from deletion upon DT injection. The compact design of IgG(tp)J opens new revenues for more effective therapeutic IgG mimicking some of the important biological functions of IgA.

Statement of Significance: Unlike secretory immunoglobulin A (sIgA), translocation of intravenously (i.v.) administrated therapeutic immunoglobulin G (IgG) onto mucosal surface is limited and, if any, is mediated by neonatal Fc receptor (FcRn). Among all the IgG-IgA hybrids, the IgG(tp)J form contains sufficient structural requirements for full binding to polymeric immunoglobulin receptor (pIgR), mimicking sIgA function in mucosa transcytosis against infectious agents and their products.

KEYWORDS: IgG; IgA; pIgR; mucosa; transcytosis

INTRODUCTION

Currently, all clinically approved therapeutic monoclonal antibodies (mAb) in the H2L2 intact form are of IgG type. Due to its large size, biodistribution studies in animals lacking the target antigen have shown that over 90% of the i.v. administrated IgG is in the plasma. The partition of extravascular IgG in normal tissues with continuous capillaries (with tight junctions) is around 5%, whereas that in tissues with fenestrated capillaries and sinusoids (liver, spleen and bone marrow) could be in the range of 30–40% [1]. In cases where the mAb binds with high affinity to extravascular sites with high-binding capacity, tissue:blood concentration ratios may be much higher [2].

One of the driving forces for extravasation of i.v. administrated therapeutic IgG is FcRn-mediated transcytosis, especially in tissues where extravasation via convection is limited. Several studies have shown a bidirectional transport of IgG in both basolateral to apical and apical to basolateral directions [3–5]. This suggests that FcRn-mediated transcytosis may play a role in the distribution of IgG from the vascular space out into tissue compartments, in addition to the well-documented transfer of maternal IgG from placenta and neonatal intestines into the circulation of the newborn. However, although IgG is detected in secretions of the human small and large intestines, lung [6] and vaginal tract [7], the antigen specificities and subclass patterns of IgG in secretions at mucosal surfaces differ from

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those found in serum [8]. Moreover, while FcRn-mediated transcytosis is responsible for both salvaging pathways of IgG and human serum albumin (HSA), the IgG/HSA ratio is much higher in secretions than in serum [8]. These observations suggest that a high percentage of IgG in secretions is of local origin and that serum IgG is poorly translocated into the mucosa.

Human diphtheria is an infection caused by *Corynebacterium diphtheriae* that affects the mucosa of the upper respiratory system and is still a public health concern in countries with insufficient vaccine coverage. Most complications of diphtheria are due to the release of diphtheria toxin (DT) that causes difficulty in breathing, heart failure, neuropathy, muscle paralysis and even death. During the development of a therapeutic anti-DT human monoclonal IgG1 for i.v. administration, we wanted to design an IgA-like IgG that not only neutralizes the toxin in the circulation, but also transcytose into the mucosa of the laryngopharynx area to prevent the intoxication of the epithelial cells in the first place. Systemic toxin neutralization as well as preservation of the epithelium integrity will be of higher therapeutic index than simple removal of the toxin from the circulation without protecting the affected mucosa. Here we report the engineering of a novel form of IgG possessing components and functional features of sIgA that can bind to pIgR for more effective epithelium transcytosis.

MATERIALS AND METHODS

DTR knockin mice

The diphtheria toxin receptor (DTR) knockin mouse used in this study has been reported previously [9]. Briefly, IRES-ZsGreen-Neo (flanked by LoxP) and IRES-DTR-2A-TdTomato expression cassettes were knocked in tandem into the mouse PD-L2 locus to make the PD-L2–ZsGreen–TdTomato–DTR (PZTD) mouse (Supplementary Fig. S1). At steady state, all the PD-L2-expressing cells are ZsGreen+. After crossing with a tissue-specific Cre transgenic mouse, e.g., CD19-Cre (Jackson Laboratories), all the PD-L2+ cells with tissue-specific Cre expression switch off ZsGreen but turn on TdTomato and DTR expression. Moreover, the red PD-L2+ cells of interest can be depleted in vivo upon DT injection [9]. All the animal studies were approved by IACUC of Boston University Medical Center.

