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## Recombinant IgA Antibodies

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### 15.1. Introduction

The production of monoclonal antibodies and the development of recombinant antibody technology have made antibodies one of the largest classes of drugs in development for prophylactic, therapeutic and diagnostic

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purposes. Currently, all of the Food and Drug Administration (FDA)-approved antibodies are immunoglobulin Gs (IgGs). However, more than 95% of the infections are initiated at the mucosal surfaces, where IgA is the primary immune effector antibody.

Immunoglobulin A has many properties that make it an attractive therapeutic agent. At mucosal surfaces, IgA is polymeric, making it more efficient at agglutinating antigens than IgG, thereby facilitating immune exclusion. IgA is anti-inflammatory due to both its inability to activate complement and its ability to inhibit complement activation by IgG and IgM (Griffiss and Goroff, 1983; Russell et al., 1998). When associated with the secretory component (SC), IgA is more resistant to proteolysis than IgG (Chintalacheruvu and Morrison, 1997; Lindh, 1975).

Studies in animals and humans suggest that passive administration of IgA could provide protection against a wide range of pathogens, including bacteria and their toxins and viruses such as HIV and respiratory syncytial virus (RSV) (Gupta et al., 2005; Hemming et al., 1987; Kozlowski and Neutra, 2003). A recombinant secretory IgA (SIgA) against *Streptococcus mutans* has been approved in Europe for topical administration to prevent dental caries (Ma et al., 1998). Antitransferrin IgA has been shown to be effective against cancers when administered intravenously (Brooks et al., 1995). However, topical administration of an anti-RSV IgA did not show significant efficacy as a prophylactic agent in clinical trials, although an IgG anti-RSV has been shown to be effective when administered intramuscularly and is approved by the FDA for use in humans (Subramanian et al., 1998). Thus, although there is much potential for IgA-based therapeutic and prophylactic agents, it is essential to use recombinant antibody technology to define the correlations between IgA structure and function, to study the biology of IgA and determine the mechanisms by which IgA and SIgA provide protection. This knowledge will facilitate the design and production of effective IgA-based immunotherapeutics for a wide range of applications.

Production of recombinant IgA poses many challenges. IgA exists as two different isotypes, with one isotype, IgA2, having three different allotypic forms. A decision must be made as to which IgA form is most appropriate for the intended application. Like IgG, production of IgA requires the expression of heavy and light chains. However, to make polymeric IgA (pIgA) a third chain, the J-chain, must also be expressed. If SIgA is needed, a fourth polypeptide, SC, must also be expressed. These chains must be appropriately assembled and secreted. IgA is a glycoprotein with two to five N-linked glycans present on the heavy chain; O-Linked glycans are present in the hinge region of IgA1. The N-linked glycans present on the  $\alpha$ -heavy-chain are exposed on the surface of the molecule (Mattu et al., 1998; Merry et al., 1992). The J-chain and SC are also glycoproteins. Because glycans on IgA play an important role in its function, particular attention must be paid to the structure of the glycans attached by different expression systems. The cost of the product is also an

important consideration. Thus, the identification of the appropriate expression system is a significant challenge when producing recombinant IgA.

## 15.2. Expression Systems for the Production of Recombinant IgA

Recombinant IgA has been produced in several different expression systems, including insect, plant, and mammalian cells and transgenic animals. Each system has its advantages, limitations, and potential applications. Large quantities of proteins can be produced in insect cells and in agricultural amounts in plants, making these expression systems an economical alternative to producing therapeutic and diagnostic reagents. Normal posttranslational modifications such as signal peptide cleavage, intrachain and interchain disulfide bond formation, and the addition of O-linked and N-linked carbohydrates are observed in plant and insect cells. However, the *N*-glycans attached by insect and plant cells differ from those normally found on human IgA (Butters et al., 1981; Jarvis and Finn, 1995, 1996; Martin et al., 1988; Ogonah et al., 1995). The *N*-glycans present on IgA produced in transgenic animals will depend on the species used. These differences in glycosylation might influence *in vivo* biologic properties such as biodistribution, half-life, and effector functions. Intact and fully assembled IgA has been produced in mammalian cells. However, in this case, cost is an important consideration. Regardless of which expression system is used, careful characterization of the *in vivo* biologic properties of the resulting IgA will be essential.

### 15.2.1. *Baculovirus*

Antibodies have been produced in insect cells. However, insect cells do not appear to contain all of the enzymes required to produce sialylated complex carbohydrates (Jarvis and Finn, 1995) although expression of glycosyltransferases can lead to proteins with more mammalianlike glycans (Jarvis and Finn, 1996). Insect cells also produce proteins with glycans such as core  $\alpha 1 \rightarrow 6$  fucosylated oligomannose (Man3GlcNAc2) that are not found on mammalian cells (Ogonah et al., 1995).

