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REVIEW

# Therapeutic immunoglobulin A antibody for dysbiosis-related diseases

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## Abstract

**Dysbiosis is alterations in the microbial composition compared with a healthy microbiota and often features a reduction in gut microbial diversity and a change in microbial taxa. Dysbiosis, especially in the gut, has also been proposed to play a crucial role in the pathogenesis of a wide variety of diseases, including inflammatory bowel disease, colorectal cancer, cardiovascular disease, obesity, diabetes and multiple sclerosis. A body of evidence has shown that intestinal polymeric immunoglobulin A (IgA) antibodies are important to regulate the gut microbiota as well as to exclude pathogenic bacteria or viral infection such as influenza and SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) at mucosal sites. Since the 1970s, trials for oral administration of therapeutic IgA or IgG have been performed mainly to treat infectious enteritis caused by pathogenic *Escherichia coli* or *Clostridium difficile*. However, few of them have been successfully developed for clinical application up to now. In addition to the protective function against intestinal pathogens, IgA is well known to modulate the gut commensal microbiota leading to symbiosis. Nevertheless, the development of therapeutic IgA drugs to treat dysbiosis is not progressing. In this review, the advantages of therapeutic IgA antibodies and the problems for their development will be discussed.**

*Keywords:* gut microbiota, IgA, mucosal immunity

## Introduction

The development of antibodies that can be used therapeutically is progressing mostly for intravenous administration. However, since most pathogens invade via mucosal sites such as the gastrointestinal or respiratory tracts where polymeric IgA plays a crucial role, direct administration of IgA to the mucosa, delivered orally or by inhalation, makes sense to prevent pathogen infection. At the mucosa, the polymeric, mostly dimeric, IgA (which consists of two heavy and two light chains with a joining chain) is secreted into gut lumen. During the secretion process, the extracellular domain of the polymeric Ig receptor (pIgR), which is known as the secretory component (SC), also attaches to the dimeric IgA, which is then termed secretory IgA (SIgA) (Fig. 1). SIgA is thought to be critical in mucosal defense and is highly resistant to the harsh intestinal environment; for example, it can resist protease-mediated cleavage.

I begin by describing the history of oral administration of immunoglobulins. In the late 1970s and 1980s, hyperimmune bovine colostrum containing pathogen-reactive IgA antibodies was orally administered to humans to treat diarrhea-associated illness caused by enterotoxigenic *Escherichia coli* (ETEC), rotavirus or cryptosporidium (1–4). In parallel, human polyclonal immunoglobulin preparations containing IgA and IgG were prepared from human serum for oral administration and were given to prevent infantile necrotizing enterocolitis (NEC) caused by ETEC, rotavirus or *Clostridioides difficile* (5–9). Those oral immunoglobulin trials focused mainly on low-birthweight infants or immunodeficient children. Although some cases showed the prevention of NEC, these studies did not strongly support oral immunoglobulin therapy for the prevention of NEC (10). Controlled trails of oral IgA alone, excluding IgG, were not performed in these studies.

Since the mid-1970s, when the method of hybridoma production was established, oral administration of monoclonal IgA antibodies in animal models demonstrated their protective effects against pathogenic bacteria such as *Vibrio cholerae* (a mouse monoclonal IgA antibody against its lipopolysaccharide; LPS) (11), *Salmonella* (a mouse monoclonal

