Secretory IgA Antibodies from Plants

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Abstract: Secretory IgA (SIgA) is the antibody type produced in both mammals and birds that protects the body from infection at mucosal surfaces. While monoclonal IgG antibodies, particularly those against tumor antigens, have received a great deal of attention, both scientific and commercial, as immunotherapeutic agents, the potential of SIgA antibodies has only recently begun to be exploited. Part of the reason for this is that SIgA production *in vivo* normally requires the cooperation of two different cell types, and single animal cell systems for monoclonal SIgA production are inefficient. Transgenic plants are currently the most productive and economical system for making SIgA. The only monoclonal SIgA to be tested therapeutically in a human clinical trial is a product called CaroRx, made in transgenic tobacco, which is designed to block adherence to teeth of the bacteria that causes cavities. This antibody accumulates to high levels in the leaves of tobacco, where it is located primarily in the endoplasmic reticulum. The antibody can be efficiently purified using the affinity reagent protein G. Topical oral treatment in human subjects was safe and effective. Characterization of the expression, secretion, purification and therapeutic use of this antibody serves as a model for additional plant-made therapeutic SIgA antibodies under development.

INTRODUCTION

Recombinant monoclonal antibodies were first made in transgenic plants more than 15 years ago [1, 2]. Since then, there have been dozens of published reports describing the expression of antibodies in plants and discussing their potential and actual application as therapeutic and prophylactic agents for the treatment or prevention of infectious disease. There have been several recent reviews that have comprehensively covered the applications and advantages of antibody production in plants [3-7]. The purpose of this review is to focus on what is known about the expression of IgA and Secretory IgA (SIgA) antibodies in plants and to shed light on the unique challenges and potential applications of this antibody type.

ASSEMBLY AND EXPRESSION OF SIGA IN ANIMALS

Most disease-causing organisms first enter the body through mucosal surfaces, which is not surprising since the surface area of human mucosae (including the gastrointestinal, genito-urinary, and respiratory tracts) exceeds that of skin by a factor of 200 [8]. Most of the antibody that is synthesized daily is secreted by epithelial cells at mucosal surfaces, where it forms a first line of defense against infection. The vast majority of all antibody-secreting plasma cells are located in the intestinal *lamina propria*, a layer of cells underlying the epithelial cell layer. These plasma cells produce more IgA than all other immunoglobulin isotypes combined (in humans, 40-60 mg/kg/day) [9, 10]. The IgA is secreted as polymeric IgA (pIgA), which is comprised mostly of IgA dimers (4 heavy chains and 4 light chains), linked together by a protein called the joining, or J chain. At the basolateral surface of epithelial cells (EC) pIgA becomes bound to a transmembrane epithelial glycoprotein called the polymeric immunoglobulin receptor (pIgR) [11]. This complex is transcytosed to the apical surface where the ligand-binding domains of the pIgR are cleaved off to become the secretory component (SC), bound *via* a disulfide bond to pIgA, thus forming secretory IgA (SIgA). Because of the high carbohydrate content of SC, SIgA becomes anchored in mucus, contributing to the exclusion of pathogens from mucosal surfaces [12]. Complexes of SIgA and antigen are removed from the body by physical mechanisms, such as peristalsis and mucociliary transport in the gastrointestinal and respiratory tracts. Secretory IgA is found in both mammals and birds, suggesting that it evolved before the divergence of birds and reptiles [13].

Immunization at mucosal surfaces, and the development of a specific SIgA response, is the basis of the success of oral vaccines against polio, rubella virus, adenoviruses, influenza A, rotavirus, salmonella, and cholera [14]. In addition to active immunization, passive mucosal immunization, involving the oral, intranasal, or intrauterine administration of antibody has been shown to prevent or cure bacterial and viral infections. The use of IgA or SIgA in passive mucosal immunotherapy has been recently reviewed by Corthesy [15]. Passive transfer of maternal SIgA through milk is the mechanism in mammals that prevents mucosal infection in newborns lacking a fully matured immune system. SIgA has a number of advantages over IgG for passive immunization at mucosal surfaces. First, the presence of four antigen-binding sites confers an avidity effect. Second, SIgA is much more resistant than IgG or monomeric IgA to the proteolytic enzymes found in the gastrointestinal tract [16-18]. Third, SIgA can block the binding of several bacterial pathogens to host epithelial cells non-specifically, because of the presence of carbohydrate on both the IgA heavy chain and SC polypeptides [19-21]. Fourth, complexes of IgA and antigen do not activate, but actually inhibit the complement cascade and the resulting inflammatory response [22].