Recombinant IgA1 has been produced in *Spodoptera frugiperda* (Sf9) insect cells by double infection with baculovirus containing chimeric mouse-human H-chain and  $\kappa$  L-chain. Yields of 0.75  $\mu\text{g}/10^6$  cells per 72 h were obtained. The secreted antibody was a H<sub>2</sub>L<sub>2</sub> heterodimer containing O-linked Gal $\beta$ (1-3) GalNAc disaccharides, and N-linked high mannose glycans. It bound antigen (*p*-azophenylarsonate), Fc $\alpha$ R on HL-60 promyelocytic leukemia cells, and complement component C3. IgA dimers were assembled inefficiently when the human J-chain was also expressed, with the majority of extracellular IgA being monomers associated with the J-chain (Carayannopoulos et al., 1994). It is not clear if the instability of the cova-

lent bond between IgA and the J-chain is due to differences in carbohydrate structure or the production level of the J-chain in the baculovirus system. In contrast to what was found for IgA produced in Chinese hamster ovary (CHO) cells (Mattu et al., 1998), insect-cell-produced IgA1 lacking the N-linked carbohydrate in C<sub>H</sub>2 did not bind to cells expressing Fc $\alpha$ R. Human SC has also been produced in Sf9 cells with yields of 50 mg/L. Although the recombinant SC was not fully glycosylated, it was shown to associate with mouse dimeric IgA (Rindisbacher et al., 1995).

### 15.2.2. Plants

Transgenic tobacco, maize, soybean, and alfalfa are expected to yield over 10 kg of therapeutic antibody per acre, with costs of about one-tenth of what would be required for the production of antibodies using mammalian cells (Larrick et al., 2001). Although plants produce proteins with both high mannose and complex N-linked glycans, they attach  $\beta$ (1 $\rightarrow$ 2)-linked xylose and  $\alpha$ (1 $\rightarrow$ 3)-linked core fucose instead of the  $\alpha$ (1 $\rightarrow$ 6)-linked core fucose found in mammals. In addition, plants do not normally add Gal to their complex N-glycans because they lack  $\beta$ (1 $\rightarrow$ 4) galactosyltransferase and the addition of sialic acid is also absent. Although Gal was added to the complex carbohydrate of a mouse IgG1 when human  $\beta$ (1 $\rightarrow$ 4) galactosyltransferase was expressed in tobacco plants, xylosylation and fucosylation were not affected (Bakker et al., 2001). The age of the leaf from which the antibodies are purified also affects the structure of N-glycans (Elbers et al., 2001). Even when a KDEL endoplasmic reticulum anchorage domain was added to a mouse IgG, N-glycans containing  $\beta$ (1 $\rightarrow$ 2) xylose and  $\alpha$ (1 $\rightarrow$ 3) fucose were present (Ramirez et al., 2003).

A significant concern is whether the glycans attached by plants will be immunogenic. Although IgE specific for plant glycoallergens  $\beta$ (1 $\rightarrow$ 2) xylose and  $\alpha$ (1 $\rightarrow$ 3) fucose has been detected in serum from allergic patients (van Ree et al., 2000), only two of six mice immunized subcutaneously with a mouse IgG1 produced in tobacco plants had a detectable immune response. However, it should be noted that the assay used detected only IgG2a, IgG2b, and IgG3, not other isotypes including IgE (Chargelegue et al., 2000).

Immunoglobulin A specific for the *Streptococcus mutans* and *S. sobrinus* antigen I/II cell surface adhesion molecule has been produced in tobacco plants (Ma et al., 1995). Hybrid IgA-producing plants were generated by crossing plants expressing a murine-human  $\alpha$ - $\gamma$  H-chain and murine  $\kappa$  L-chain. Dimeric IgA was produced by subsequent crossing with plants expressing the murine J-chain. Finally, crossing with rabbit SC-producing plants resulted in the expression of SIgA in a single plant cell. However, only about 50% of IgA was covalently associated with SC. In addition, the SC bound to IgA was 50 kDa, which is smaller than the expected size of 66.5 kDa. Antibody yields of 10–80 mg of antibody/kg of fresh plant material were obtained (Ma et al., 1998).

### 15.2.3. *Transgenic Animals*

Transgenic animals are attractive expression systems for the large-scale production of antibodies. However, mammals differ in their sialic acid structure, ability to add bisecting GlcNAc and Gal, and core fucosylation, making the choice of species an important consideration (Raju et al., 2000). Transgenic mice secreting a chimeric murine–porcine IgA against transmissible gastroenteritis coronavirus (TGEV) have been engineered (Sola et al., 1998).  $\beta$ -Lactoglobulin regulatory sequences were used to target IgA synthesis to the mammary glands. The concentration of porcine IgA in the milk was 0.01–6 mg/mL, with no detectable levels in the serum of the transgenic animals. Transgenic mice overexpressing murine pIgR in mammary gland epithelial cells were shown to have increased levels of IgA in milk as compared with control mice (De Groot et al., 2000). Mouse IgA against phosphorylcholine has been expressed in transgenic mice, pigs, and sheep (Lo et al., 1991).

