Chapter

Engineering Secretory IgA against Infectious Diseases

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Abstract

The dawn of antibody therapy was heralded by the rise of IgG therapeutics. However, other antibody classes are at our disposal—one of the most exciting is IgA and is the most abundant antibody class within humans. Unlike IgG, it is uniquely specialized for mucosal applications due to its ability to form complex Secretory IgA (SIgA) molecules. Since the mucosa is constantly exposed to potential infectious agents, SIgA is pivotal to disease prevention as an important component of the mucosal barrier. Compared to IgG, SIgA has proven superior effectiveness in mucosal surfaces, such as the airway epithelium or the harsh gut environment. Despite this, hurdles associated with low yield and challenging purification have blocked SIgA therapeutic advancement. However, as a result of new antibody engineering strategies, we are approaching the next generation of (IgA-based) antibody therapies. Strategies include fine-tuning SIgA assembly, exploring different production platforms, genetic engineering to improve purification, and glycoengineering of different components. Due to its stability in mucosal environments, SIgA therapeutics would revolutionize passive mucosal immunotherapy—an avenue still underexploited by current therapeutics. This chapter will focus on the current perspectives of SIgA engineering and explore different approaches to unlocking the full therapeutic potential of SIgAs.

Keywords: antibody engineering, infectious diseases, mucosal immunity, glycoengineering, mucosal antibodies, secretory IgA, IgA

1. Introduction

Out of all the antibody classes, the most abundant isoform in the human body is IgA [1]. It is the predominant antibody found in the mucosa—a vast extracellular environment constantly exposed to antigens from both pathogens and commensal bacteria—where it is secreted in the form of secretory IgA (SIgA). This is a multicomponent molecule comprised of two IgA monomers linked together by a joining chain (J chain) and covalently attached to the secretory component (SC) [2]. The secretory component wraps around the antibody complex and confers resistance to proteolytic degradation, along with protection in low pH environments. The ability to form this stable structure is exclusive to mucosal antibodies IgA and IgM and presents a unique advantage to traditional IgG therapy which currently dominates the immunotherapy market [3]. Despite this, there are currently no licenced SIgA products available for use.

Being adapted to mucosal secretions means SIgA has an intrinsic advantage as an oral therapeutic targeting mucosal pathogens compared to other antibody classes. In airway infections, SIgA has been shown to neutralise influenza virus and prevent virus-induced pathology in the upper respiratory tract, at times better than IgG [4, 5]. This is likely to be due to its ability to bind antigens with high avidity on the mucosal surface and prevent adherence to the epithelium, a method called immune exclusion [3]. SIgA is anchored to the mucosal surface by interacting with mucins (glycoproteins which make up the mucus layer) via the antibody's secretory component, ensuring a layer of protection against potential pathogens [6]. More recently, the respiratory application of SIgA therapy was highlighted against COVID-19, with a monoclonal SIgA, but not IgG, potently neutralising SARS-CoV-2 virus [7]. This was attributed to the increased avidity of SIgA arising from its polymeric and flexible Fab regions.

SIgA is also a key immune component of gut mucosa, where it is secreted as the first line of defence [8]. This is an exceedingly complex environment where part of the interplay between host and gut microbiota can be disrupted by intestinal pathogens to establish infection (such as *Escherichia coli*, *Campylobacter jejuni* and *Clostridium difficile*) [9]. There is a global need to engineer therapeutics for diarrhoeal diseases which are a leading cause of death in developing countries, the majority being of young children [10]. In the search for therapeutics against such infections, SIgA has emerged as an attractive candidate. Administered via the oral route, SIgA against enterotoxigenic *E. coli* (ETEC) reduced instances of diarrheal disease by hindering bacterial adhesion to the gut lumen in a nonhuman primate model [11]. When compared to an IgG counterpart, SIgA neutralised a *C. difficile* toxin up to 100 times more effectively, although this was not replicated by all SIgA subtypes [12]. SIgA has also been demonstrated to be more stable in a low pH simulated intestinal fluid environment than the IgG1 variant [13]. The protective barrier arising from the interaction between SIgA and the mucus is effective at preventing bacteria from anchoring and colonising the gut lumen—the neutralised bacteria are then unable to cause disease.