Generation of stable CHO cell line expressing surface pIgR

A stable Chinese hamster ovary (CHO) cell line expressing human pIgR was generated with the 'Toggle-In' system (Antagen). The pIgR open reading frame was cloned by polymerase chain reaction (PCR) from the complementary DNA library of human peripheral blood mononuclear cells (PBMC) into pTOG3 vector, and 1.0 μg of this construct was co-transfected with 20 ng Cre-encoding pOG231 plasmid (Addgene) into CHO-E1 cells at a transcriptional 'hot-spot' via Cre-LoxP recombination-mediated cassette exchange, followed by Hygromycin B selection (800 μg/mL). Single CHO clones were picked and confirmed by reverse transcription PCR. All clones were isogenic with the same genomic integration by the 'Toggle-In' method. A clonal line #5 was chosen for assays.

Expression of various anti-DT IgG-IgA hybrids

Genes of interest were cloned into pDirect CHO expression vectors (Antagen) and electroporated (1620 V, pulse width 10 ms, three pulses) with Neon electroporation system (LifeTech) into CHO-K1 cells, followed by Zeocin selection (400 μg/mL) for 2 weeks. Drug-resistant cells were pooled together and transferred to shaking culture in HyCell serum-free medium (GE Health Sciences). Culture supernatants were passed through Protein A columns to purify various anti-DT IgG with IgA components.

Protection of DT-mediated depletion of peritoneal PD-L2 + DTR+ cells by anti-DT antibodies

CD19-Cre$^{+/-}$PZTD$^{+/+}$ mice were used, whose peritoneal B1a B cells are PD-L2+TdTomato+DTR+ [9]. Various anti-DT IgG-IgA hybrids were i.v. injected 1–6 h before intraperitoneal (i.p.) injection of 25 ng of DT (Sigma-Aldrich, D0564) per gram of body weight in 200 μL Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS). After 20 h, mice were sacrificed. Ten milliliters of Hank's balanced salt solution in 2% fetal bovine serum was injected into the mouse abdominal cavity and peritoneal cells were retrieved with a syringe. Peritoneal cells were washed, incubated with Fc Blocker™ and stained with anti-mCD5-PE-Cy7 (BioLegend, 100622), anti-mIgM-APC (BioLegend, 406509) and Brilliant Violet 421™ anti-mPD-L2 (BioLegend, 107219) for fluorescence-activated cell sorting (FACS) analysis.

RESULTS AND DISCUSSION

Fusing IgA tail piece and J chain to IgG Fc is sufficient for IgG binding to pIgR

During diphtheria infection, bacteria-released DT first destroys the mucosal epithelial cells in the laryngopharynx area and then finds its way into the circulation. We have cloned a pair of heavy chain variable region (VH) and light chain variable region (VL) sequences from the peripheral blood lymphocytes of DTaP (diphtheria, tetanus, and acellular pertussis)-vaccinated humans and reconstituted into a full IgG1 (Antagen 802131) expressed by CHO cells, which shows a strong DT neutralization activity (EC_{50} = 3.54 ng/mL) in Vero cell assay (Supplementary Fig. S2). In executing a Phase II Small Business Innovative Research grant from the Centers for Disease Control and Prevention in developing a therapeutic anti-DT IgG antibody, we wanted to design an IgA-like IgG that when injected i.v. not only neutralizes the toxin in the circulation, but also transcytose into the mucosa to protect the epithelium in the first place.