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For passive immunization at mucosal surfaces to afford protection against infection, it is likely that a few milligrams/kg will be required daily during some susceptible period, whereas clearance of an existing infection might require much higher amounts. Thus, for highly specific immunotherapy based on SIgA to be practical, an efficient and inexpensive production system is needed. Transgenic plants are likely to be the most economical system for the production of many recombinant proteins, including SIgA antibodies [23].

ASSEMBLY AND EXPRESSION OF SIGA IN PLANTS

All of the published work on SIgA in plants is related to immunoglobulins derived from a single monoclonal antibody, called Guy's 13. This murine IgG1 binds to streptococcal antigen I/II (SA I/II), a major cell surface glycoprotein of *Streptococcus mutans*, the principal causal agent of tooth decay. This antibody was shown, in several clinical trials, to prevent colonization by *S. mutans* in the human oral cavity [24-27]. It recognizes a conformational epitope comprised of residues 45 to 457, in the N-terminal half of SA I/II, and residues 816 to 983, in the C-terminal half [28].

The genes for the heavy and light chains of this antibody were cloned from cDNAs and assembled into plant expression vectors [29]. The heavy chain was constructed as a hybrid molecule: it consists of the signal peptide, variable region, Cγ1 and Cγ2 domains of the Guy's 13 heavy chain fused to the Cα2 and Cα3 domains from an IgA secreting hybridoma. The presence of Cα2 and Cα3 domains confer on the antibody the ability to assemble with J chain and secretory component, while retaining the very useful ability of the Cγ2 domain to bind to the affinity chromatography reagent Protein G [30]. The kappa light chain clone was not complete at the 5' end, so the signal peptide of a different murine immunoglobulin was used to ensure that the kappa chain would be co-translationally inserted into the endoplasmic reticulum (ER). Tobacco plants transformed with these two constructs were selected and crossed together (Fig. **1**), producing progeny that expressed an assembled IgA/G antibody (of about 210 kDa) that bound to SA I/II [29]. The expression level of IgA/G in these plants was about 1% of total soluble protein.

Although normal assembly of SIgA in mammals requires the cooperation of two cell types (plasma and epithelial

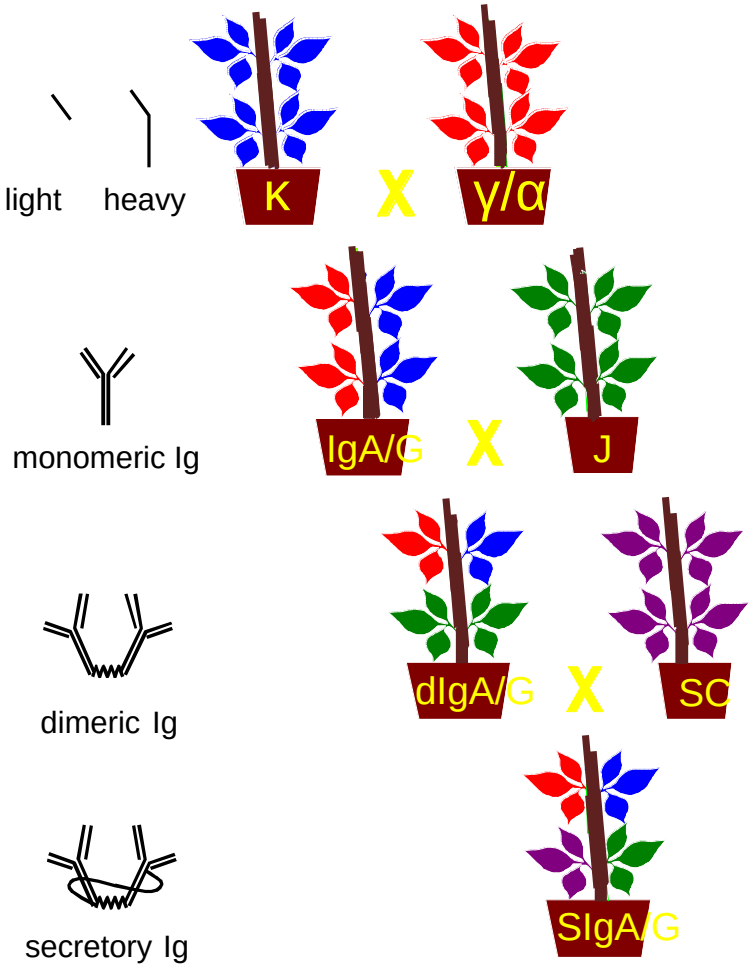

Fig. (1). Schematic showing the sequential crossing of individual plants expressing kappa chain, the chimeric IgA/G heavy chain, J chain and secretory component to produce IgA/G, dimeric IgA/G and secretory IgA/G.