### 15.2.4. *Mammalian Cells*

Mammalian expression systems have been used extensively for the production of IgA and SIgA. To date, nonimmunoglobulin producing murine myeloma and CHO cell lines have been the expression systems of choice. However, the glycans attached by these expression systems are not identical to those present on normal human IgA. In addition, we have observed incomplete assembly of IgA2 produced in CHO cells (Chintalacheruvu et al., unpublished data).

Murine carbohydrates differ from those present in the human. Proteins produced in murine myelomas lack the bisecting GlcNAc linked to the trimannosyl core of N-linked glycans (Fukuta et al., 2000), which has been implicated as being important for antibody function (Davies et al., 2001; Lifely et al., 1995; Umana *et al.*, 1999). In addition, sialic acid structure varies among mammalian species, with human cells attaching *N*-acetylneuraminic acid (NANA), whereas mouse cells attach *N*-glycolylneuraminic acid (NGNA) (Raju et al., 2000). Mouse cells also synthesize Gal $\alpha$ 1 $\rightarrow$ 3Gal $\beta$ 1 $\rightarrow$ 4GlcNAc. A significant amount of circulating IgG in humans is against Gal $\alpha$ 1 $\rightarrow$ 3Gal $\beta$ 1 $\rightarrow$ 4GlcNAc, an epitope abundant on glycoproteins from nonprimate mammals, prosimians, and New World monkey, but absent from Old World monkeys, apes, and man (Galili, 1989). The presence of this carbohydrate structure on therapeutic proteins would have significant consequences. The presence of this epitope on recombinant antibodies is quite variable, with a humanized IgG1 produced by GS-NS0 cells containing no Gal $\alpha$ 1 $\rightarrow$ 3Gal $\beta$ 1 residues (Hills et al., 2001) and H-chain glycans produced in J558 possessing the epitope (Lund et al., 1990, 1993). However, much of the N-linked glycans on human IgA1 and IgA2 produced in murine myeloma cells appear to contain Gal $\alpha$ 1 $\rightarrow$ 3Gal $\beta$ 1 (Morrison et al., unpublished observation). The exposed nature of the glycans on IgA might facilitate processing to this glycan form.

Chinese hamster ovary cells appear to lack the glycosyltransferases necessary for generating both the bisecting GlcNAc and  $\alpha(2\rightarrow6)$  linked sialic acid on N-linked glycans (Routier et al., 1997). Transfection of exogenous glycosyltransferases into CHO cells has made it possible to express proteins that contain more humanized glycans with less glycan heterogeneity (Lee et al., 1989; Weikert *et al.*, 1999). *In vitro* galactosylation and sialylation has also been used for glyco-engineering of antibody molecules (Raju *et al.*, 2001). In addition, CHO cells have been engineered to overexpress protein disulfide isomerase, facilitating the assembly of recombinant proteins (Davis et al., 2000).

### 15.3. Vectors for Expression of Recombinant IgA

The general strategy has been to design immunoglobulin expression vectors as cassettes to facilitate manipulation of the antibody genes. The vectors for expression of H- and L-chains usually contain different selectable markers, although bicistronic expression vectors containing human  $\kappa$  L- and J-chain genes and human IgA1 and murine dihydrofolate reductase (DHFR) genes have also been described (Wolbank et al., 2003). Because the amount of L-chain produced by a transfectant appears to limit the amount of antibody produced, an effective method for generating transfectants is to first isolate good L-chain-producing transfectants. A second round of transfections with the H-chain and selection using a different marker yields transfectants producing large amounts of antibody (Chintalacharuvu et al., 1994; Chintalacharuvu and Morrison, 1996; Morton et al., 1993). Alternatively, cotransfection of L- and H-chains can be performed, but only one marker is usually used for selection (Berdoz *et al.*, 1999). Variable and constant region genes were initially obtained by genomic cloning but now are more routinely amplified by the polymerase chain reaction (PCR) from cDNA made from antibody-producing cells (Berdoz et al., 1995; Campbell et al., 1992; Coloma et al., 1992; Gavidondo-Cowley *et al.*, 1990; Gillies et al., 1989; Larrick et al., 1989; Orlandi et al., 1989). PCR has been used successfully for rapid cloning and modification of V regions from antibodies of many different specificities. If the sequence of the variable region to be cloned is known, specific PCR primers can be produced. If the sequence is unknown, degenerate primer sets that anneal to the relatively conserved leader or first framework sequences at the 5' end are used. A limited number of primers will amplify all of the heavy-chain isotypes; there are only two isotypes of the light chain. By including appropriate restriction sites in the primers, it is straightforward to clone the PCR product into an expression vector. In addition, fully human IgAs have also been produced by cloning V regions from single-chain Fv fragments selected by phage display (Berdoz and Corthesy, 2004; Boel et al., 2000; Huls et al., 1999b).