The prerequisite for sIgA transcytosis is its binding to pIgR. We purified various anti-DT IgG-IgA constructs, and stained CHO-pIgR cells for FACS. While IgG does not bind to CHO-pIgR cells (not shown), fusion of IgG

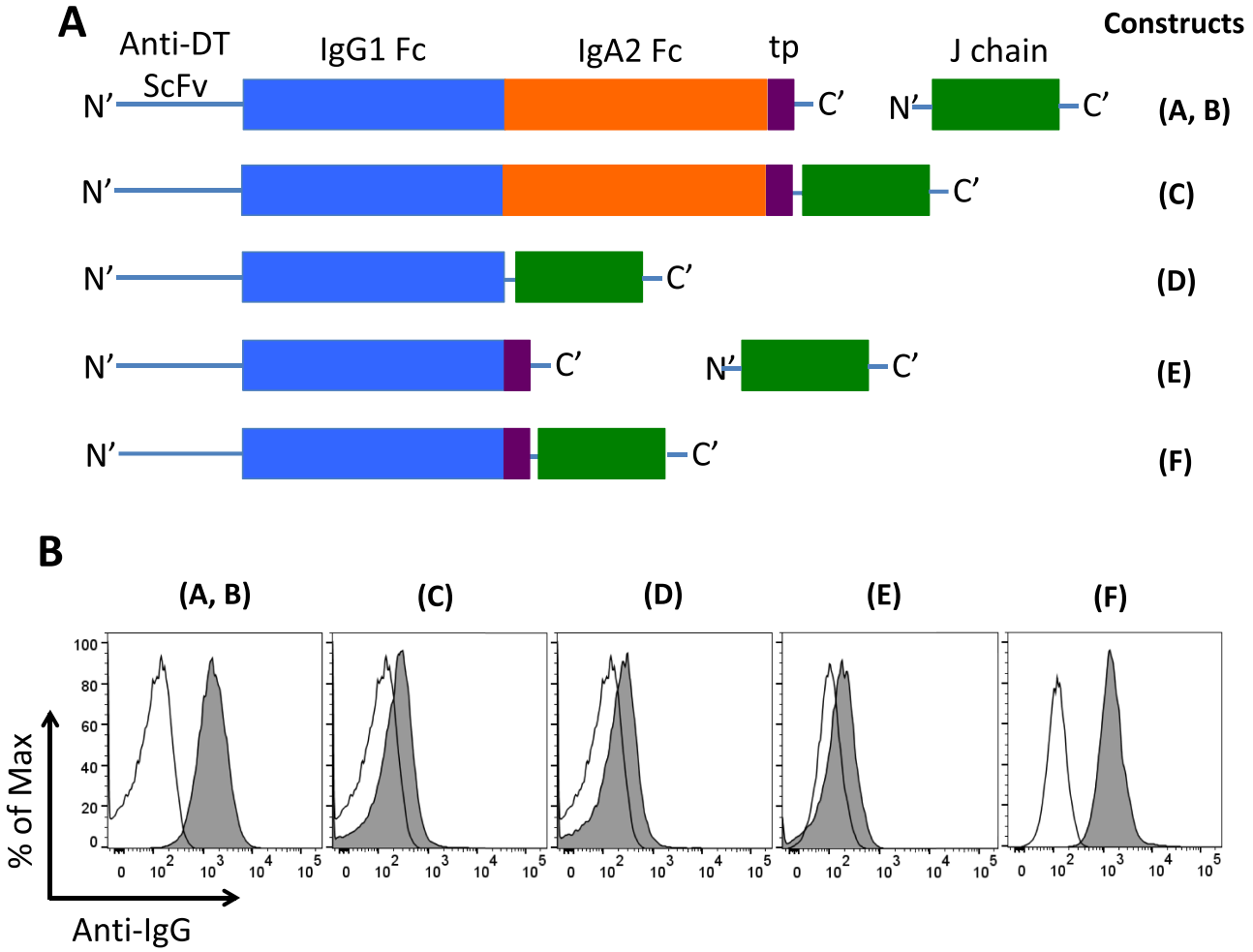

Figure 1. Differential binding of various IgG-IgA hybrids to pIgR. (A) Illustration of Constructs A–F. The N-terminal anti-DT single-chain variable fragment (ScFv) is all the same. Constructs A and B encode and assemble into the same products except that J chain is expressed either by pDirect7.0 vector or in a pre-inserted site via 'Toggle-In', as is in Construct E. The staining intensities for Constructs C and D are >60-fold weaker than those for A, B and F. The protein sequences of these constructs are disclosed in Supplementary data. (B) FACS staining of CHO-pIgR cells with Protein A purified Constructs A–F (1.0 μg/mL), followed by anti-human IgG secondary antibody. Empty area: control staining; shaded area: staining with each IgG-IgA hybrid, respectively. Data represent at least three different experiments.

