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REVIEWS

The prospect of orally administered monoclonal secretory IgA (SIgA) antibodies to prevent enteric bacterial infections

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ABSTRACT

Eliminating diarrheal diseases as a leading cause of childhood morbidity and mortality in low- and middle-income countries (LMICs) will require multiple intervention strategies. In this review, we spotlight a series of preclinical studies investigating the potential of orally administered monoclonal secretory IgA (SIgA) antibodies (MAbs) to reduce disease associated with three enteric bacterial pathogens: *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* (ETEC), and invasive *Salmonella enterica* serovar Typhimurium. IgA MAbs targeting bacterial surface antigens (flagella, adhesins, and lipopolysaccharide) were generated from mice, humanized mice, and human tonsillar B cells. Recombinant SIgA1 and/or SIgA2 derivates of those MAbs were purified from supernatants following transient transfection of 293 cells with plasmids encoding antibody heavy and light chains, J-chain, and secretory component (SC). When administered to mice by gavage immediately prior to (or admixed with) the bacterial challenge, SIgA MAbs reduced infection *C. jejuni*, ETEC, and *S.* Typhimurium infections. Fv-matched IgG1 MAbs by comparison were largely ineffective against *C. jejuni* and *S.* Typhimurium under the same conditions, although they were partially effective against ETEC. While these findings highlight future applications of orally administered SIgA, the studies also underscored the fundamental challenges associated with using MAbs as prophylactic tools against enteric bacterial diseases.

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Diarrheal diseases remain a leading cause of morbidity and mortality in young children in low- and middle-income countries (LMICs). In 2013, the landmark Global Enteric Multicenter Study (GEMS) identified the pathogens most frequently associated with moderate-to-severe diarrhea (MSD) in infants and young children under the age of five at seven sites across Africa and Asia.[1] Topping the list were rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* (ETEC) and *Shigella*, followed by an array of other agents, including *Vibrio cholerae* O1 and *Campylobacter jejuni*. The same culprits were implicated in a separate multinational and multidisciplinary birth cohort study called MAL-ED, which reported on the incidence and etiology of diarrhea in children under the age of two in eight low resource locations around the globe.[2] While a leading cause of MSD, the food-and water-borne pathogen, *Salmonella enterica* serovar Typhimurium (STm), also deserves attention because it has emerged in parts of Saharan and sub-Saharan Africa as a cause of fatal bloodstream infections in young children.[3,4]

For newborns, breastfeeding is one of the most effective defenses against diarrheal diseases.[5–7] Colostrum and breast milk each contain an array of bioactive compounds, including cytokines, defensins, lactoferrin, and human milk oligosaccharides that collectively hinder opportunistic and pathogenic bacteria from colonizing the neonatal intestinal epithelium.[6,8] Human colostrum and breast milk are also enriched in secretory IgA (SIgA), with concentrations ranging from 10 to 50 mg/ml in colostrum and 0.5–1 mg/ml in breastmilk.[8–10] SIgA is the primary class of antibodies in mucosal secretions, including the fluids that coat the human gastrointestinal tract, where it acts locally to protect epithelial surfaces from viral and bacterial infections. SIgA also plays an important role in sculpting the gut microbiome and promoting intestinal homeostasis.[11] SIgA, in colostrum and breastmilk, originates from IgA-antibody secreting cells that were primed at distal mucosal sites (e.g., intestinal mucosa), then preferentially homed to the lactating mammary gland.[12,13] As such, maternally derived SIgA has immunological specificity for an array of enteric pathogens. Therefore, SIgA plays an important role in protecting the newborn gastrointestinal tract from a range of diarrheal diseases during the first years of life.

Recognizing the impact of enteric diseases on childhood health in LMICs, the Bill and Melinda Gates Foundation (BMGF) has invested in efforts to develop effective vaccines against leading causes of childhood diarrhea, including rotavirus.[14] In addition, the Innovative Technology Solutions group recently awarded a series of research grants to develop low-cost supplements to human colostrum to combat MSD within at-risk populations. Of particular interest is the prospect of oral delivery of recombinant human SIgA monoclonal antibodies (MAbs) to target the handful of bacterial pathogens most frequently associated with MSD.[2,15,16] The prospect of preventing infections by *C. jejuni*, ETEC, and STm in infants with SIgA MAbs is a tall order, considering the myriad of challenges that have been encountered over the past decades of enteric vaccine development.[17,18] Nonetheless, as proof of

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concept, Virdi and colleagues reported that monomeric immunoglobulin A (IgA)-like antibody was sufficient to prevent ETEC-like infection in piglets.[19] In this brief review, we highlight a first series of reports from BMGF's so-called "synthetic colostrum" investment.