cells), SIgA expression and assembly in a single cell was first achieved in plants by sequentially crossing plants expressing the IgA/G antibody with plants expressing murine J chain and the rabbit secretory component (Fig. **1**) [29, 31]. Just as in mammals, assembly of SC with antibody required the presence of J chain. When plants expressing monomeric IgA/G were crossed to SC-expressing plants, protein immunoblot analysis revealed no change in assembly of the IgA/G. When J chain was included as well, about 50% of the assembled antibody was found in a molecule of ~470 kDa. The remainder was either IgA/G (210 kDa) or dIgA/G (400 kDa) (Fig. **2**). Co-expression of J chain and SC led to a substantial increase in accumulation of antibody, with the total accumulation of all three forms of assembled IgA/G exceeding 5% of total soluble protein.

At Planet Biotechnology we have subsequently transformed plants with all four of these chains simultaneously, and in addition have produced SIgA that is fully human with the exception of murine variable regions (unpublished results). We have also determined that mammalian signal peptides are not required, and plant signal peptides can be used instead. More recently others have produced chimeric [32] or fully human SIgA in mammalian cells [17, 33], but production of SIgA from these cells in culture remains low.

TRANSPORT OF SIGA WITHIN PLANT CELLS

Immunogold localization of SIgA/G heavy chain in transgenic tobacco leaves showed that the antibody primarily accumulated in the highly vacuolated cells of the mesophyll, particularly in bundle sheath cells. At the light microscopic level the antibody appeared to be localized to the periphery of the cells, but did not penetrate the cell wall [31]. In contrast, murine IgG expressed in tobacco plants appears to penetrate the cell wall easily, as it has been found to be secreted from intact roots [34]. Work in this laboratory (unpublished) has confirmed that SIgA/G antibody is not secreted from intact roots.

Subsequent work has compared the fates of SIgA/G and IgG in plant cells, and found some intriguing differences. Protoplasts derived from leaves of plants expressing SIgA/G and IgG were subjected to pulse-chase analysis [35]. Protoplasts, labeled with [^{35}S]Met and [^{35}S]Cys, and the corresponding incubation media were homogenized, immunoprecipitated with anti-IgG antiserum, and analyzed by SDS-PAGE and fluorography. It was determined that secretion of SIgA/G from transgenic tobacco protoplasts was slow, with only about 10% of the antibody found in the surrounding medium after 12 hours. Secretion of IgG was more rapid, with about 70% of the molecules secreted by 16 hours. This difference was independent of the assembly state of the antibody, as secretion of monomeric (IgA/G) and dimeric (dIgA/G) antibody was just as slow as the SIgA/G. After 12 hours of chase, several polypeptides that appeared to be breakdown products of the heavy or light chains were detected in the protoplasts. The appearance of these degradation products was inhibited by brefeldin A, a molecule that