The SC is produced by proteolytic cleavage of pIgR during epithelial transcytosis (see Chapter 4). Initially, SIgA was produced by coculturing

IgA-producing hybridomas with Madin-Darby canine kidney (MDCK) cells expressing pIgR (Hirt et al., 1993). In another approach, polymeric murine IgA produced in mice ascites was injected into Lewis rats, where it associated with the rat pIgR, and SIgA with rat SC was collected from bile (Renegar et al., 1998). As an alternative approach, SC was produced by introducing a stop codon into the position corresponding to the natural cleavage site in the pIgR (Rindisbacher et al., 1995). Purified pIgA from hybridoma cell lines can then combine with the purified recombinant SC in the test tube (Crottet et al., 1999; Lullau et al., 1996; Rindisbacher et al., 1995). These SIgA complexes had the same affinity for antigen as the native molecule, and formed the correct covalent bonds between the two molecules (Lullau et al., 1996). Although SIgA is normally a product of two different cell types, recombinant SIgA has been produced in a single cell. When the gene for human SC was transfected into a myeloma cell producing chimeric mouse-human pIgA containing the endogenous murine J-chain, SIgA was produced. Virtually all of the SC was covalently bound to dimeric IgA, and SIgA assembly appeared to take place in the Golgi apparatus (Chintalacharuvu and Morrison, 1997). Subsequently, CHO (Berdoz et al., 1999; Johansen et al., 1999) and Baby Hamster Kidney (BHK) (Vidarsson et al., 2001) cells have been used to produce SIgA by transfecting in genes for the human J-chain and SC into cells already expressing chimeric IgA.

The controlling elements used to direct IgA expression depend on the cell type to be transfected. In B-lineage cell lines, the expression of the recombinant H- and L-chain genes is usually driven by the immunoglobulin promoter and enhancer elements (Bruggemann et al., 1987; Chintalacharuvu et al., 1994; Chintalacharuvu and Morrison, 1996; Knight et al., 1988). Expression of IgA in B-lineage cell lines is advantageous in that the endogenous J-chain is incorporated into the recombinant IgA. Strong heterologous promoters such as the human cytomegalovirus (CMV) promoter can also be used to direct IgA expression. These viral controlling elements have led to high antibody yields and have the advantage that they are versatile and can function in a variety of cell types such as CHO, BHK, and COS cells as well as lymphoid cells. Chimeric murine-human IgA1 and IgA2 have been produced in these cell lines (Berdoz et al., 1999; Berdoz and Corthesy, 2004; Chintalacharuvu et al., 1994; Chintalacharuvu and Morrison, 1996; Morton et al., 1993; Trill et al., 1995; Vidarsson et al., 2001). Chimeric murine-porcine IgA (Sola et al., 1998) and 12 isotypes of chimeric murine-rabbit IgA (Schneiderman et al., 1989) have been produced in Sp2/0 cells; IgA2m(1) has also been produced in plasmacytoma J558L cells (Braathen et al., 2002; Bruggemann et al., 1987) and chimeric murine-bovine IgA has been produced in the murine hybridoma 27/44 (Knight et al., 1988).

Vaccinia virus expression systems are advantageous in that the extended host range of the virus makes it possible to express the heterologous gene in almost any mammalian cell type. Viral promoters such as the vaccinia late promoter P11K flanked by the vaccinia thymidine kinase sequence, which directs

recombination into the viral genome, and viral or mammalian transcriptional and translational elements have been used to regulate expression. In the case of human SC, equivalent amounts of protein (5–10 mg/L) were produced when human SC or vaccinia 11K regulatory sequences were used in HeLa and HeLaS3 (human epithelial), CV-1 (monkey fibroblast) and TK<sup>-</sup> 143B (human fibroblast) cell lines (Rindisbacher et al., 1995). The recombinant SC produced was capable of associating with dimeric mouse IgA and contained complex *N*-glycans with cell-line-specific processing. Rabbit SC (Corthesy et al., 1996) and murine SC have also been produced using a vaccinia virus expression system in a non-Ig-producing hybridoma cell line (Crottet *et al.*, 1999). The glycosylation profile for the recombinant murine SC was the same as that found on SC from murine milk or bile.