With functional efficacy and applications against a wide range of mucosal pathogens, it is understandable why SIgA emerges as an attractive candidate in the growing field of antibody therapy. However, there are a host of factors which must be addressed before this antibody type can fulfil its therapeutic potential. Issues relating to SIgA production, purification and glycosylation must be addressed—the good news is there are already extensive efforts to do so.

2. Structure and assembly

SIgA is a complex molecule which relies on all 4 components assembling to form a functional antibody. This can be an issue when producing SIgA *in vitro* as partially assembled forms will also be produced (such as monomers and dimers). Furthermore, there are 2 subtypes of IgA which mainly differ in their hinge region: IgA1 with a long O-glycosylated hinge and IgA2 with a short non-glycosylated hinge [14]. Hinge length is associated with differences in flexibility and the ability to reach more distant epitopes at the expense of increased protease-mediated breakdown susceptibility [15]. As a result, the IgA subtype used for a specific therapeutic may be dependent on its application—for example, a potential SIgA therapeutic administered to the bacteria-rich gut mucosa might benefit from being IgA2 in order to evade hinge

degradation by bacterial proteases. On the other hand, IgA1 may offer high stability due to the presence of strong covalent bonds between its heavy and light chains, otherwise absent in IgA2.

Engineering the IgA heavy chain in order to reduce sensitivity to bacterial proteases has highlighted which antibody domains are necessary for protease activity [13]. For example, three amino acids in the CH3 domain were found to confer susceptibility to breakdown by a *Neiseria meningitidis* protease. Efforts to engineer a IgA1/IgA2 hybrid with half of IgA1's long hinge has elucidated the specific proteases which bind to each half of the long hinge [16]. For example, proteases produced by Neisseria *gonorrhoeae* and *Neisseria meningitidis* are able to cleave the hybrid hinge, whereas the *Haemophilus influenzae Type 1* protease did not.

Protein structure can be stabilised by covalent bonds or non-covalent forces. A strategy in antibody engineering is introducing covalent bonds to antibody domains in the form of disulphide bridges. These make the antibody complex more stable and less prone to breakdown. For example, a single amino acid mutation (P221R) will sterically allow new covalent bonding in IgA2 between the heavy and light chain interaction—this leads to a more robust antibody and less free light chain [17].

The incorporation of J chain has been identified as a bottleneck in SIgA assembly [18]. This small 15 kDa polypeptide covalently attaches to the Fc region of opposite IgA monomers via two key cysteines to make dimeric IgA. However, excess J chain can lead to high molecular weight aggregation due to the protein's two free thiol groups [19]. Fine-tuning the expression of J chain relative to the other SIgA components, for example by putting it under the control of a stronger promoter, can help optimise dimeric and secretory forms of the antibody [20].

The final step to make a SIgA molecule is attachment of the secretory component. *In vivo* this happens as dimeric IgA is trancytosed from the lamina propria into mucosal secretions after binding to the polymeric immunoglobulin receptor [21]; *in cellulo* strategies rely on the SIgA complex assembling through co-expression of multiple genes or *in vitro* by incubating free secretory component with purified dimeric IgA [22].

There has been interest in engineering IgA fused with other immunoglobulin forms, particularly with camelid VHH nanobodies, replacing the variable light and heavy chains. These nanobodies are small (~15 kDa compared to the ~55 kDa Fab domain) and eliminate the need for a light chain. This enables the targeting of epitopes in deeper antigenic clefts and also simplifies production [23]. VHH-IgA fusions demonstrated increased functionality whilst retaining the ability to dimerise via the J chain and bind to secretory component. For example, secretory VHH-IgA fusions were protective against ETEC infection unlike related VHH-IgG fusions [24]. Another engineered fusion antibody which has been explored is an IgG backbone with IgA Fc sequences inserted—this demonstrated binding to both the IgA receptor (FcαRI) and IgG receptors (FcγRI/FcγRIIa/ FcγRIIb), possessing both classes of effector function [25].