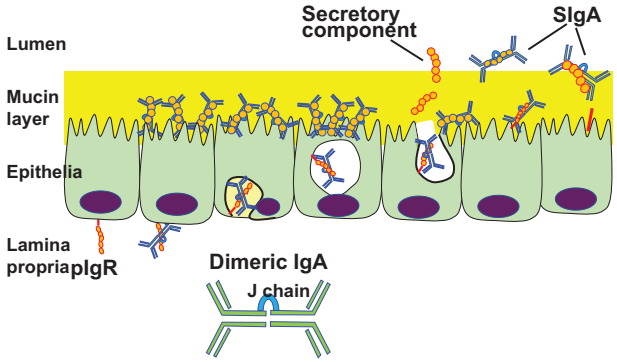


**Fig. 1.** IgA secretion into the gut lumen. On the contrary to serum IgA, which is mainly in a monomeric form, polymeric or dimeric IgA with a joining (J) chain is produced by IgA-producing plasma cells in the lamina propria. It binds to the pIgR on the basolateral surface of epithelial cells and is transcyotosed to the apical surface of epithelial cells, where the receptor is cleaved by proteolysis. As a result, the secreted form of IgA (SIgA) is released into the gut lumen. The cleaved extracellular portion of pIgR is known as the secretory component.

IgA antibody against its O5 antigen) (12) or ETEC (a human monoclonal IgA antibody against its surface material known as colonization factor) (13). In these studies, polymeric IgA antibodies were used because they were more effective for bacterial infection because of their avidity and increased stability in the gut lumen than IgG antibodies.

For humans, only three monoclonal antibodies have been approved to treat or prevent infectious diseases—respiratory syncytial virus (a human monoclonal IgG antibody against a viral protein) (14), anthrax (a human monoclonal IgG antibody against an anthrax toxin) (15) and *C. difficile* (a human IgG monoclonal antibody against its toxin B) (16)—and all three antibodies are administered intravenously, but not orally. More recently, a novel, orally delivered ovine polyclonal antibody (mostly IgG) therapy against the toxins of *C. difficile* has been applied in clinical trials (17). However, at present, no oral IgA antibody drug has been successfully developed for clinical trials.

## Dysbiosis associated with reduced IgA quantity or quality

Selective IgA deficiency is relatively common in humans (about 1 in 500 Caucasian individuals) (18). It was considered asymptomatic, but recent studies have shown it to be symptomatic in 80% of patients (19, 20). A cohort study compared the susceptibility to immune-related diseases between IgA-deficient patients and healthy controls. For example, IgA deficiency showed a large impact on patients with inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis as well as celiac disease, type 1 diabetes and rheumatoid arthritis (21). Other studies also have demonstrated that human IgA deficiency is associated with recurrent infections and autoimmunity (22, 23). Moreover, dysbiosis (altered microbial composition) and reduced microbial diversity were common between human IgA deficiency and mouse models with IgA deficiency (24, 25). Immunoglobulin M (IgM) did not completely compensate dysbiosis in IgA-deficient humans, although the levels of serum/mucosal IgM are higher in IgA-deficient individuals (24, 26). Thus, IgA has a significant role in regulating the gut microbiota composition.

In addition, it should be noted that not only the quantity but also the quality of IgA is important for gut homeostasis. As we age, our gut microbial composition changes to slight dysbiosis (27, 28). As reported previously, we found that healthy elderly people (the mean age: 76 years old) had a reduced relative abundance of 'beneficial' bacteria, Bifidobacteriaceae, whereas Enterobacteriaceae increased, even though the subjects were all healthy and produced a comparable amount of intestinal IgA to healthy adults (the mean age: 35 years old) (29). Our IgA-seq analysis (comparative analysis between IgA-bound and -unbound bacterial taxa by 16S rRNA sequencing) demonstrated that the IgA responses to Enterobacteriaceae significantly decreased in elderly individuals compared with younger adults (29).

As we age, the immune response such as the germinal center (GC) reaction that is crucial for the generation of high-affinity antibodies becomes senescent, resulting in compromised IgA responses against certain species bound strongly by IgA in adults, like Enterobacteriaceae. In mice with altered IgA repertoires because of T-cell functional deficiency (30) or a somatic hypermutation defect (31), gut microbiota perturbations have been reported. Taken together, the quality and quantity of IgA are important in regulating the gut microbiota.