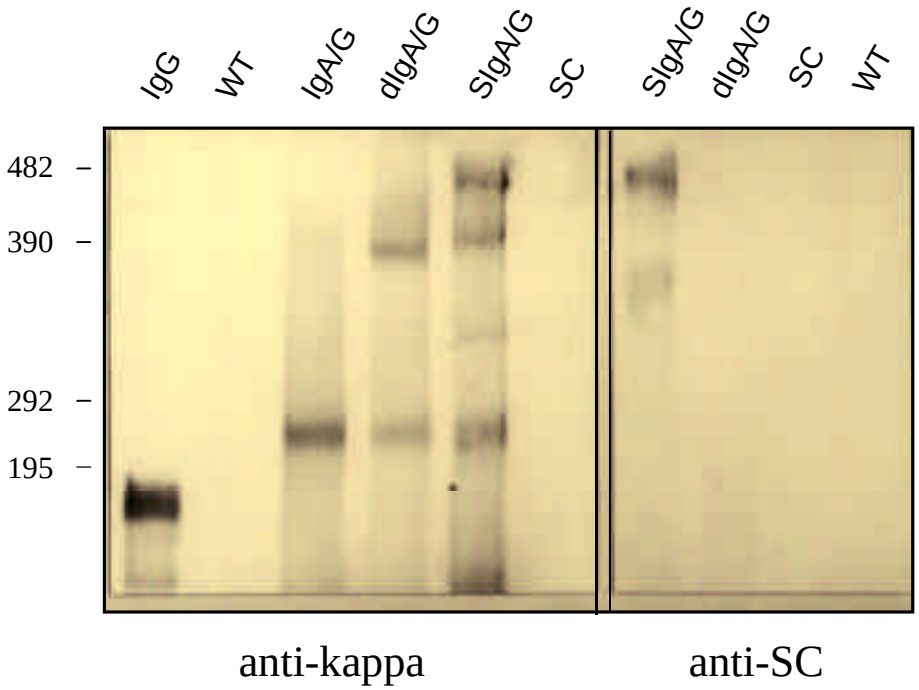

Fig. (2). Protein immunoblot (western) of plant extracts prepared under nonreducing conditions, detected with Antisera to the mouse k light chain (lanes 1 to 6) or to rabbit SC (lanes 7 to 10). Samples were separated on 4% SDS-PAGE. IgG, Guy's 13 mAb prepared from hybridoma cell culture supernatant; WT, nontransformed wild-type plant; IgA-G, transgenic plant expressing chimeric heavy and light chain genes of Guy's 13; dIgA-G, transgenic plant expressing chimeric heavy and light chain genes of Guy's 13 and the J chain; SIgA-G, transgenic plant expressing chimeric heavy and light chain genes of Guy's 13, the J chain, and SC; SC, transgenic plant expressing SC. Molecular masses are indicated in kilodaltons.

inhibits membrane traffic from the ER, which indicated that the degradation occurred in a compartment past the ER in the secretory pathway. These breakdown products were found in purified vacuoles, and immunogold staining of leaf sections identified large vacuolar aggregates that stained with gold-conjugated anti-SC and anti-IgG antibodies.

Plants expressing just the light chain and chimeric heavy chain were compared to plants that additionally expressed J chain or J chain plus SC. In this way it was determined that the re-routing of a percentage of antibody to the vacuole in the SIgA/G plants did not require J chain or SC. The tetrameric IgA/G form was just as inefficiently secreted. Antibodies could be detected within the endomembrane system by immunofluorescence confocal laser scanning microscopy on fixed, permeabilized protoplasts. This method confirmed that IgG was being exported while SIgA/G was being retained: staining near the plasma membrane was more intense in the IgG transgenics than the SIgA/G transgenics.

Protoplasts transiently transfected with IgA/G and kappa showed the same pattern of slow secretion and fragmentation in the vacuole as stably transformed plants [36]. This was not due to general overloading of the secretory pathway caused by the high levels of protein accumulation, since a different secreted protein transiently expressed in the same protoplasts was not affected. In addition, fractionation of leaf extracts from transgenic plants showed that the fragments found in the vacuole were not monomeric, but consisted of multiple peptides that were partially assembled. One of the fragments was shown to have N-glycans of the complex type, indicating that it had passed through the Golgi complex on its way to the vacuole.

The possibility that vacuolar delivery was the result of endocytosis of secreted proteins was ruled out, since increasing the volume of the incubation medium did not decrease the intensity of the fragments [35]. Nor did incubation of non-transgenic protoplasts in medium containing secreted IgA/G result in uptake of labeled intracellular fragments [36].

The investigators hypothesized that the slow secretion and sorting to the vacuole might be related to the IgA tailpiece. The IgA tailpiece is an 18 amino acid sequence (in mice: PTNVSVSVIMSEGDGICY) found at the C-terminal end of IgA heavy chains that does not have a homolog on IgG (although there is a homologous IgM tailpiece). The tailpiece contains the cysteine residue required for J chain binding [37]. To test this hypothesis, they deleted the tailpiece to produce a truncated γ/α heavy chain (denoted ΔC18). Protoplasts were co-transfected with plasmids encoding the κ light chain together with the γ/α, γ, or ΔC18 heavy chain. Deletion of the C-terminal 18 amino acids of the IgA/G heavy chain eliminated vacuolar targeting. The rate of secretion of this construct was comparable to or even higher than that of IgG.