With the recent increased application of advanced data science and machine learning in biotechnology, the coming years will potentially be very exciting for antibody engineering. Increased access to technology such as AlphaFold by Google, an open-source software to predict the 3D structure of protein sequences, will facilitate the bridging between *in vitro* and digital modelling [26]. Future approaches to improve antibody stability may employ computational models to identify antibody conformations with increased stability [27]. Indeed, proof-of-concept studies that apply different types of modelling software to antibody design are already underway,

either by designing new proteins using an existing sequence dataset, or improving a 3D model and predicting the sequence which would give rise to it [28]. Such strategies have the potential to take native antibody sequences and generate novel sequences with better antigen binding affinity, for example [29]. It is important to note that this strategy requires a robust data set to base new designs on, and that improved antibody characteristics are based on simulation data which may not completely translate *in vitro/in vivo.* However, this is a relatively new technology. More experimental real-world data will both help guide and validate algorithms—unfortunately this information is sparse for IgA compared to IgG. Nevertheless, *in silico* antibody engineering has the power to drastically reduce the hours spent in laborious real-world antibody screening [30]. It is only a matter of when, not if, it will be applied to mainstream SIgA production.

3. Production: mammalian cells, plants and beyond

Production platforms for monoclonal antibodies determine the cost effectiveness and hence their viability as a therapeutic product. Due to the need to transcribe and assemble 4 components (IgA heavy & light chain, J chain and secretory component), SIgA production is a complex multi-step process which has been attempted in different protein production systems.

Mammalian cells, specifically CHO (Chinese hamster ovary cells), are the industry standard in therapeutic IgG monoclonal antibody production. SIgA manufacture has been achieved either through multiple gene transfection or in vitro reconstitution (ie. dimeric IgA incubated with secretory component) [22]. However, the technology is associated with very high production costs which is reflected in the expensive price of monoclonal antibody therapy, even for IgGs [31]. This problem is exacerbated for SIgA as the yields in mammalian cells are still low.

Plants have emerged as an attractive alternative platform for SIgA production. Plant-derived therapeutics are coming of age—previously against Ebola, Gaucher's disease and more recently the plant-based SARS-CoV-2 vaccine Covifenz® has been approved for use by Health Canada [32–35]. Plants are well suited to produce SIgA by expressing the four components either transiently or by the sequential crossing of plant lines stably expressing each component [36]. A plant-specific issue, however, is the apparent cleavage of the IgA Fc tailpiece required for J chain incorporation—which may be due to differential glycosylation in plants [37]. Efforts to circumvent this include the co-expression of the N-glycosylation facilitating enzyme oligosaccharyltransferase from *Leishmania major* [38].

Furthermore, plant-associated glycans (non-human modifications) on recombinant antibodies present a challenge to therapeutic advancement. For example, plants lack branched and sialylated N-glycosylation, and produce plant-specific xylose and fucose residues [39]. Plant O-glycosylation also differs from humans with the presence of complex arabinogalactans on hydroxyproline residues (extensively found on the IgA1 long hinge [40]) and galactosylated serine which does not appear in humans [41]. This is being addressed by using glycoengineered plant lines—these can produce antibodies which function as well as a their CHO-produced counterpart, but with more controlled and homogenous glycan profiles [42, 43].

Overall, SIgA production in different production platforms carry specific advantages and disadvantages—the overarching issue being the production of multiple assembly intermediates (single chains, monomers and dimers), and it is troublesome

to isolate the desired fully assembled SIgA complex [16, 44]. In order to increase the therapeutic potential of SIgA, it is necessary to increase yield (expression levels) and optimise downstream processing.

4. Purification

Downstream purification is the major determinant of cost of goods and monoclonal antibody therapies are currently regarded as some of the most expensive drugs in the world [45]. For SIgA to enter the immunotherapy market, the cost of downstream processing must be comparable to that of IgG. However, there are hurdles associated with SIgA purification which must be addressed beforehand.