with the Fc portion of IgA2 (including the tail piece [tp] corresponding to residues 455–472 of IgA2m1, Genbank: AAT74071.1) plus an independent J chain expressed either by the same bicistronic pDirect7.0 vector (Construct A) or in a pre-inserted site via 'Toggle-In' (Construct B) demonstrates strong pIgR binding. To simplify the construction of production cell line, we further fused the J chain after A2(tp) with a flexible linker, but this Construct C shows very low, if any, binding to pIgR, possibly due to structural constraints or distortions. Removing the entire IgA Fc, simply fusing J chain with IgG1 Fc (Construct D) does not improve pIgR binding over Construct C. As tp of IgA is important in complex with J chain, we preserved tp and fused it directly after IgG1 Fc in Constructs E and F. When J chain is expressed separately (Construct E), there is no binding to pIgR, in line with previous findings that structure in IgA Fc other than tp is required to bring J chain to form stable complex [10,11]. Indeed, when J chain is directly fused after IgG1(tp) (Construct F), this requirement for IgA Fc is obviated and full binding to pIgR is restored (Fig. 1A, B). Thus, we created an IgG fusion that can recapitulate sIgA's binding capacity to pIgR.

In vivo protection of DT-mediated depletion of DTR+ cells by anti-DT antibodies

To test the potential mucosa transcytosis brought by pIgR binding, we utilized CD19-Cre$^{+/-}$PZTD$^{+/+}$ mice, whose peritoneal B1a B cells are >50% PD-L2+ [12] and turn on linked expression of TdTomato and DTR [9] (Fig. 2A). When DT is injected (i.p.), the gated IgM + CD5^{int}PD-L2+ cells can be specifically depleted (DT, 3.2% vs. PBS, 45.5%) (Fig. 2A). We used this model to test whether i.v. injected antibody can transcytose from the circulation through the omentum and into the peritoneal cavity to protect the DTR+ cells from DT-mediated depletion. This is to mimic the potential transcytosis of i.v. injected antibody from the circulation into the mucosa of the laryngopharynx area during diphtheria infection.