## The pathogenesis of IBD

The pathogenesis of IBD has been proposed to be a combination of host factors and environmental factors including gut dysbiosis. Genome-wide association studies (GWAS) have identified more than 200 loci related to IBD susceptibility, mostly associated with anti-microbial defense such as the nuclear factor-kappa B (NF-kB) and interleukin 17 (IL-17) pathway (32–34). Accordingly, many drugs have been developed to treat the inflammatory conditions by modifying our immune system, specific cytokines or signaling molecules (35). However, unlike infectious pathogens, the target microbial species in IBD have not been defined. As a typical example, decreased taxa in IBD patients include short-chain fatty acid (SCFA)-producing bacteria such as Bifidobacteriaceae and Clostridiaceae, which are considered as beneficial bacteria; increased taxa in IBD patients include Enterobacteriaceae and Fusobacteriaceae, which are considered as pathobionts (36).

Even if the target is not specified, changing the gut microbiota composition is a promising approach to prevent or treat IBD. At present, there is almost no effective therapy to modulate gut microbiota. Together with fecal microbial transplantation (FMT), probiotics (live bacteria that provide health benefits) or prebiotics (food compounds that help bacterial growth), oral IgA antibody is expected to emerge as a therapeutic drug for dysbiosis in the future.

## The difference in IgA responses between rodents and humans

As discussed above, IgA antibody is important to modulate the human gut microbiota. Because most of the gut bacteria are common between humans and mice, the experimental results in mice are expected to be applicable for humans. However, considering the function of IgA in the host, we should note the dissimilarity between IgA systems in humans compared with mice as reviewed in ref. (37), especially in

terms of the inflammatory functions. IgA is generally accepted as a non-inflammatory antibody so that it can maintain gut mucosal homeostasis.

IgA can, however, potently activate inflammatory responses through cross-linking of one of its receptors, FcαRI, which mice lack. On the other hand, the SC that attaches to dimeric IgA upon secretion into the gut lumen inhibits the binding of secreted IgA (SIgA) to FcαRI because of steric hindrance. Therefore, the drug formulation of IgA should be carefully considered. Since mouse IgA binds poorly to human FcαRI, to prevent unnecessary inflammatory responses, dimeric mouse IgA is a simple formulation as a human gut microbial modulator.

## The potential of therapeutic intestinal IgA antibodies to modulate gut microbes

Considering that the intestinal IgA antibodies are known to react to gut bacteria in a poly-reactive manner (38, 39), it is not necessary to specify the target microbe causing dysbiosis before treatment. Indeed, our mouse study supported this idea (31). We have generated the mutant mouse strain, AID$^{G23S}$ mice, which have only low-affinity IgA because of a specific defect in somatic hypermutation. They produce normal levels of intestinal IgA, but the mutation frequency of their intestinal IgA is significantly reduced.

In AID$^{G23S}$ mice, the absence of high-affinity IgA caused dysbiosis, and the lipocalin 2 level in feces (a biomarker of gut inflammation) increased especially in aged mutant mice even under specific-pathogen-free conditions, supporting the idea that the quality of IgA is important to regulate the gut commensal microbiota (40). Moreover, AID$^{G23S}$ mice are more susceptible to Yersinia invasion into mesenteric lymph nodes as well and are more severely affected by oral cholera toxin challenge than wild-type mice are, indicating that high-affinity IgA produced in wild-type mice can protect from pathogen invasion more efficiently than low-affinity IgA produced in AID$^{G23S}$ mice does, even without immunization against those pathogens (31). Since intestinal IgA is well known to be poly-reactive, the high-affinity IgA in wild-type mice protects from Yersinia or cholera toxin via a cross-reactive recognition. Taken together, as a gut microbial modulator, the best intestinal IgA should be high-affinity and poly-reactive against a wide range of gut microbiota.

To translate an oral IgA drug into clinical application, cost-effective antibody-production platforms such as mammalian cell systems, transgenic animals, plants or filamentous fungi should be considered and developed in the future. Because it is not completely known whether the glycosylation of IgA, which diversified in different species, is essential for its protective ability against bacteria, future studies together with the recent remarkable advances in antibody engineering technology are necessary to solve them.