#### 15.4. Purification and Characterization of IgA and SIgA Proteins

A variety of methods have been used to purify recombinant IgA and SIgA. Purification protocols based on selection by antigen are widely used. In addition, a synthetic peptide of 50 residues derived from a streptococcal M protein has been shown to bind to human IgA. The peptide, designated Sap for streptococcal IgA-binding peptide, was shown to deplete >99% of IgA from human serum but only 45% of SIgA from saliva. Sap appears to bind both IgA1 and IgA2 with similar affinities (Sandin et al., 2002), making it an attractive reagent for isolation and purification of recombinant human IgA. Lectins such as concavalin A have been used to purify SC (Rindisbacher et al., 1995) and the lectin Jacalin binds the O-linked glycans in the hinge of IgA1. Tags can also be added to aid the purification of the recombinant proteins; if separated from the protein by a protease cleavage site, these tags can then be removed following protein purification. A 6xHis tag at the C-terminus of human SC did not alter its secretion by either the baculovirus or vaccinia virus expression systems (Rindisbacher et al., 1995) and a FLAG tag has been used to purify murine SC (Crottet et al., 1999).

##### 15.4.1. Assembly of Human IgA and SIgA

Recombinant technology has made it possible to shed light on the requirements necessary for IgA assembly and secretion, polymer formation, and association with other proteins such as the J-chain and pIgR/SC. Studies using site-directed mutagenesis and domain-exchanged proteins have identified some of the structural elements important for assembly of IgA and SIgA.

The  $\alpha$  H-chains and L-chains, which are synthesized on polysomes, are translocated into the lumen of the endoplasmic reticulum, where they are assembled into monomers and higher polymers. The H- and L-chains first



associate noncovalently and then covalently through disulfide bonds. The predominant covalent assembly pathway for human IgA1, IgA2m(2), and IgA2(n) molecules is through an HL intermediate in which the H- and L-chains are first disulfide bonded before forming the covalently associated H<sub>2</sub>L<sub>2</sub> monomer. In contrast, the major intermediate in the assembly of IgA2m(1) is H<sub>2</sub>, and the majority of the IgA2m(1) molecules lack disulfide bonds between the H- and L-chains (Chintalacharuvu and Morrison, 1996). Cys133 in C<sub>H</sub>1, which is absent in IgA2, participates in disulfide bond formation with the L-chain in IgA1 (Chintalacharuvu and Morrison, 1996). In IgA2, the L-chain is covalently associated to the hinge proximal Cys241 or Cys242 in C<sub>H</sub>2, with the structure of the C<sub>H</sub>1 influencing the efficiency of disulfide bond formation (Chintalacharuvu et al., 2002).

Immunoglobulin A possesses an 18-amino-acid extension in the C-terminus called the tailpiece, which participates in polymerization; IgM, which also forms polymers, also possesses a tailpiece. The tailpiece contains an N-linked carbohydrate addition site, which is required for IgA dimer formation (Atkin et al., 1996), and a conserved penultimate Cys residue, which is involved in polymerization. Studies using domain-exchanged proteins have revealed that in addition to the tailpiece, structural motifs in C<sub>H</sub> are critical for polymer assembly and J-chain incorporation. Assembly of dimers containing the J-chain requires C<sub>α</sub>3 and the α tailpiece, with more efficient dimer assembly in the presence of C<sub>α</sub>2 (Braathen et al., 2002; Chintalacharuvu et al., 2001; Yoo et al., 1999).

Only polymers containing the J-chain can bind to the pIgR (Brandtzaeg and Prydz, 1984) (see Chapter 4). Both covalent and noncovalent interactions are found between pIgA and pIgR. Human pIgR forms disulfide bonds with human, rat, and rabbit dimeric IgA (Tamer et al., 1995); 11 rabbit IgA-f isotypes were shown to bind to rabbit pIgR covalently, whereas 1 rabbit IgA-g isotype was noncovalently associated (Schneiderman et al., 1989). Disulfide bond formation does not appear to facilitate transport but, rather, might be important in stabilizing pIgA–SC complexes. Although the J-chain promotes IgA polymer assembly, human IgA1 and IgA2m(1) produced in CHO cells were able to form dimers in its absence (Morton et al., 1993). The J-chain contains eight Cys residues, two of which (Cys15 and 69) form disulfide bonds with the α-chain and six of which (Cys13, 72, 92, 101, 109, and 134) form intrachain disulfide bridges. The C-terminus of the J-chain as well as two of the three intrachain disulfide bridges were found to be dispensable for formation of IgA polymers but were required for binding to SC (Johansen et al., 2001). Either Cys15 or Cys69 of the J-chain was required for IgA polymerization, but these polymers were stabilized by noncovalent interactions. Although the noncovalently associated polymers bound free SC, transcytosis by pIgR-expressing MDCK cells was greatly diminished (Johansen et al., 2001).