Secretory IgA (SIgA) in mucosal immunity

The mucosal surfaces that line the upper and lower airways, the female genital tract, and the entire length of the alimentary tract, represent points of entry for viral and bacterial pathogens. As a defense mechanism, humans secrete a myriad of complex proteinaceous and gelatinous substances that form a physical barrier against particles and pathogenic agents. In addition, a network of lymphoid tissues and specialized leukocyte populations form the so-called mucosal immune system that gives rise to pathogen-specific cellular and humoral responses associated with clearing active infections and preventing future recurrences. From the perspective of mucosal antibodies, SIgA is the most well recognized because of its sole distribution in external secretions and its unique biological attributes.

SIgA is an assemblage of two or more IgA monomers linked at their C-termini by joining (J) chain and associated with secretory component (SC) (Figure 1).[20–22] Humans have two IgA subclasses (IgA1, IgA2) that differ in the length of the hinge regions (Figure 1).[23] B cells that express J chain and, therefore, secrete dimeric (dIgA) and/or polymeric IgA (pIgA) are induced specifically within mucosa-associated lymphoid tissues (MALT), such as Peyer's patches of the small intestine.[24] MALT-derived plasmablasts home specifically to distant mucosal tissues and the lactating mammary gland.[12,13] Dimeric and pIgA are transported across polarized epithelia in the gut and mammary glands by the polymeric immunoglobulin receptor (pIgR) and then released into intestinal secretions and breast milk, respectively. Prior to release, the pIgR is proteolytically cleaved to liberate an ~80 kDa fragment known as secretory component (SC), which remains associated with IgA after its release and, by definition, gives rise to SIgA (Figure 1).[9] While the overall organization of SIgA has been known for decades, only recently has high-resolution cryo-electron microscopy revealed the molecular interactions between IgA, J chain and SC.[20–22]

A multitude of functional activities have been ascribed to SIgA, with immune exclusion at the top of the list. Immune exclusion refers to the ability of SIgA to crosslink (agglutinate) antigens, entrap them in mucus or other matrices, and promote their clearance from the intestinal lumen through peristalsis.[25,26] Immune exclusion occurs in the context of the gut and the airways.[27] SIgA's other activities include toxin neutralization, inhibition of virus uptake, suppression of bacterial virulence factors, and interference with bacterial division processes ("enchained growth").[28,29] In addition to its effects against pathogens, SIgA also shapes the composition of the commensal microbiota[30] and is postulated to play an important role in maintaining stability and microbial diversity on mucosal surfaces.[31] However, the precise mechanisms by which SIgA influences the host microbiome remains unclear.[32]

Inhibition of *C. jejuni* infection with recombinant SIgA MAbs

C. jejuni is a primary etiological agent of MSD in children under the age of five in the developing world, according to the GEMS.[1,16,33] *C. jejuni* infection is associated with multiple post-infectious sequelae, including reactive arthritis, Guillain-