## A mouse monoclonal IgA as a candidate oral drug for dysbiosis

According to our criteria, W27—a monoclonal IgA (mostly dimeric IgA) derived from an IgA-producing hybridoma established from mouse small intestine—was selected and administered orally to AID$^{G23S}$ mice in the drinking water for 4 weeks (41). A cocktail of antibiotics in the drinking water showed an inhibitory effect on GC B-cell hyperplasia in these mice because of the total eradication of gut bacteria including beneficial bacteria. Of note, W27 oral treatment also reduced hyperplasia of GC B cells and changed the gut microbe composition: there was an increase in *Clostridium* species, beneficial ones, which are known as regulatory T cell (Treg cell) inducers. Correspondingly, the number of colonic Treg cells increased after W27 oral treatment in AID$^{G23S}$ and AID$^{-/-}$ mice (40).

Another study has shown that oral W27 given to IgA-deficient mice decreased Enterobacteriaceae and increased Lactobacillaceae in their feces, indicating clearly the beneficial effect of W27 IgA on the gut microbial balance (42). More recently, we demonstrated that W27 IgA suppressed the growth of *Escherichia* in an *in vitro* anaerobic culture of the healthy human intestinal microbiota. In addition, *Bifidobacterium* increased significantly in same *in vitro* culture, supporting the proposed beneficial effect of W27 IgA as a human gut microbial modulator (43).

## The biological functions of IgA against bacteria

In the gut lumen, SIgA plays an important role mainly by inhibiting the mucosal adherence of microorganisms; IgA agglutinates microbes, inhibits bacterial motility by interacting with their flagella and neutralizes bacterial enzymes and toxins. In addition, SIgA can change bacterial gene expression, thereby helping the colonization of certain bacterial species (reviewed in ref. (44)). How SIgA can regulate these oppositely directed functions (immune exclusion and inclusion) on each bacterium has not been clarified yet. For example, Slack's group has demonstrated that high-avidity IgA that was produced after vaccination against *Salmonella* induced 'enchained' bacterial growth, in which bacteria grew at normal speed but in a block (45). Therefore, even though they grew, the pathogens were discarded safely as a block with the help of IgA. In contrast, W27 IgA inhibited *E. coli* cell growth in *in vitro* culture (41). Future studies will reveal the more complex IgA biology, to answer how IgA can access microbial metabolic enzymes or modify their gene expression.

## Conclusions

Dysbiosis has been shown to associate with a wide range of diseases such as IBD, obesity, allergic disorders, type 1 diabetes mellitus, autism and so on, in both humans and animal models. Defective IgA responses result in dysbiosis as well as intestinal infections. Although pathogen-specific vaccination can induce protective IgA responses, aged individuals or those with defects in the production of high-affinity antibody cannot. Moreover, the causative bacterium for dysbiosis is usually not diagnosed easily. Therefore, the oral administration of selected high-affinity poly-reactive dimeric IgA is a promising approach to re-set dysbiosis. In addition to prevention against intestinal infection, dysbiosis is a good target for oral IgA therapy, since the target of IgA exists in the gut lumen.