Because the J-chain is required for pIgR binding and both C<sub>α</sub>3 and the tailpieces are required for J-chain incorporation, it is not surprising that studies using domain-exchanged proteins showed that C<sub>α</sub>3 is required for binding and for transport by pIgR-expressed on MDCK cells (Braathen et al., 2002;

Hexham et al., 1999). An exposed loop of the C $_{\alpha}$ 3 domain containing amino acids 402–410 (QEPSQGTTT) was predicted to be the binding site for pIgR (Hexham et al., 1999), with amino acids 430–443 also participating in pIgR binding (White and Capra, 2002). However, a naturally occurring mutant that lacks 36 amino acids in C $_{\alpha}$ 3, including amino acids 402–410, was found to associate with rat SC and be transported from blood into bile in a manner indistinguishable from pIgA (Switzer et al., 1992). Nevertheless, green fluorescent protein (GFP) fusion proteins containing monomeric and dimeric forms of amino acids 402–410 and a peptide from a phage display library resembling amino acids 402–410 were transported by the pIgR (Hexham et al., 1999), suggesting that such peptides might be used to target and deliver therapeutics to mucosal sites. Although the addition of C $_{\alpha}$ 3 from IgA2m(2) to IgG1 resulted in proteins that incorporated the J-chain and bound to pIgR, the hybrid molecules were transported by pIgR-expressing MDCK cells only with low efficiency (Chintalacharuvu et al., 2001).

#### 15.4.2. Stability of IgA and SIgA

The environment at mucosal surfaces is a hostile one, with the pH in the gastrointestinal tract ranging from 2.0 in the stomach to 8.0 in the intestinal lumen. IgA has evolved to function in this hostile environment, and the addition of C $_{\alpha}$ 3 of IgA2m(2) to IgG1 resulted in proteins that were more stable than wild-type IgG1 at most pHs. Replacement of C $_{\gamma}$ 1 with C $_{\alpha}$ 1 further increased the stability of the hybrid antibodies, especially at low pH (Chintalacharuvu et al., 2001). SC serves to increase the stability of IgA (see Chapter 8). In mice, SIgA1 was cleared less readily than dimeric IgA (Chintalacharuvu and Morrison, 1997), and SIgA was more resistant to degradation than dimeric IgA when exposed to intestinal washes (Berdoz et al., 1999; Chintalacharuvu and Morrison, 1997; Crottet and Corthesy, 1998). In addition, recombinant SIgA produced in plants was found to be more stable in the human oral cavity than an IgG containing identical V regions (Ma et al., 1998).

Many bacteria that cause disease at mucosal surfaces secrete proteases that cleave IgA1 but not IgA2. Type 1 proteases cleave between Pro-Ser residues, whereas type 2 cleave between Pro-Thr residues. Streptococcal species produce type 2 metalloproteases, whereas *Neisseria meningitidis*, *N. gonorrhoeae*, and *Haemophilus influenzae* produce both type 1 and type 2 proteases. The O-linked glycans in the hinge appear to be important in sensitivity to streptococcal proteases (Batten et al., 2003). The length of the hinge has been shown to be important for recognition and cleavage by proteases (Senior and Woof, 2005b) (see Chapter 1). Studies using human IgA1 hinge mutants revealed that the bacterial proteases require specific amino acid sequences for cleavage (Batten et al., 2003), with many but not all proteases able to cleave a hybrid IgA2/IgA1 with a shortened hinge (Senior et al., 2000). The proteases are able to cleave alternative peptide bonds if the wild-type sites are not present (Senior and Woof, 2005a). In addition to sequence requirements in the hinge,

both C<sub>H</sub>2 and C<sub>H</sub>3 of IgA1 are required for the cleavage by proteases from *H. influenzae* and *N. gonorrhoeae* (Chintalacharuvu et al., 2003).

#### 15.4.3. Binding to Fc $\alpha$ RI (CD89)

Immunoglobulin A-mediated immune effector mechanisms such as phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), respiratory burst, and cytokine release are mediated through Fc $\alpha$ RI, an IgA-specific receptor present on human macrophages, monocytes, eosinophils, and neutrophils (Monteiro et al., 1990) (see Chapter 4). No comparable receptor is expressed in the mouse. Both monomeric and dimeric IgA can bind the Fc $\alpha$ R and activate the immune effector cells (Vidarsson et al., 2001). IgA forms a 2:1 complex with the receptor by binding to each C<sub>H</sub>2/C<sub>H</sub>3 interface (Herr et al., 2003). Initial studies using recombinant human IgA1/IgG1 domain-exchanged proteins revealed that C $\alpha$ 2 and C $\alpha$ 3 are necessary and sufficient for binding to Fc $\alpha$ RI (Carayannopoulos et al., 1996). Specifically, residues Leu257 and Leu258 in the C<sub>H</sub>2 loop and Pro440, Leu441, Ala442, and Phe443 in the C<sub>H</sub>3 loop are important for binding and signaling through the receptor (Carayannopoulos et al., 1996; Pleass et al., 1999). Electrostatic interactions of acidic residues Glu254, Asp255, and Glu437 on IgA1 do not seem to play a major role in binding to Fc $\alpha$ RI because mutation of these residues had little effect on affinity for the receptor (Pleass et al., 2003a). The crystal structure of Fc $\alpha$ RI in complex with the Fc of IgA has confirmed and extended these conclusions (Herr et al., 2003). The Fc $\alpha$ RI:Fc $\alpha$  interface is composed of a central hydrophobic core composed of Leu257, Leu258, Met433, Leu441, Ala442, Phe443, and the aliphatic portion of the Arg382 side chain flanked by charged residues (Arg382, Glu389, and Glu437). The *N*-glycans approach within 8 Å of Fc $\alpha$ RI but do not directly contact the receptor. Fc $\alpha$  is bound to Fc $\alpha$ RI in an “upright” orientation such that its C-terminus would be oriented near the cell membrane when bound to cell-associated Fc $\alpha$ RI. Bound SC would appear to occlude the binding site, and SIgA does not enhance or stimulate phagocytosis (Vidarsson et al., 2001). Indeed, activation of neutrophil effector functions can be prevented by SIgA (Motegi and Kita, 1998).