Firstly, there is no established gold standard purification resin for SIgA or IgA antibodies. Protein A (the well-established resin used for industry scale IgG purification) cannot be used for SIgA due to the lack of suitable binding sites on the antibody complex. Alternative purification resins that can be used for circumventing this are jacalin [46], SSL7 [47] or generic anti-kappa affinity resins such as protein L [48]; each method is associated with different limitations.

Jacalin is an O-linked glycan-specific lectin derived from jackfruit which is used to purify monoclonal IgA1, and can demonstrate purification efficiency similar to IgG and protein A [49]. Specifically binding to α-D-galactose on the antibody surface, jacalin will not bind to IgA2 or IgA1 with modified glycosylation (e.g. without the O-glycosylated hinge), which may be a benefit or a hindrance depending on the desired product [49]. Inconveniently, Jacalin will also bind to host cell proteins exhibiting O-linked glycosylation, which presents a problem if the target antibody is not present at high concentrations [46].

Protein L is a bacterial cell wall molecule with high binding affinity to certain kappa light chain sequences [48]. This can purify any IgA subtype provided the target sequences are present in the light chain. Unfortunately, protein L will also bind SIgA assembly intermediates and fragments, single light chains, along with monomers and dimeric IgA. This complicates downstream processing since the eluted sample must be further processed (by size exclusion chromatography, for example) to isolate fully formed SIgA. Other purification options, such as CaptureSelect® using llama antibody Fab fragments against single alpha or kappa chain, also suffer from this limitation [50].

Antibody engineering efforts directed at facilitating IgA purification have highlighted the sequences required for affinity purification binding. For example, engineering SIgA light chains to gain protein L binding ability has been described [48]. In addition, murine IgA was made purifiable by SSL7 (another IgA binding protein derived from bacteria) using a 2-amino acid mutation in the Fc region [51], showing that engineering novel purification methods of IgAs are feasible and potentially able to simplify downstream processing.

5. Glycoengineering: finding the sweet spot with complex sugars

Glycosylation is a post-translational protein modification which involves the incorporation of complex sugar molecules (N-linked or O-linked glycans) to specific amino acid residues. It is a common but complex process due to the potential heterogeneity of glycan composition and site occupancy [52]. Antibody glycosylation is

dependent on the expression system, as post-translation modifications differ between cell types and production platforms [41]. Glycoengineering approaches in plants for immune modulation usually focuses on changes in galactosylation, fucosylation and sialylation [53]. The glycans associated with an antibody can significantly impact functional characteristics and is a parameter which must be closely monitored in potential therapeutic antibodies [54].

The singular N-linked Fc glycosylation site in IgG has been extensively studied and engineered for a tailored immune response. For example, removing the core fucose increases antibody-dependent cell-mediated cytotoxicity (ADCC) by facilitating the antibody's binding to FcγRIIIA [55]. Conversely, the inflammatory response mediated by IgG effector functions can be reduced by using sialylated glycans, as this impairs complement-dependent cytotoxicity [55]. It is clear even a single glycan site is influential to IgG function. In comparison, a fully assembled SIgA complex can harbour as many as 26 sites.

Every component of SIgA has potential N-linked glycosylation sites: 4 to 9 per IgA monomer, 1 on the joining chain and 7 on the secretory component. In addition, the IgA1 subtype has an extended hinge region with up to 6 clustered O-glycan sites which make investigations into glycosylation status and the resulting effect on function difficult [56]. Despite this, it is clear that glycosylation is important for effective SIgA function. For instance, SIgA with unglycosylated secretory component is subject to rapid breakdown in the gut [57]. Indeed, the glycan repertoire of IgA is markedly different to that of the well-established IgG and requires extensive further study for levels of familiarity to be comparable [58].

6. Applications: engineering SIgA for passive immunisation?

The route of administration of monoclonal antibody therapy is a determining factor in its effectiveness. Vaccination via the systemic route, whilst effective in generating serum responses, often does not mount a sufficient mucosal response [59]. As a result, most systemic vaccines will not protect against initial infection, since host-pathogen interactions mainly occur directly via the mucosa. This highlights a need for mucosal immunisation and by extension passive immunisation with antibodies.