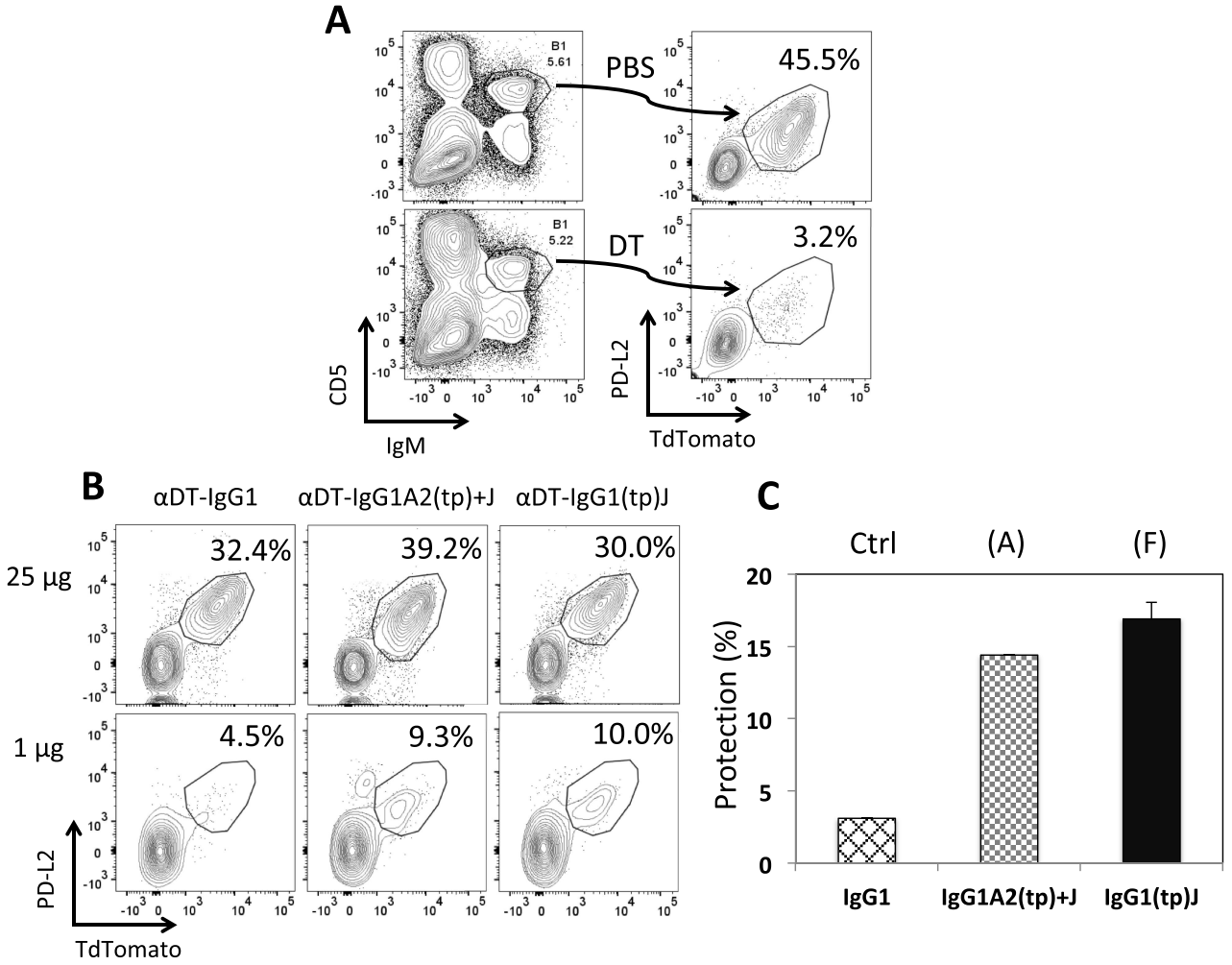

Figure 2. In vivo protection of DT-mediated depletion of PD-L2 + DTR+ cells by IgG-IgA hybrids in a defined window of a peritoneal transcytosis model. (A) Peritoneal IgM + $CD5^{int}$ B1a B cell population contains PD-L2+ cells that are genetically tagged with TdTomato and DTR in CD19-$Cre^{+/-}$PZTD$^{+/+}$ mice [9]. DT treatment (i.p., 25 ng/g of body weight) depleted PD-L2+TdTomato+ population from 45.5 to 3.2% of the gated B1a population. (B) i.v. injection of 25 μg (upper panel) or 1 μg (lower panel) of anti-DT antibodies in 0.1 mL PBS 1 h before DT injection protected PD-L2+DTR+ cells from depletion. Due to the confounding omental vascular leakage, the differential effect of different antibody forms can only be shown with the least amount of antibody administrated (1 μg, low panel). (C) Protection efficiency by anti-DT antibodies (1 μg) on DT-mediated depletion of PD-L2+TdTomato+DTR+ cells is calculated based on depletable PD-L2+TdTomato+ population size as: [antibody treated−DT only (3.2%)]/[PBS only (45.5%)−DT only (3.2%)] × 100%.