#### 15.4.4. Complement Fixation

Whereas IgA does not fix complement via the classical pathway, it is debatable whether it can activate the alternative pathway and might depend on the species producing the IgA (see Chapter 6). Chimeric murine–human IgA1 against *Pseudomonas aeruginosa* lipopolysaccharide (LPS) did not mediate deposition of C3 onto the surface of purified LPS or whole bacteria (Preston et al., 1998); although chimeric mouse–human IgA1 specific for the hapten dansyl bound C3, it did not appear to activate the alternative pathway because subsequent steps in the complement cascade (factor B cleavage and

terminal complex binding) were not observed (Chuang and Morrison, 1997). However, human serum IgA has been shown to activate the alternative pathway to enhance the killing of bacteria under some circumstances (Janoff et al., 1999), and 12 recombinant rabbit IgA isotypes activated the alternative pathway (Schneiderman et al., 1990). The absence of glycosylation on IgA resulted in a reduction in C3 binding (Zhang and Lachmann, 1994).

#### 15.4.5. Role of Carbohydrates in IgA and SIgA

Immunoglobulin A and SIgA are heavily glycosylated proteins with oligosaccharide side chains accounting for >10% of the molecular mass of the IgA H chain (Tomana et al., 1976), 8% of the J-chain (Baenziger, 1979; Royle et al., 2003), and 15–20% of SC (Hughes et al., 1999; Phalipon et al., 2002). The presence or absence of glycans as well as their structure can affect IgA assembly, secretion, and function. Deletion of N-linked glycans in C<sub>H</sub>2 and the tailpiece did not affect assembly and secretion of human IgA1 (Chuang and Morrison, 1997) but did inhibit the secretion of murine IgA (Taylor and Wall, 1988). The absence of N-linked carbohydrate in human IgA1 did not affect its binding to pIgR (Chuang and Morrison, 1997). When IgA was produced in insect cells, glycosylation in C<sub>H</sub>2 was found to be critical for interaction with Fc $\alpha$ RI (Carayannopoulos et al., 1996); however, this was not the case when the IgA was produced in CHO cells (Mattu et al., 1998), possibly reflecting structural differences that occur because of the altered structure of either the N-linked carbohydrate at Asn459 or the O-linked glycans in the hinge.

The N- and/or O-linked oligosaccharides of IgA and SC have been proposed to function as ligands for bacterial adhesions and appear to be important in providing protection by serving as receptor analogues in innate immunity (Dallas and Rolfe, 1998; Mantis et al., 2004; Royle et al., 2003) (see Chapter 8). In addition, the N-linked glycans on recombinant human SC were shown to be critical for directing the appropriate localization of IgA and conferring protection against *Shigella flexneri* infection in mice (Phalipon et al., 2002). Secretion of recombinant human SC requires that it be glycosylated because cells grown in the presence of tunicamycin, which inhibits N-linked glycosylation, were unable to secrete recombinant human SC (Cottet and Corthesy, 1997).

Carbohydrate structure also influences the pharmacokinetic properties of glycoproteins. The asialoglycoprotein-receptor (ASGPR) mediates the rapid clearance of glycoproteins bearing terminal  $\beta$ -linked *N*-acetylgalactosamine (GalNAc) or Gal. In mice, all three allotypes of IgA2 were rapidly cleared from the serum by ASGPR expressed in the liver, whereas much less IgA1 was cleared from circulation by this route (Rifai et al., 2000). IgA1 has been found to be highly sialylated (Mattu et al., 1998) and increasing the amount of sialic acid content on the carbohydrate was found to decrease the interaction of IgA1 and IgA2 with the ASGPR (Basset et al., 1999). Greater serum levels of IgA1 than IgA2 might be explained in part by this rapid clearance of IgA2 by ASGPR.