Passive immunisation would involve the topical administration of pathogen-specific SIgA directly to the oral, nasal, respiratory or gastro-intestinal mucosal. This would immediately form an enhanced protective barrier. For example, there has been significant efforts in the search for artificial colostrum—an oral formulation containing mucosal antibodies against disease in neonates, emulating the protective qualities of breastmilk [60].

In animals, orally-administered VHH-SIgA fusions have provided protection against *E.coli* infection superior to their IgG counterparts [61]. This proof-of-concept study shows the exciting applications of passive immunisation and highlights the importance of IgA-based therapy, although it is yet to be replicated in humans. Furthermore, SIgA's heterologous glycan profile is less of a complication if taken via the oral route due to the body's natural oral tolerance to exogenous antigens [62]. However, the pharmacokinetics and stability of SIgA in the human gastric environment may need further consideration and optimization before implementation [63]. Although our understanding of IgA-mucin interactions is still limited, it is a promising field of study nonetheless.

7. Summary

The mucosal system is the body's first line of defence against infection and SIgA is the primary immunological weapon. Although there have been significant advancements in the understanding of this antibody complex, SIgA has been overshadowed by the therapeutic success of IgG antibodies. Renewed interest in mucosal vaccines and oral immunotherapy has highlighted that existing therapeutics may not be best suited for mucosal therapy, ushering a potential new era of SIgA-based therapy. This chapter has highlighted that:

- Fine-tuning the correct balance of antibody effector function is critical for therapeutic viability. Further engineering of SIgA's structure, glycans and production will continue to push it closer to joining IgG in the mainstream immunotherapy market.

- Novel engineering of IgA-based therapeutics are attractive due to new intellectual property opportunities in the rapidly growing and lucrative field of immunotherapy.

- As machine learning applications to antibody engineering become more accessible and continues to inform the design on future antibodies, SIgA will surely benefit. However, more real-world experimental data may be required for accurate computational design of SIgA in particular.

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References

[1] Russell MW. Biological functions of IgA. In: Mucosal Immune Defense: Immunoglobulin A. Boston, MA: Springer; 2007. pp. 144-172

[2] Wang Y, Wang G, Li Y, Zhu Q, Shen H, Gao N, et al. Structural insights into secretory immunoglobulin A and its interaction with a pneumococcal adhesin. Cell Research. 2020;**30**(7):602-609

[3] de Sousa-Pereira P, Woof JM. IgA: Structure, function, and developability. Antibodies. 2019;**8**(4):57

[4] Renegar KB, Small PA, Boykins LG, Wright PF. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. The Journal of Immunology. 2004;**173**(3):1978-1986

[5] de Fays C, Carlier FM, Gohy S, Pilette C. Secretory immunoglobulin A immunity in chronic obstructive respiratory diseases. Cell. 2022;**11**(8):1324

[6] Rogier EW, Frantz AL, Bruno ME, Kaetzel CS. Secretory IgA is concentrated in the outer layer of colonic mucus along with gut bacteria. Pathogens. 2014;**3**(2):390-403

[7] Ejemel M, Li Q, Hou S, Schiller ZA, Tree JA, Wallace A, et al. A cross-reactive human IgA monoclonal antibody blocks SARS-CoV-2 spike-ACE2 interaction. Nature Communications. 2020;**11**(1):1-9

[8] Corthesy B. Role of secretory IgA in infection and maintenance of homeostasis. Autoimmunity Reviews. 2013;**12**(6):661-665

[9] Vecchio AL, Conelli ML, Guarino A. Infections and chronic diarrhea in children. The Pediatric Infectious Disease Journal. 2021;**40**(7): e255-e258

[10] Farthing M, Salam MA, Lindberg G, Dite P, Khalif I, Salazar-Lindo E, et al. Acute diarrhea in adults and children: A global perspective. Journal of Clinical Gastroenterology. 2013;**47**(1):12-20