The authors suggest that the 18 amino acid tailpiece of IgA, by chance, contains a sequence recognized by plant cells as a C-terminal vacuolar sorting signal. Although there is no consensus for C-terminal vacuolar sorting signals (Ctvss), the sequence VSVSV in the tailpiece is a repetition of dipeptides (VS or SV) that are present multiple times in a tobacco and a potato Ctvss [38, 39]. The presence of a cryptic vacuolar sorting signal in the IgA tailpiece does not adequately explain the slow trafficking out of the ER. However, retention in the ER, rather than an ability of SC to protect the antibody from proteolysis in the apoplast, may explain why this SIgA accumulates to much higher levels than the parental IgG. It is now well established that antibodies retained in plant ER by the addition of the C-terminal KDEL accumulate to higher levels than antibodies that are secreted into the apoplast [40, 41]. What characteristic of the tailpiece may be responsible for slow secretion and partial transport to the vacuole remains unknown. It is also unknown whether the tailpiece can be modified so that it retains positive characteristics, such as binding to J chain and retention in the ER, but loses negative characteristics such as transport to the vacuole. In experiments done in this laboratory, removal of the tailpiece resulted in significantly lower levels of IgA/G accumulation when transiently expressed in *Nicotiana benthamiana* leaves [42], as did addition of two terminal glycine residues, a modification that abolished vacuolar targeting of the barley lectin in transgenic tobacco [43] (unpublished).

PURIFICATION OF SIGA FROM PLANTS

A primary advantage of using genetically engineered green plants to produce antibodies, compared to conventional animal cell bioreactors, is the vastly lower cost of goods that plants provide. The lack of human pathogenic viruses or bacteria in plants is often cited as an advantage of plants, since expensive validation of virus-removal steps during purification may not be necessary. However, one might anticipate the presence of a diverse bioburden on or in plants grown outdoors in non-sterile conditions. Processes to eliminate or minimize contamination with endotoxins and mycotoxins will be necessary in any commercial process to purify antibodies from plants. Indeed, the cost of purifying antibodies from plant tissue may not be all that different from the cost of purifying antibodies from cell culture supernatant. The processing challenges will be different depending on the plant tissue used, such as leaves vs seeds vs other storage organs like roots.

Phenolics are a major concern when extracting stems and leaves of tobacco, and they are best removed early in the process. They can interact with proteins in ways that can dramatically and irreversibly alter the properties of proteins [44]. Fortunately, most of the phenolics released during extraction are small in size, water soluble, and removable by ultrafiltration/diafiltration steps.

As already mentioned, the inclusion of two domains of IgG1 (Cγ1 and Cγ2), in the Guy's 13 SIgA/G (now known as CaroRx™) allowed it to be recognized by the bacterial binding proteins, protein A and protein G. In practice, however, the affinity of Protein A for murine IgG1 is too low, and protein G must be used for affinity chromatography. Currently protein G chromatography is the step that provides the greatest purification of CaroRx. The added advantage of protein G is that it captures all forms of the antibody that are effective in blocking the binding of mutans streptococci to teeth, including SIgA/G, dIgA/G, IgA/G, and $F(ab')^2$. The final product has very little contamination by Fc

fragments, possibly because they are unstable and rapidly degraded within the plant. Even though it is an expensive purification step, protein G (or A) affords such a large purification factor, that it continues to be used in purification of therapeutic antibodies made in animal cell bioreactors. This may be the same with CaroRx. To date, several other chromatographic media have shown little potential as purification steps for SIgA/G from tobacco leaf extracts.

An affinity chromatography step of some kind will likely remain critical to purifying antibodies from green plant tissue. For antibodies, like human SIgA, that do not bind to Protein A or G, this will likely involve one of several alternative chromatography reagents. Protein L, from the bacterial species *Peptostreptococcus magnus* binds specifically to the variable domain of kappa light chains. On human kappa chains, binding is limited to variable region subgroups V_{kI}, V_{kIII} and V_{kIV} [45, 46]. While these subgroups represent the majority of human kappa chains, many murine kappa variable regions do not bind protein L, nor do any lambda light chains. There are also various streptococcal binding proteins that specifically recognize human IgA Fc regions. These include Protein B and sir22, from *Streptococcus pneumoniae* [47, 48]. A 50 amino acid peptide derived from sir22 was found to bind as well as the whole protein [49]. SpsA, a surface protein from *Streptococcus pneumoniae*, interacts specifically with human SIgA *via* the secretory component (SC) [50, 51]. The binding motif was identified as a hexapeptide (YRNYPT). Other than Protein L, none of these alternative streptococcal binding proteins are available commercially, although identification of defined peptide sequences from some of them raises the possibility of direct synthesis of an affinity reagent. Another alternative for SIgA purification is a peptido-mimetic derived *via* screening of multimeric combinatorial peptide libraries for molecules with affinity for immunoglobulins [52]. This reagent is available under the name KaptivAE.