Although there is a confounding element in this model, i.e., both the omental capillary endothelium and the mesothelium either are discontinuous or contain fenestrations [13], it is possible to titrate down the factors to demonstrate the difference between IgG vs. IgG-IgA hybrids in transepithelial influx into peritoneum. After a series of in vivo experiments on antibody dosing (200 × 3, 100 × 1, 50 × 1, 25 × 1 and 1 × 1 μg/mouse), we finally titrated down the antibody dose to be only 1 μg/mouse. Also, the time intervals between antibody and DT injections were reduced from 3 days to 6, 4, 2 h and finally to 1 h. We also reduced the i.v. injection volume from 0.5 to 0.1 mL to avoid non-specific leakage of fluids from blood stream into the peritoneum. Indeed, under such optimized conditions, IgG1 antibody did not show transcytosis activity (PD-L2+DTR+ population: 4.5% as shown in Fig. 2B vs. PBS-treated 45.5% and DT-injected 3.2% as shown in Fig. 2A), whereas IgG1(tp)J is as effective as the whole dimeric IgG1A2(tp)+J (Constructs A and B) in transcytosis, as measured by protection of DTR+ cells from DT-mediated depletion in the first 1 h window (PD-L2+DTR+ population: all at about 10%) (Fig. 2B). The calculated protection efficiency is 3.1%, 14.4% and 16.9%, respectively (Fig. 2C). In the above short-coursed experimental system where vascular leakage due to capillary fenestrations plays a dominant role, we had to titrate down the antibody doses and shorten the time intervals between antibody and DT injection, in order to show the difference between IgG1 and IgG1A2(tp)+J or IgG1(tp)J. It can be envisioned that in situations when large amounts of antibodies are adoptively transferred via i.v. route over a relatively long period, such as during neutralization antibody therapy for mucosal infections, the IgG1(tp)J format would demonstrate superior ability over