[11] Stoppato M, Gaspar C, Regeimbal J, Nunez RG, Giuntini S, Schiller ZA, et al. Oral administration of an anti-CfaE secretory IgA antibody protects against Enterotoxigenic Escherichia coli diarrheal disease in a nonhuman primate model. Vaccine. 2020;**38**(10):2333-2339

[12] Bhaskara V, Leal MT, Seigner J, Friedrich T, Kreidl E, Gadermaier E, et al. Efficient production of recombinant secretory IgA against Clostridium difficile toxins in CHO-K1 cells. Journal of Biotechnology. 2021;**331**:1-3

[13] Wallace AL, Schneider MI, Toomey JR, Schneider RM, Klempner MS, Wang Y, et al. IgA as a potential candidate for enteric monoclonal antibody therapeutics with improved gastrointestinal stability. Vaccine. 2020;**38**(47):7490-7497

[14] Su CT, Lua WH, Ling WL, Gan SK. Allosteric effects between the antibody constant and variable regions: A study of IgA Fc mutations on antigen binding. Antibodies. 2018;**7**(2):20

[15] Senior BW, Woof JM. Sites in the CH3 domain of human IgA1 that influence sensitivity to bacterial IgA1 proteases. The Journal of Immunology. 2006;**177**(6):3913-3919

[16] Senior BW, Dunlop JI, Batten MR, Kilian M, Woof JM. Cleavage of a

recombinant human immunoglobulin A2 (IgA2)-IgA1 hybrid antibody by certain bacterial IgA1 proteases. Infection and Immunity. 2000;**68**(2):463-469

[17] Lohse S, Brunke C, Derer S, Peipp M, Boross P, Kellner C, et al. Characterization of a mutated IgA2 antibody of the m (1) allotype against the epidermal growth factor receptor for the recruitment of monocytes and macrophages. Journal of Biological Chemistry. 2012;**287**(30):25139-25150

[18] Westerhof LB, Wilbers RH, van Raaij DR, van Wijk CZ, Goverse A, Bakker J, et al. Transient expression of secretory IgA in planta is optimal using a multi-gene vector and may be further enhanced by improving joining chain incorporation. Frontiers in Plant Science. 2016;**6**:1200

[19] Redwan ER, Matar SM, Serour IA. Recombinant human J-chain: Fix the protein aggregations and yield maximize. Human Antibodies. 2006;**15**(3):95-102

[20] Juarez P, Huet-Trujillo E, Sarrion-Perdigones A, Falconi EE, Granell A, Orzaez D. Combinatorial analysis of secretory immunoglobulin A (sIgA) expression in plants. International Journal of Molecular Sciences. 2013;**14**(3):6205-6222

[21] Wei H, Wang JY. Role of polymeric immunoglobulin receptor in IgA and IgM transcytosis. International Journal of Molecular Sciences. 2021;**22**(5):2284

[22] Virdi V, Juarez P, Boudolf V, Depicker A. Recombinant IgA production for mucosal passive immunization, advancing beyond the hurdles. Cellular and Molecular Life Sciences. 2016;**73**(3):535-545

[23] Hoey RJ, Eom H, Horn JR. Structure and development of single domain antibodies as modules for therapeutics and diagnostics. Experimental Biology and Medicine. 2019;**244**(17):1568-1576

[24] De Greve H, Virdi V, Bakshi S, Depicker A. Simplified monomeric VHH-Fc antibodies provide new opportunities for passive immunization. Current Opinion in Biotechnology. 2020;**61**:96-101

[25] Kelton W, Mehta N, Charab W, Lee J, Lee CH, Kojima T, et al. IgGA: A "cross-isotype" engineered human Fc antibody domain that displays both IgG-like and IgA-like effector functions. Chemistry & Biology. 2014;**21**(12):1603-1609

[26] Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;**596**(7873):583-589

[27] Warszawski S, Borenstein Katz A, Lipsh R, Khmelnitsky L, Ben Nissan G, Javitt G, et al. Optimizing antibody affinity and stability by the automated design of the variable light-heavy chain interfaces. PLoS Computational Biology. 2019;**15**(8):e1007207