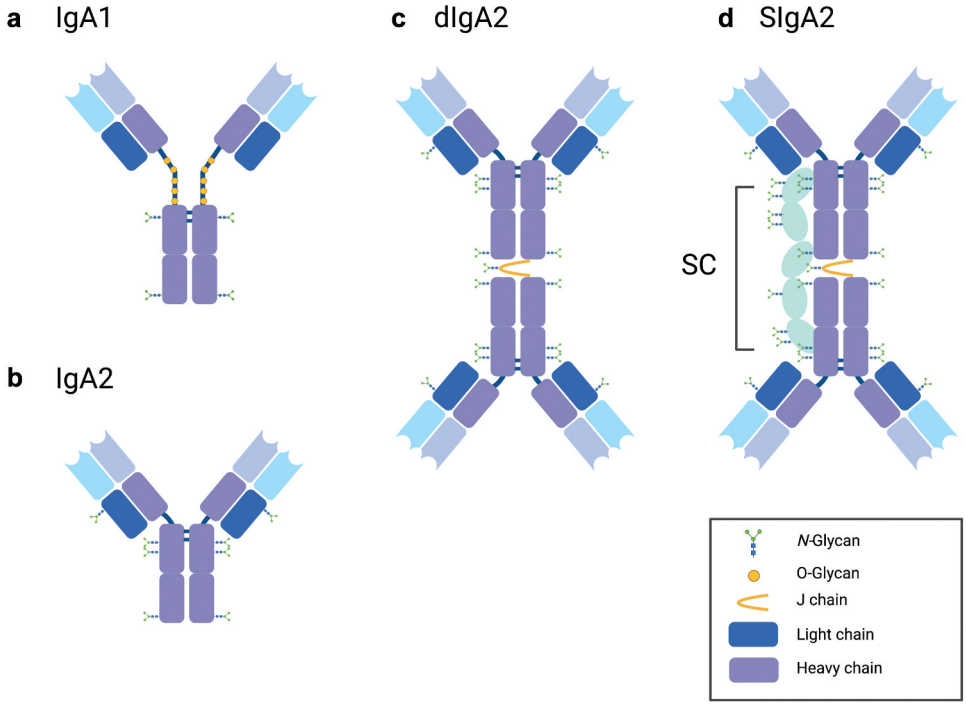

Figure 1. Structure of human IgA and SIgA. Cartoons depicting human (a) monomeric IgA1, (b) monomeric IgA2, (c) dimeric IgA2, and (d) SIgA2. SIgA2 contains multiple *N*-glycans, with one *N*-glycan found on J chain. Graphic generated using BioRender.com.

Barré syndrome, and irritable bowel disease.[34] Recently published longitudinal studies conducted in seven developing countries have implicated *Campylobacter* as causing permanent growth stunting, a finding that has intensified the call by public health officials for measures to control *Campylobacter* in regions where the disease remains endemic.[15,35] At the same time, there is considerable evidence that SIgA is important in immunity to *C. jejuni*. For example, fecal IgA antibody responses were associated with reduced illness in human subjects that underwent a primary and secondary challenge with *C. jejuni* strain 81–176.[36] In children, immunity to *C. jejuni* infection correlated with levels of anti-*Campylobacter* SIgA in breast milk.[37]

C. jejuni virulence factors include capsular polysaccharide (CPS),[38] cytolethal distending toxin (CDT),[39] and lipo-oligosaccharide (LOS). In addition, infection of the human intestinal mucosa by *C. jejuni* is dependent on the bacterium's two polar flagella, which contribute to motility, as well as adherence to and invasion of intestinal epithelial cells (Figure 2).[40–44] In cell and animal models, strains of *C. jejuni* lacking flagella are unable to colonize the intestinal mucosa,[44–46] while motility-deficient strains are severely attenuated in human subjects.[47] The *C. jejuni* flagellar filament consists of a major flagellin subunit, FlaA, and a minor subunit, FlaB, and is capped by FliD.[42] The flagellin subunits of *C. jejuni* are unusual in that they are heavily glycosylated, possibly to evade host innate and adaptive immunity.[48] The flagellar-capping protein FliD, also known as hook-associated protein 2 (HAP2), is a 70-kDa protein with high sequence conservation across the *C. jejuni* species, making it an appealing target for MAbs.[49,50]

Perruzza and colleagues isolated FliD-reactive MAbs from IgA+ and IgG+ B memory cells from 50 human tonsillar samples.[50] B memory cells were immortalized and screened for clones expressing FliD-reactive antibodies, and the corresponding V_H regions were cloned into human IgA1 or IgA2 vectors that were used to transiently co-transfect Expi293 cells in conjunction with vectors encoding V_L, J-chain and SC. Properly assembled SIgA was purified from cell supernatants by affinity and size exclusion chromatography. In the end, the