ROLE OF GLYCOSYLATION

N-linked glycans are sugar structures that are covalently attached to certain asparagine residues of glycoproteins. The N-linked glycans that have been identified on plant-made immunoglobulins reflect general differences in the glycosylation of plant and mammalian glycoproteins. In plants, complex N-glycans are characterized by the presence of a β(1, 2)-Xylose residue linked to the Man-3 and/or an α(1, 3)-Fucose residue linked to GlcNAc-1, linkages not found in mammals. Plant glycans lack the β(1, 4)-Gal and terminal α(2, 6)-sialic acid residues, and the α(1, 6)-Fuc residue linked to the GlcNAc-1 found on human IgG. In addition to the complex glycans, plant-produced IgGs carry a significant percentage of "high mannose" glycoforms, containing 4 to 9 mannose residues (Fig. **3**) [53, 54]. The exact nature of N-linked glycans on plant-made SIgA is yet to be determined, but is likely to be similar to what has been found on IgGs expressed in plants.

There are two reasons that some consideration of N-glycan structure of plant-made SIgA is important. The first relates to the effect of glycans on the function of immunoglobulins. Human SIgA is highly glycosylated, being about 10% carbohydrate by weight. Human IgA1 contains two

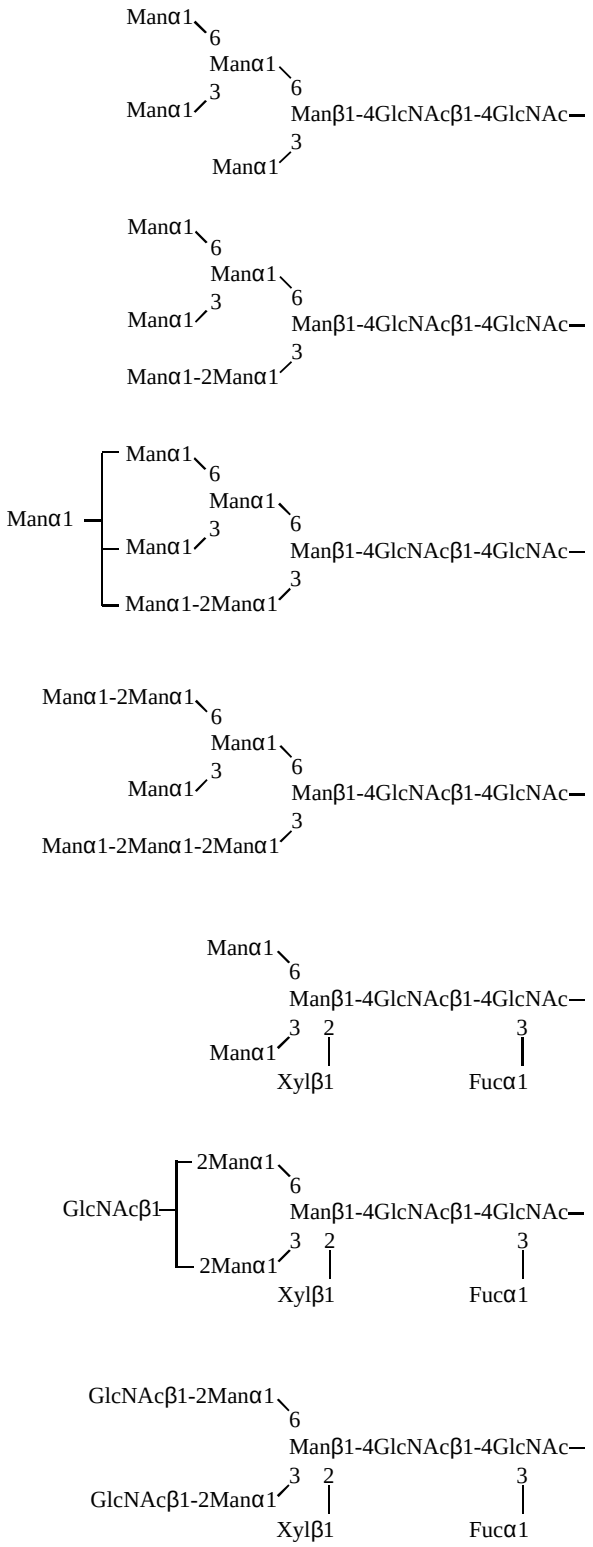

Fig. (3). Structures of the high-mannose (Man-5 to Man-8) and complex (xylose- and fucose-containing) N-glycans isolated from plantibody Guy's 13 [54].