[28] Gao W, Mahajan SP, Sulam J, Gray JJ. Deep learning in protein structural modeling and design. Patterns. 2020;**1**(9):100142

[29] Akbar R, Robert PA, Weber CR, Widrich M, Frank R, Pavlović M, et al. In silico proof of principle of machine learning-based antibody design at unconstrained scale. MAbs. 2022;**14**(1):2031482

[30] Hanning KR, Minot M, Warrender AK, Kelton W, Reddy ST. Deep mutational scanning for therapeutic antibody engineering. Trends in Pharmacological Sciences. 2022;**43**(2):123-135

[31] Hernandez I, Bott SW, Patel AS, Wolf CG, Hospodar AR, Sampathkumar S, et al. Pricing of monoclonal antibody therapies: Higher if used for cancer. The American Journal of Managed Care. 2018;**24**(2):109-112

[32] Group TP, Multi-National PREVAIL II Study Team. A randomized, controlled trial of ZMapp for Ebola virus infection. The New England Journal of Medicine. 2016;**375**(15):1448

[33] Fox JL. First plant-made biologic approved. Nature Biotechnology. 2012;**30**(6):472-473

[34] Ibtisam T. Effectiveness of currently authorized vaccines against SARS-CoV-2 variants of concern and the future of COVID-19 vaccines. Undergraduate Journal of Experimental Microbiology and Immunology. 2022;**6**:1

[35] Ward BJ, Gobeil P, Séguin A, Atkins J, Boulay I, Charbonneau PY, et al. Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19. Nature Medicine. 2021;**27**(6):1071-1078

[36] Paul M, Reljic R, Klein K, Drake PM, Van Dolleweerd C, Pabst M, et al. Characterization of a plant-produced recombinant human secretory IgA with broad neutralizing activity against HIV. MAbs. 2014;**6**(6):1585-1597

[37] Dicker M, Tschofen M, Maresch D, König J, Juarez P, Orzaez D, et al. Transient glyco-engineering to produce recombinant IgA1 with defined N-and O-glycans in plants. Frontiers in Plant Science. 2016;**7**:18

[38] Göritzer K, Goet I, Duric S, Maresch D, Altmann F, Obinger C, et al. Efficient N-glycosylation of the heavy chain tailpiece promotes the formation of plant-produced dimeric IgA. Frontiers in Chemistry. 2020;**8**:346

[39] Mercx S, Smargiasso N, Chaumont F, De Pauw E, Boutry M, Navarre C. Inactivation of the β (1, 2)-xylosyltransferase and the α (1, 3)-fucosyltransferase genes in Nicotiana tabacum BY-2 cells by a multiplex CRISPR/Cas9 strategy results in glycoproteins without plant-specific glycans. Frontiers in Plant Science. 2017;**8**:403

[40] Karnoup AS, Turkelson V, Anderson WK. O-linked glycosylation in maize-expressed human IgA1. Glycobiology. 2005;**15**(10):965-981

[41] Strasser R. Plant protein glycosylation. Glycobiology. 2016;**26**(9):926-939

[42] Montero-Morales L, Steinkellner H. Advanced plant-based glycan engineering. Frontiers in Bioengineering and Biotechnology. 2018;**6**:81

[43] Teh AY, Cavacini L, Hu Y, Kumru OS, Xiong J, Bolick DT, et al. Investigation of a monoclonal antibody against enterotoxigenic Escherichia coli, expressed as secretory IgA1 and IgA2 in plants. Gut Microbes. 2021;**13**(1):1859813

[44] Chintalacharuvu KR, Gurbaxani B, Morrison SL. Incomplete assembly of IgA2m (2) in Chinese hamster ovary cells. Molecular Immunology. 2007;**44**(13):3445-3452

[45] Samaranayake H, Wirth T, Schenkwein D, Räty JK, Ylä-Herttuala S. Challenges in monoclonal antibody-based therapies. Annals of Medicine. 2009;**41**(5):322-331