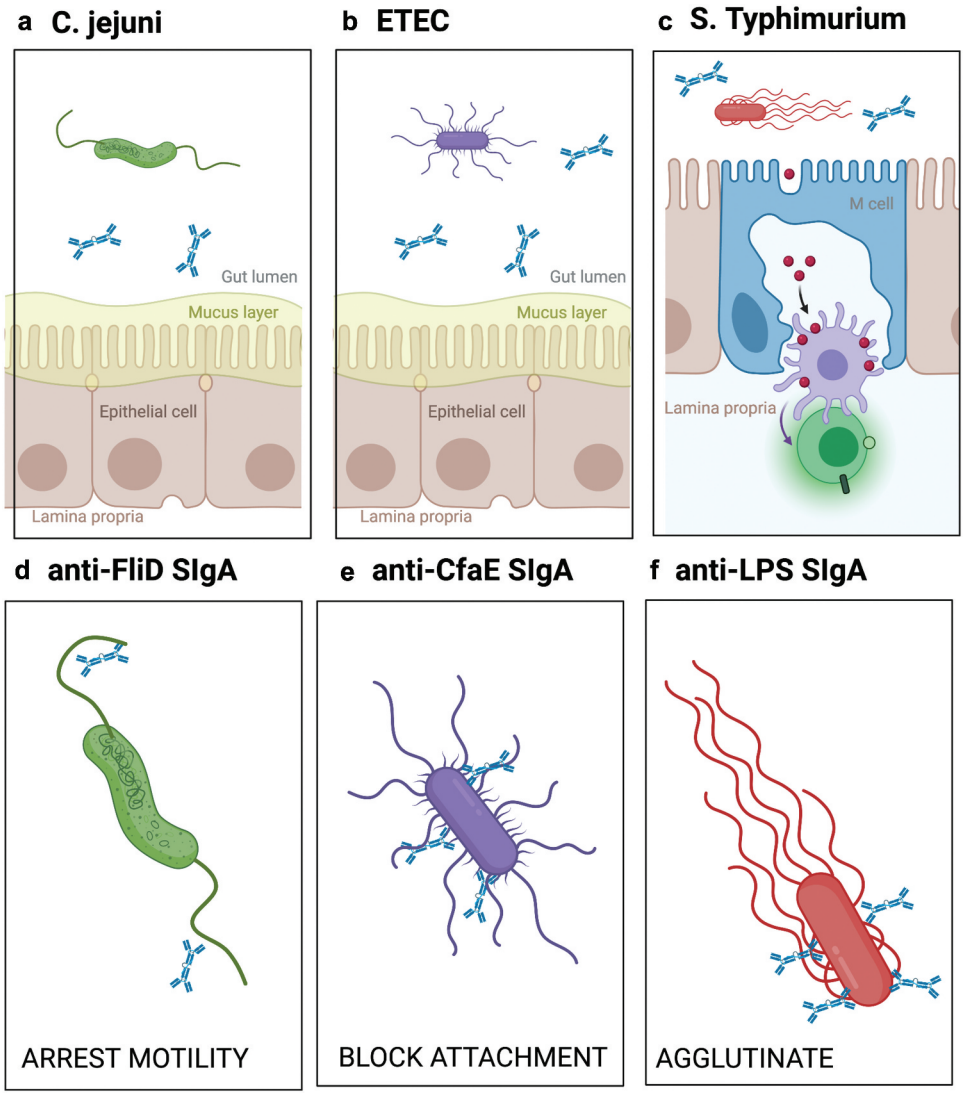

Figure 2. Proposed mechanisms by which SIgA MAbs prevent *C. jejuni*, ETEC and STm from interacting with the intestinal epithelium. Schematic showing interactions of SIgA and (a) *C. jejuni*, (b) ETEC, (c) and STm with the context of the intestinal lumen, mucus, enterocytes and M cells. The lower panels (d–f) depict recombinant SIgA interactions with their target antigens and proposed modes of action. Graphic generated using BioRender.com.

investigators evaluated two MAbs, CAA1 and CCG4, targeting different epitopes on FliD in a mouse model of *C. jejuni* colonization.