conserved *N*-glycosylation sites in each α-chain (Asn263 and Asn459), while the IgA2 subclass contains an additional

two (IgA2m(1)) or three (IgA2m(2)) conserved *N*-glycans. Secretory component, with five to seven N-glycans, is 22% carbohydrate [55]. A detailed picture of the N-glycans found on normal human SIgA has only recently been determined, revealing that SC carries a wide range of glycan structures [21]. These include galactose linked both β1–4 and β1–3 to GlcNAc; fucose linked β1–3 and β1–4 to GlcNAc and α1–2 to galactose, as well as both α2–3- and α2–6-linked sialic acids. The majority of structures were fully galactosylated, biantennary structures. Over 70% of the glycans were at least mono-sialylated. As well as having a role in protecting both SC and the SIgA from proteases [16], the glycans on SC can specifically interact with bacterial adhesins and lectins. SC has been shown to bind to a range of bacteria *via* its glycans (e.g. *Helicobacter pyroli* [56, 57], *E. coli* [58-60] and *Clostridium difficile* toxin A [61]), thereby inhibiting attachment to and infection of epithelial surfaces. The glycans of SC are also involved in anchoring the SIgA to the mucus lining epithelial surfaces, where it may have a role in facilitating the growth of normal enteric bacteria [62]. In cases where the attachment is through high-mannose glycans, plant-made SIgA should function in the same way, but in cases where galactose and sialic acid residues are involved plant-made SIgA may not perform as well. Interestingly, the majority (66%) of the SIgA H chain N-glycans on both SIgA1 and SIgA2 terminate in GlcNAc residues and 12% are high mannose structures. Only 15% contain a single galactose, and less than 15% contain any sialic acid [21]. Thus, an abundance of high-mannose glycans and lack of sialic acid or galactose on plant-made SIgA seems less likely to cause problems with the function of the heavy chain than with SC.

Human J chain contains a single N-glycan site, and deletion of this site by substitution of Asn with Ala prevents IgA dimer formation, indicating that the N-glycan plays an important role in this process [63, 64] In normal human SIgA, over 75% of the glycans on J chain are sialylated. This led to the speculation that the charge on this glycan is important in maintaining the correct conformation of the J chain for presentation to pIgR [21]. However, since SC assembles with J chain and IgA in plant cells, the charge may not be as important as the mere presence of N-glycan on the J chain.

The second reason for considering N-glycan structure relates to the potential immunogenicity/allergenicity of plant N-glycans [54, 65]. It has been known for years that fucose and xylose are involved in IgE antibody recognition of plant and invertebrate animal N-glycans [66, 67]. BALB/c mice immunized subcutaneously with a recombinant mouse monoclonal antibody produced in tobacco plants, together with alum as an adjuvant, did not develop detectable levels of antibody directed against either the protein or the glycan part of the plantibody [68]. A subsequent study found a small but significant immune response in C57BL/6 mice and a stronger response in rats [69]. That study also found that 50% of serum samples from non-allergic blood donors carry IgG or IgM to core β(1, 2)-xylose, and 25% carry IgG against core α(1, 3)-fucose (low titers of 1:100 to 1:1000). Carbohydrate-reactive IgE is predominantly found in serum only from patients allergic to pollen or insect venom [70]. Despite this, there is no convincing evidence that anti-carbohydrate IgE is clinically relevant to food or insect allergies [70].

Attempts have been made to make plant glycoproteins more "human-like", with some success [71-74]. Tobacco cells transformed with human β1, 4-galactosyl-transferase secreted glycoproteins with galactose extended glycans, and without α(1, 3)-linked fucose, but with β(1, 2)-xylose [74]. Antibodies from transgenic tobacco plants expressing human β1, 4-galactosyltransferase were also galactosylated, but still contained both fucose and xylose linkages [73]. The moss *Physcomitrella patens* is the only land plant known that efficiently undergoes homologous recombination in its nuclear DNA [75]. Using the appropriate gene sequences, it may be possible in this plant to not only add β1, 4-galactosyl-transferase, but to "knock out" xylosyl- and fucosyl-transferases [76], resulting in N-glycans that would not be considered foreign to the human immune system. There is a private company, Greenovation, that is currently pursuing this strategy (www.greenovation.com).