[46] Puligedda RD, Vigdorovich V, Kouiavskaia D, Kattala CD, Zhao JY, Al-Saleem FH, et al. Human IgA monoclonal antibodies that neutralize poliovirus, produced by Hybridomas and

recombinant expression. Antibodies. 2020;**9**(1):5

[47] Langley R, Wines B, Willoughby N, Basu I, Proft T, Fraser JD. The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-FcαRI binding and serum killing of bacteria. The Journal of Immunology. 2005;**174**(5):2926-2933

[48] Åkerström B, Björck L. Protein L: An immunoglobulin light chain-binding bacterial protein: Characterization of binding and physicochemical properties. Journal of Biological Chemistry. 1989;**264**(33):19740-19746

[49] Roque-Barreira MC, Campos-Neto AN. Jacalin: An IgA-binding lectin. The Journal of Immunology. 1985;**134**(3):1740-1743

[50] Hermans P, Adams H, Detmers F. Purification of antibodies and antibody fragments using CaptureSelect™ affinity resins. In: Monoclonal Antibodies. Totowa, NJ: Humana Press; 2014. pp. 297-314

[51] Bakshi S, Depicker A, Schepens B, Saelens X, Juarez P. A two-amino acid mutation in murine IgA enables downstream processing and purification on staphylococcal superantigen-like protein 7. Journal of Biotechnology. 2019;**294**:26-29

[52] Ferrara C, Grau S, Jäger C, Sondermann P, Brünker P, Waldhauer I, et al. Unique carbohydrate–carbohydrate interactions are required for high affinity binding between FcγRIII and antibodies lacking core fucose. Proceedings of the National Academy of Sciences. 2011;**108**(31):12669-12674

[53] Göritzer K, Strasser R. Glycosylation of plant-produced immunoglobulins. In: Antibody Glycosylation. Cham: Springer; 2021. pp. 519-543

[54] Stelter S, Paul MJ, Teh AY, Grandits M, Altmann F, Vanier J, et al. Engineering the interactions between a plant-produced HIV antibody and human Fc receptors. Plant Biotechnology Journal. 2020;**18**(2):402-414

[55] Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, et al. Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity. The Journal of Clinical Investigation. 2015;**125**(11):4160-4170

[56] de Haan N, Falck D, Wuhrer M. Monitoring of immunoglobulin N-and O-glycosylation in health and disease. Glycobiology. 2020;**30**(4):226-240

[57] Pabst O, Slack E. IgA and the intestinal microbiota: The importance of being specific. Mucosal Immunology. 2020;**13**(1):12-21

[58] Chandler KB, Mehta N, Leon DR, Suscovich TJ, Alter G, Costello CE. Multi-isotype Glycoproteomic characterization of serum antibody heavy chains reveals isotype-and subclass-specific N-glycosylation profiles*[S]. Molecular & Cellular Proteomics. 2019;**18**(4):686-703

[59] Kurono Y. The mucosal immune system of the upper respiratory tract and recent progress in mucosal vaccines. Auris, Nasus, Larynx. 2022;**49**(1):1-0

[60] Richards A, Baranova D, Mantis NJ. The prospect of orally administered monoclonal secretory IgA (SIgA) antibodies to prevent enteric bacterial infections. Human Vaccines & Immunotherapeutics. 2021;**18**:1-8

[61] Virdi V, Coddens A, De Buck S, Millet S, Goddeeris BM, Cox E, et al. Orally fed seeds producing designer IgAs protect weaned piglets against enterotoxigenic Escherichia coli infection. Proceedings of the

National Academy of Sciences. 2013;**110**(29):11809-11814

[62] Juarez P, Virdi V, Depicker A, Orzaez D. Biomanufacturing of protective antibodies and other therapeutics in edible plant tissues for oral applications. Plant Biotechnology Journal. 2016;**14**(9):1791-1799

[63] Hu Y, Kumru OS, Xiong J, Antunez LR, Hickey J, Wang Y, et al. Preformulation characterization and stability assessments of secretory IgA monoclonal antibodies as potential candidates for passive immunization by oral administration. Journal of Pharmaceutical Sciences. 2020;**109**(1):407-421