In a twenty-one-day old mouse model, animals were gavaged with 200 µg of CAA1 or CCG4 as SIgA1 or SIgA2 and challenged 2 h later with 10^8 CFU of virulent *C. jejuni* 81–176. Readouts of *C. jejuni* infection included bacterial shedding in stool and mucosal inflammation in the cecum, as measured by lipocalin-2 release, neutrophil infiltration, and a 24-point histopathology scoring system (e.g., crypt hyperplasia, goblet cell depletion, epithelial desquamation). The investigators found that CAA1 and CCG4 expressed as SIgA1 or SIgA2 were equally as effective at reducing bacterial shedding and suppressing intestinal inflammation in the mouse model. At early times points (i.e., 24 h and 48 h), shedding of *C. jejuni* from SIgA-treated mice was greater than the control mice, suggesting that targeting the bacterial flagella accelerates the clearance of *C. jejuni* from the gut lumen. At later time points (i.e., 72 h), *C. jejuni* shedding in the stools of CAA1 and CCG4 SIgA-treated mice were lower than controls, indicative of SIgA treatment having prevented the bacterium from establishing a niche in the cecum. Unfortunately, neither dose response or time course experiments were conducted, so it is unclear whether SIgA1 and SIgA2 variants of CAA1 and CCG4 have different efficacies when antibody levels are limiting.

One additional notable finding by Peruzza and colleagues relates the role of antibody isotype. The authors reported that 2 h pre-treatment of mice with IgG1 versions of CAA1 and CCG4 (200 µg each) had no demonstrable effect on *C. jejuni* colonization or campylobacter-induced inflammation, compared to the same amount of SIgA2.[50] Although the reasons why IgG1 failed to confer mucosal immunity against *C. jejuni* were not investigated, they did report that intestinal clearance ("half-life") of IgG1 was faster than SIgA.

Targeting ETEC colonization factors with SIgA

Enterotoxigenic *E. coli* (ETEC) is a motile Gram-negative bacterium that is transmitted via the fecal-oral route. ETEC is ubiquitous in LMICs and infections can occur across all age groups. For tourists and military personnel traveling or stationed in countries where ETEC is endemic, the disease presents as an acute, self-limiting bout of severe watery diarrhea commonly known as Traveler's diarrhea. Unfortunately, things are more problematic for young children who live in these same regions. A recent global survey of LMICs implicated ETEC as a leading cause of MSD in children under the age of five, with evidence that prolonged or repeated bouts of ETEC and/or other leading causative agents of diarrhea can have repercussions for growth and cognitive development.[2,51]

ETEC disease pathogenesis is attributed to a handful of virulence factors (Figure 2).[52] In humans, ETEC adheres to the proximal small intestinal epithelium using a panoply of adhesins (pili or fimbriae) and colonization factors, after which the bacterium secretes a cholera-like toxin known as heat-labile toxin (LT) and/or a small heat stable (ST) toxin.[52] While the exact correlates of protection are not known, inhibition of colonization and toxin-neutralization are considered important determinants. Moreover, as ETEC is noninvasive and resides within the gut lumen, it is safe to assume that SIgA is important (and possibly indispensable) in preventing and clearing ETEC infections.[53]

In a series of studies, orally administered recombinant SIgA MAbs were evaluated in mouse and NHP models for the ability to limit the severity and duration of experimental ETEC infection.[54–58] A collection of MAbs reactive with the ETEC adhesin CfaE were isolated from humanized mice and a subset expressed as recombinant SIgA or IgG1. Following down-selection based on *in vitro* functional assays, three SIgA MAbs were tested in mice. In those studies, ETEC (10^7) were incubated for 1 h with the equivalent of 10 mg/kg of each MAb as SIgA1 and SIgA2, then administered to mice by gavage as a single bolus. Bacterial burden was assessed 24 h later by measuring ETEC colony forming units (CFUs) from intestinal homogenates. The investigators found that irrespective of IgA subclass, each MAb reduced bacterial load by 10–100-fold in the mouse model.

One of those MAbs, 68–61 SIgA2, was also shown to afford a degree of protection against intragastric ETEC challenge when administered to non-human primates (*Aotus nancymaae*) at a dose of 9 mg/kg on days −1, 0 and +1. The benefit of 68–61 SIgA2 was apparent in terms of reduced severity of diarrhea, even though antibody treatment did not affect shedding of ETEC in stool. These results demonstrate the that a single orally administered SIgA MAb is able to at least partially protect mice and NHPs against ETEC colonization and disease.