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## References


1 Mietens, C., Keinhorst, H., Hilpert, H. *et al.* 1979. Treatment of infantile *E. coli* gastroenteritis with specific bovine anti-*E. coli* milk immunoglobulins. *Eur. J. Pediatr.* 132:239.
2 Tzipori, S., Roberton, D. and Chapman, C. 1986. Remission of diarrhea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. *Br. Med. J.* 293:1276.
3 Hilpert, H., Brüssow, H., Mietens, C. *et al.* 1987. Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. *J. Infect. Dis.* 156:158.
4 Tacket, C. O., Losonsky, G., Link, H. *et al.* 1988. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. *N. Engl. J. Med.* 318:1240.
5 Barnes, G. L., Doyle, L. W., Hewson, P. H. *et al.* 1982. A randomised trial of oral gammaglobulin in low-birth-weight infants infected with rotavirus. *Lancet* 1:1371.
6 Losonsky, G. A., Johnson, J. P., Winkelstein, J. A. *et al.* 1985. Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis. A pharmacokinetic and functional analysis. *J. Clin. Invest.* 76:2362.
7 Eibl, M. M., Wolf, H. M., Fürnkranz, H. *et al.* 1988. Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA-IgG feeding. *N. Engl. J. Med.* 319:1.
8 Tjellström, B., Stenhammar, L., Eriksson, S. *et al.* 1993. Oral immunoglobulin A supplement in treatment of *Clostridium difficile* enteritis. *Lancet* 341:701.
9 Wolf, H. M. and Eibl, M. M. 1994. The anti-inflammatory effect of an oral immunoglobulin (IgA-IgG) preparation and its possible relevance for the prevention of necrotizing enterocolitis. *Acta Paediatr. Suppl.* 396:37.
10 Foster, J. P., Seth, R. and Cole, M. J. 2017. Oral immunoglobulin for preventing necrotizing enterocolitis in preterm and low birth weight neonates. *Cochrane Database Syst. Rev.* 4:CD001816.
11 Lee, C. K., Weltzin, R., Soman, G. *et al.* 1994. Oral administration of polymeric immunoglobulin A prevents colonization with *Vibrio cholerae* in neonatal mice. *Infect. Immun.* 62:887.
12 Richards, A. F., Doering, J. E., Lozito, S. A. *et al.* 2020. Inhibition of invasive *Salmonella* by orally administered IgA and IgG monoclonal antibodies. *PLoS Negl. Trop. Dis.* 14:e0007803.
13 Stoppato, M., Gaspar, C., Regeimbal, J. *et al.* 2020. Oral administration of an anti-CfaE secretory IgA antibody protects against enterotoxigenic *Escherichia coli* diarrheal disease in a nonhuman primate model. *Vaccine* 38:2333.
14 The IMpact-RSV Study Group. 1998. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 102:531.
15 Migone, T.-S., Subramanian, G. M., Zhong, J. *et al.* 2009. Raxibacumab for treatment of inhalational anthrax. *N. Engl. J. Med.* 361:135.
16 Babcock, G. J., Broering, T. J., Hernandez, H. J. *et al.* 2006. Human monoclonal antibodies directed against toxins A and B prevent *Clostridium difficile*-induced mortality in hamsters. *Infect. Immun.* 74:6339.
17 Roberts, A. K., Harris, H. C., Smith, M. *et al.* 2020. A novel, orally delivered antibody therapy and its potential to prevent *Clostridioides difficile* infection in pre-clinical models. *Front. Microbiol.* 11:578903.
18 Yel, L. 2010. Selective IgA deficiency. *J. Clin. Immunol.* 30:10.
19 Jorgensen, G. H., Gardulf, A., Sigurdsson, M. I. *et al.* 2013. Clinical symptoms in adults with selective IgA deficiency: a case-control study. *J. Clin. Immunol.* 33:742.
20 Koskinen, S. 1996. Long-term follow-up of health in blood donors with primary IgA deficiency. *J. Clin. Immunol.* 16:165.
21 Ludvigsson, J. F., Neovius, M. and Hammarström, L. 2014. Association between IgA deficiency & other autoimmune conditions: a population-based matched cohort study. *J. Clin. Immunol.* 34:444.