People are exposed daily to plant N-glycans in food without ill effect, so it seems unlikely that the same glycans on orally administered antibodies would be a problem. It is still possible that a therapeutic plantibody carrying these epitopes may be immunogenic, or may sensitize humans or animals to protein epitopes on the antibodies. Future clinical trials with plant-made SIgA antibodies, especially if they are administered nasally or parenterally, will continue to examine this question closely.

CURRENT AND FUTURE APPLICATIONS OF PLANT-MADE SIGA

CaroRx (the SIgA/G derived from Guy's 13) was the first plant-made antibody, and currently the only monoclonal SIgA, that has been tested in human clinical trials [77]. In a randomized, double-blind, placebo controlled trial at Guy's Hospital, London, subjects were treated with chlorhexidine (a broad-spectrum oral antiseptic) followed by routine dental examination and cleaning, which reduced the oral colony counts of *S. mutans* to zero. Subsequently, groups of four subjects were given six topical oral applications of either saline, the original Guy's 13 IgG, or CaroRx over a period of three weeks. Subjects receiving either antibody maintained no counts of *S. mutans* over 4 months of follow-up. In a later trial at the UCSF Dental School, groups of 12 subjects received saline or two, four or six applications of CaroRx. Initial antisepsis failed in 1/3 of those subjects, and complete protection from colonization by CaroRx was not observed, however, subjects receiving 6 doses of CaroRx had significantly lower counts of S. *mutans* at 6 months, while the placebo group did not (unpublished).

Oral topical treatment of this kind with plant-made SIgA appeared to be completely safe. No adverse events were reported in either trial, and no signs of local or systemic side effects or hypersensitivity reactions were noted after any of the interventions or at any of the follow-up visits. No human anti-mouse antibody (HAMA) response was detected in post-treatment serum samples. In the published trial, pre-and post-immunization sera were assayed by ELISA for antibodies

that bound to the plant SIgA/G preparation. There was no significant change in the specific IgG, IgA or IgM antibody titers of subjects receiving plantibody [77]. This suggests that plant-made SIgA/G is not immunogenic when given orally. Overall, none of the safety-related measurements identified differences between subjects who received plantibody and those who did not.

There were no differences between the protective effect of the IgG and the SIgA/G version of the antibody tested in the first trial. There were, however, two observations that suggest potential advantages of the SIgA/G version. The first was the observation that while the two antibody forms had very similar dissociation constants (as measured by surface plasmon resonance), the SIgA/G antibody had a higher functional affinity (or avidity) as measured by a competition ELISA. The second observation was that the SIgA/G was detectable for at least 72 hours on teeth and for up to 48 hours in saliva, while the IgG was undetectable after 24 hours [77]. Both these observations suggest that SIgA/G could be effective at a lower concentration and for a longer time than the IgG, thus being potentially less expensive.

There are numerous passive mucosal immunotherapies involving IgA or SIgA that make attractive targets for SIgA made in plants. Readers are referred to the excellent recent review by Corthesy [15] for a thorough discussion of a number of these therapies. There is one more immunotherapy that merits further mention, because it involves some of the advantages of SIgA, though it is not, strictly speaking, a SIgA. Planet Biotechnology is developing a new approach for blocking infection by rhinovirus, a major cause of the common cold. Human rhinoviruses account for approximately half of all common colds [78]. Rhinovirus infection is initiated when virus binds intercellular adhesion molecule 1 (ICAM-1), on the surface of cells in the nasal mucosa. It has been shown that rhinovirus infection can be significantly impeded, both *in vitro* [79] and *in vivo* [80], by overwhelming the virus with soluble ICAM. A chimeric molecule consisting of the extracellular domains of ICAM-1 fused to the Fc of IgA was 200 times more effective than soluble ICAM-1 at blocking viral killing of HeLa cells [81]. Molecules of this type, in which the Fc region of an immunoglobulin and the ligand-binding region of a receptor or adhesion molecule are fused, are known as immunoadhesins. The improved effectiveness in blocking virus infection is attributed to the bi-valency of the molecule. Expression of an IgA-ICAM chimera along with human J chain and secretory component has resulted in the assembly of an SIgA-ICAM in transgenic tobacco. Preliminary *in vitro* tests indicate that this molecule is at least as active in preventing rhinovirus infection of cells as ICAM-IgG (unpublished), and the presence of secretory component is anticipated to prolong the life of the molecule in the nasal mucosa.

CONCLUSIONS

Secretory IgA is the body's first line of defense against infection. Under certain circumstances, passive immunotherapy with SIgA may be desirable. Plants appear to be a safe and economical way to produce the quantities of SIgA that are likely to be required for mucosal immunotherapy.

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