When assessing ETEC vaccine efficacy in human Phase I clinical trials, the primary endpoint is defined as a reduction in episodes of moderate to severe diarrhea.[59] It should be noted that bacterial shedding in stool samples does not necessarily correlate with disease severity (*e.g.*, individuals that do not experience MSD can still shed ETEC in high numbers), an observation that can confound interpretation of preclinical studies in mice and NHPs.

Inhibition of invasive *S.* Typhimurium by recombinant anti-LPS SIgA

STm is a leading cause of enteric disease in children and adults worldwide. While infection normally manifests as self-limiting gastroenteritis, the emergence invasive non-typhoidal STm (iNTS) isolates such as sequence type 313 (ST313) in sub-Saharan Africa capable of causing fatal systemic infections in children and immunocompromised individuals has raised alarms.[3,4,60] Furthermore, the increase of iNTS isolates carrying resistance to one or more commonly used antibiotics has prompted investigations into vaccines and alternative biologics, such as SIgA, to prevent *Salmonella* infections.

STm is a highly versatile pathogen that employs a range of metabolic pathways and virulence factors to successfully colonize and invade the intestinal mucosa.[61] In mice, STm initially breaches the intestinal barrier by invading M cells (Figure 2), a specialized epithelial cell type overlying gut-associated lymphoid tissues such as Peyer's patches in the ileum.[62] M cell invasion is an active process that involves flagella-based motility and a type-three secretion system (T3SS) encoded by a specialized genomic island called SPI-1.[63] Due to the rarity

of M cells along the length of the GI tract, M cell uptake is considered a bottleneck in STm infection process.[64] Following M cell uptake, STm resides within macrophages and dendritic cells as it spreads systemically, largely hidden from circulating antibodies. With this in mind, blocking STm invasion of M cells constitutes the most desirable point in which to interfere with infection.

In two recent reports, Richards and colleagues investigated the ability of orally administered mouse and human SIgA MAbs to prevent invasion of Peyer's patch tissues by STm in a mouse model.[65,66] The antibody of choice for these studies was Sal4, a well-studied murine IgA MAb directed against the immunodominant O5-antigen of STm lipopolysaccharide.[67–71] In pioneering studies, Michetti and colleagues demonstrated that Sal4 IgA alone was sufficient, when transported into intestinal secretions as the result of a backpack tumor implant, to significantly reduce STm entry into Peyer's patch tissues.[70,71] To evaluate whether oral administration of Sal4 is also effective, Richards and colleagues purified dimeric Sal4 mouse IgA, complexed it with recombinant SC in vitro, and delivered it in the form of SIgA across a range of doses (0.4–50 μg per mouse; 0.02–2.5 mg/kg) to mice by gavage.[65] Mice were euthanized 24 h later and bacterial numbers within the Peyer's patch tissues were determined. When admixed with STm (10^7 CFUs), Sal4 SIgA reduced bacterial uptake into Peyer's patch tissues in a dose-dependent manner. At the equivalent of 2.5 mg/kg Sal4 SIgA, for example, bacterial uptake was reduced by several orders of magnitude. While not unexpected, a class-switched IgG1 variant of Sal4 had no demonstrable effect on STm uptake into Peyer's patch tissues, even at relatively high doses (10 mg/kg). It was proposed that the stark difference in efficacy between Sal4 SIgA and IgG1 was due to differences in antibody stability in the gastrointestinal environment and/or functionality due to SIgA multivalency.

In a follow-up study, Richards and colleagues generated a human Sal4 IgA2 variant and expressed it in Expi293 cells as a monomer, dimer or SIgA.[66] All three forms of Sal4 IgA2 were able to reduce STm uptake into Peyer's patch tissues when administered to mice by gavage at the time of challenge, although at lower doses SIgA and dIgA proved superior to mIgA, possibly revealing the importance of avidity and cross-linking in antibody functionality *in vivo*. The ability of Sal4 SIgA to promote large and densely packed aggregates of STm within the intestinal lumen was cited as the culprit in limiting bacterial uptake via M cells.