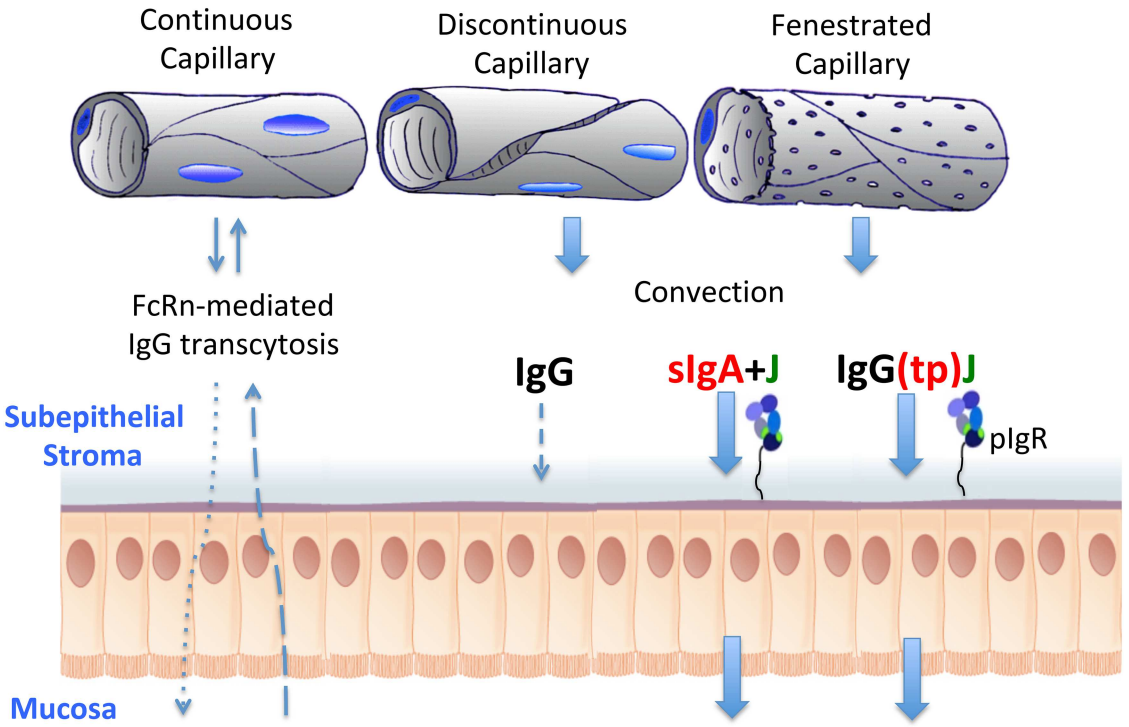

Figure 3. Model of cross-epithelium transcytosis of IgG(tp)J by pIgR. As normal IgG, extravasation of intravusly administrated IgG(tp)J also relies on convection (more at discontinuous and fenestrated capillaries) and/or FcRn-mediated transcytosis from circulation into interstitial space. At subepithelial stroma of tissues with rich blood flow, serum IgG could transcytose epithelium via FcRn, although in a bidirectional manner. Locally produced secretory dimeric IgA in complex with J chain can be specifically bound by pIgR on the basolateral side of epithelial cells, and unidirectionally transcytose the epithelium onto mucosa surface. Likewise, the novel form of IgG(tp)J can bind to pIgR equally well and efficiently transcytose the epithelium; hence, the biodistribution of IgG(tp)J includes not only circulation, but also mucosa.

time in transcytosis from the lamina propria across the mucosal barrier.

Humoral immunity in human secretions is mainly associated with dimeric IgA bound to the secretory component (SC) as sIgA. This isotype is synthesized in the subepithelial stroma (e.g., lamina propria in gut-associated lymphoid tissue) and is then actively transcytosed through epithelial cells with the help of the transmembrane form of the SC, i.e., pIgR. Although FcRn-mediated extravascular transcytosis into interstitial space is reported, the same process plays a minor role in net transcytosing IgG into secretions. Our finding showed that the much efficient transcytosis from subepithelial stroma onto mucosa surface by pIgR as unidirectional molecular 'IgA pump' could be piggybacked by IgG with simple fusion of the 18-aa tp and 137-aa J chain plus a flexible 15-aa $(G_4S)_3$ linker (see Fig. 3 for a working model. Protein sequences of IgG-IgA constructs are disclosed in Supplementary data).

Up till now, there has been no IgA therapeutic antibody approved. Efforts have been tried to convert neutralizing IgG into sIgA for mucosal applications, but the formidable challenge of co-expression and purification of correctly assembled multi-chain sIgA (H, L, J, SC) has yet to be overcome for a viable manufacture scale-up. Converting IgG into IgA also renders a much shorter half-life in vivo. Our compact and novel design of IgG(tp)J in fusion not only simplifies the production and purification, but also preserves FcRn-dependent long half-life in circulation. In addition, the faster clearance of IgA caused by asialoglycoprotein receptor binding to hyposialylated glycan on IgA Fc [14] could be less severe a problem when only the tp of IgA and not the full Fc portion is adopted.

Our finding is particularly relevant to the imminent application of neutralizing antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as a potential therapy for COVID-19. At this time, more and more potent SARS-CoV-2 neutralizing antibodies are being discovered [15–17] and are under expedited development for clinical use. If these neutralizing antibodies could be engineered into the IgG(tp)J format that possess both the characteristics of IgG and IgA, then neutralization would occur in the circulation, as well as at the mucosal area of the respiratory system. Such antibodies would be more effective than the traditional IgG in blocking the transmission and ensuing deadly pathologies of this highly contagious virus. Thus, the IgG(tp)J format could expand the frontiers for i.v. administrated therapeutic IgG in fighting against various human pathogens, fortifying the first-line defense at the mucosa.

SUPPLEMENTARY DATA

Supplementary data are available at the online website of the journal.

ACKNOWLEDGMENTS

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AUTHORS' CONTRIBUTIONS

C.M. performed most experiments including animal studies and analyzed the data. R.N. generated pIgR CHO cell line. V.S. provided technical assistance and animal husbandry. X.Z. and W.G. designed PZTD knockin mouse, monitored and financially supported the study. W.G. wrote the manuscript. All authors critically reviewed the drafts of the manuscript and approved the final version.

CONFLICT OF INTEREST STATEMENT

C.M. and W.G. are employees of Antagen Pharmaceuticals, Inc., which owns the proprietary rights on IgG1(tp)J format.

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