22 Ludvigsson, J. F., Neovius, M. and Hammarström, L. 2016. Risk of infections among 2100 individuals with IgA deficiency: a nationwide cohort study. *J. Clin. Immunol.* 36:134.
23 Aghamohammadi, A., Cheraghi, T., Gharagozlou, M. *et al.* 2009. IgA deficiency: correlation between clinical and immunological phenotypes. *J. Clin. Immunol.* 29:130.
24 Fadlallah, J., Kafsi, H. E., Sterlin, D. *et al.* 2018. Microbial ecology perturbation in human IgA deficiency. *Sci. Trans. Med.* 10:eaan1217.
25 Kubinak, J. L. and Round, J. L. 2016. Do antibodies select a healthy microbiota? *Nat. Rev. Immunol.* 16:767.
26 Catanzaro, J. R., Strauss, J. D., Bielecka, A. *et al.* 2019. IgA-deficient humans exhibit gut microbiota dysbiosis despite secretion of compensatory IgM. *Sci. Rep.* 9:13574.
27 Mitsuoka, T. and Hayakawa, K. 1973. The fecal flora in man. Composition of the fecal flora of various age groups. *Zbl. Bakt. Hyg. I. Abt. Orig.* 223:333.
28 Odamaki, T., Kato, K., Sugahara, H. *et al.* 2016. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiol.* 16:90.
29 Sugahara, H., Okai, S., Odamaki, T. *et al.* 2017. Decreased taxon-specific IgA response in relation to the changes of gut microbiota composition in the elderly. *Front. Microbiol.* 8:1757.
30 Kawamoto, S., Tran, T. H., Maruya, M. *et al.* 2012. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. *Science* 336:485.
31 Wei, M., Shinkura, R., Doi, Y. *et al.* 2011. Mice carrying a knock-in mutation of Aicda resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense. *Nat. Immunol.* 12:264.
32 de Lange, K. M., Moutsianas, L., Lee, J. C. *et al.* 2017. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. *Nat. Genet.* 49:256.
33 Jostins, L., Ripke, S., Weersma, R. K. *et al.*; International IBD Genetics Consortium (IIBDGC). 2012. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 491:119.
34 Liu, J. Z., van Sommeren, S., Huang, H. *et al.*; International Multiple Sclerosis Genetics Consortium; International IBD Genetics Consortium. 2015. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.* 47:979.
35 Anonymous. *The facts about inflammatory bowel diseases*. New York, NY: Crohn's and Colitis Foundation of America; 2014. Available at: http://www.crohnscolitisfoundation.org/assets/pdfs/updatedibdfactbook.pdf.
36 Gevers, D., Kugathasan, S., Denson, L. A. *et al.* 2014. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 15:382.
37 Bakema, J. E. and van Egmond, M. 2011. Immunoglobulin A: a next generation of therapeutic antibodies? *MAbs* 3:352.
38 Rollenske, T., Szijarto, V., Lukasiewicz, J. *et al.* 2018. Cross-specificity of protective human antibodies against *Klebsiella pneumoniae* LPS O-antigen. *Nat. Immunol.* 19:617.
39 Sterlin, D., Fadlallah, J., Adams, O. *et al.* 2020. Human IgA binds a diverse array of commensal bacteria. *J Exp. Med.* 217:e20181635.
40 Okai, S., Usui, F., Ohta, M. *et al.* 2017. Intestinal IgA as a modulator of the gut microbiota. *Gut Microbes* 8:486.
41 Okai, S., Usui, F., Yokota, S. *et al.* 2016. High-affinity monoclonal IgA regulates gut microbiota and prevents colitis in mice. *Nat. Microbiol.* 1:16103.
42 Xiong, E., Li, Y., Min, Q. *et al.* 2019. MZB1 promotes the secretion of J-chain-containing dimeric IgA and is critical for the suppression of gut inflammation. *Proc. Natl Acad. Sci. USA* 116:13480.
43 Sasaki, K., Mori, T., Hoshi, N. *et al.* 2021. W27 IgA suppresses growth of *Escherichia* in an *in vitro* model of the human intestinal microbiota. *Sci. Rep.* 11:14627.
44 Breedveld, A. and Egmond, M. 2019. IgA and FcalphaRI: pathological roles and therapeutic opportunities. *Front. Immunol.* 10:553.
45 Moor, K., Diard, M., Sellin, M. E. *et al.* 2017. High-avidity IgA protects the intestine by enchaining growing bacteria. *Nature* 544:498.