Summary and perspectives

Combatting diarrheal diseases on a global scale will require a holistic approach that includes improved water and sanitation, vaccine deployment, and targeted preventative measures for high-risk individuals. With the successful implementation of highly effective oral vaccines against rotavirus over the past decade, attention is now turned toward the other etiologic agents of MSD in certain populations, the incidence of many enteric bacterial pathogens is unabated with significant public health consequences.

The demonstration in mice and NHPs that orally administered recombinant SIgA MAbs targeting single epitopes on *C. jejuni*, ETEC, and STm were able to curtail intestinal infection contributes to an emerging field aimed at the development of effective prophylactics against diarrheal diseases.[19,72] Nonetheless, notable challenges remain. Foremost is the need for sustained or repeated delivery of SIgA to afford protection for prolonged periods. In the mouse studies highlighted in this review, protection was achieved only when SIgA MAbs were administered shortly before or at the time of bacterial challenge. For example, Perruzza demonstrated protection against *C. jejuni* infection within 2 h following SIgA MAb treatment,[50] while Richards reported that the window for STm was <20 min after Sal4 SIgA treatment.[65] In the case of ETEC, a reduction in intestinal ETEC burden was only observed when the bacteria were premixed with anti-CfaE HuMAbs prior to oral delivery.[54] Moreover, dose-response experiments were not conducted, except in the case of Sal4.[65] Therefore, the exact amount of SIgA required for protection against ETEC and *C. jejuni* and the frequency of SIgA dosing for the three pathogens remains to be determined.

The economics of SIgA production and formulation will be major determinants of the practicability of prophylactic oral antibody treatment. Existing mammalian cell-based expression systems are considered prohibitively expensive for the purpose of manufacturing SIgA at-scale, although advances in continuous downstream processes are changing the landscape to some degree.[73] Options for SIgA include a range of yeast- and plant-based systems.[58,72,74,75] As a case in point, Virdi and colleagues reported that camelid single-chain-derived IgA antibodies could be produced in soybean seeds or secreted from the yeast Pichia pastoris, freeze- or spray-dried, and orally delivered within food in a pig model.[19] One non-traditional platform being pursued (at least for single chain antibody production) is Spirulina.[76] Alternative strategies such as transgenic animals that express human MAbs including human IgA in colostrum and breast milk are also worthy of investigation, especially considering their track record with other biologics.[77,78]

While oral administration of MAbs and MAb cocktails has obvious benefits over intravenous and subcutaneous routes of delivery, the pharmacokinetics and stability of SIgA in the human gastric and intestinal environments remain unknown and need to be taken in consideration.[55,57] Within the context of the gut, the benefit of SIgA over IgG is obvious and is likely due to SIgA glycosylation and resistance to intestinal proteases.[79] Considering the importance of IgA MAb stability to ensure adequate local concentrations in the gut, antibody engineering approaches have been employed to increase the serum half-life of polymeric IgA for a systemic administration strategy, assuming efficient luminal transport via pIgR.[80] Other strategies include extending SIgA's retention time within the protective mucus barrier using SIgA carrying bacterial-derived mucin-binding proteins.[81] One caveat to that approach is that it has yet to be determined whether mucus affinity promotes or hinders mucosal protection, as recent studies on IgG in cervical vaginal mucus demonstrate that the ability to rapidly diffuse through mucus is advantageous over entrapment.[82]

Another approach is the use of multivalent or combination MAb cocktails to target a single pathogen of interest or to broaden the efficacy of a single prophylactic formulation. Shrestha and colleagues demonstrated that multivalent IgGs

had enhanced sperm agglutinating activity in a mucin matrix designed to mimic human cervix environment.[83] Others have opted to engineer camelid-derived single-chain antibodies carrying IgA Fc regions, with great success.[19,72] In summary, the prospect of oral antibody prophylaxis, especially with SIgA, is of great interest as an adjunct to vaccination or antibiotic treatment. Even short-term interventions have the potential to have long-term impacts on in childhood health in LMICs. The studies highlighted in this review constitute a first step toward a new and targeted applications in combating enteric diseases.

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