## 15.5. Applications of Recombinant IgA

A growing public health concern is the increased presence of antibiotic-resistant disease-causing microbes. The increased number of these bacteria has led to renewed interest in the use of passive antibodies for the treatment of infectious disease. Passive antibodies were widely used in the preantibiotic era but were abandoned when the widespread use of antibiotics became common (reviewed in Casadevall et al., 2004). Currently, most of the antibodies in use and in clinical trials are for systemic administration. However, possibly the greatest use for antibodies might lie in their administrations for both the prevention and treatment of infections at the mucosal surfaces, the major portal of entry for infectious agents. From a public health perspective, prevention is very important and direct application of antibody to the site of infection can block pathogen entry. Local administration of antibodies provides effective concentrations of drug immediately, and topical application is an easy and attractive mode of administration. Therapeutics based on IgA would appear to hold great promise for this route of treatment. SIgA can confer protection when administered passively, as in breast milk. In addition, recombinant IgAs have been used in a variety of disease models.

### 15.5.1. Bacterial Diseases

Recombinant IgA specific for a wide range of bacterial products has been produced. Fully human dimeric and pIgA and SIgA produced in CHO cells bind *Helicobacter pylori* urease (Berdoz and Cortesey, 2004) and a chimeric murine-human IgA1 recognizes *Pseudomonas aeruginosa* serogroup O6 lipopolysaccharide (Preston et al., 1998). Recombinant IgG (1–4), IgA1, and IgA2 against porin A from *Neisseria meningitidis* have been produced in BHK cells (Vidarsson et al., 2001). A mixture of monomeric and dimeric IgA2 containing the J-chain was shown to trigger a respiratory burst in polymorphonuclear leukocytes (PMNs) and monomeric IgA but not SIgA was shown to activate phagocytosis of heat-killed *Neisseria*. Polymeric chimeric IgA2m(1) specific for the enterotoxin A of *Clostridium difficile* showed increased avidity, which enhanced both the efficacy and duration of protection of human colonic epithelial T84 cell monolayers against the destructive effects of toxin A in comparison to IgG and monomeric IgA containing the same V regions (Stubbe et al., 2000). The protection in *Shigella flexneri* infection conferred by IgA specific for serotype 5a LPS was shown to be enhanced when pIgA is associated with SC. SC effectively localized IgA to the epithelial surface of the nasal cavity and the bronchial mucus, whereas IgA by itself was distributed diffusely in tissue. (Phalipon et al., 2002).

The first human trial of monoclonal secretory antibody has been performed using tobacco plant-derived SIgA against *Streptococcus mutans*

(Ma et al., 1998). The SIgA/IgG hybrid antibody was found to be more stable than murine IgG in the human oral cavity and SIgA was shown to prevent oral colonization by *S. mutans* for at least 4 months.

### 15.5.2. *Viral Diseases*

About 75% of human immunodeficiency virus (HIV)-resistant women expressed HIV-1-specific IgA in their genital tract as opposed to 26% of infected women (Kaul et al., 1999) (see Chapter 12). IgA appears to play an important role in preventing sexual transmission of HIV-1 because the IgA from these persistently seronegative individuals not only neutralizes HIV-1 but also prevents its transcytosis across tight epithelial cell layers (Devito et al., 2000a, 2000b). Human IgA1 against a nonimmunodominant epitope on the HIV-1 gp41 produced in CHO cells was shown to neutralize transepithelial HIV-1 entry *in vitro* using Me180 or HT-29 polarized epithelial monolayers, although it appears to be less effective than IgG and IgM at virus neutralization through complement and protection of human peripheral blood mononuclear cells (PBMCs) with HIV primary isolates (Wolbank et al., 2003).

Transmissible gastroenteritis coronavirus (TGEV) infects both enteric and respiratory tissues and causes close to 100% mortality in newborn pigs. Recombinant chimeric murine–porcine IgA produced in Sp2/0 cells neutralized TGEV 50-fold more efficiently than IgG1 and presumably due to the increased avidity of dimeric IgA. Transgenic mice expressing this same porcine IgA in the mammary gland were shown to secrete virus neutralizing antibodies into milk, suggesting that transgenic technology can be used to prevent neonatal infection and disease in livestock (Sola et al., 1998).

### 15.5.3. *Parasitic Diseases*

Recombinant IgA also shows promise in the treatment of parasitic diseases such as malaria. Therapeutics based on IgA might be advantageous over IgG because parasites initiate hypergammaglobulinemia as a smokescreen to evade the immune system (Miller et al., 1994); IgG also binds to the inhibitory receptor Fc $\gamma$ RIIb, thereby interfering with the immune response to the parasite (Clynes et al., 2000). Pleass et al (2003b) produced a recombinant murine–human chimeric IgA1 and IgG1 against MSP1<sub>19</sub>, merozoite surface protein 1, an antigen on *Plasmodium yoelii*. IgA1 was more effective than IgG1 in stimulating an oxidative burst in human neutrophils *in vitro*. However, in a murine model of malaria, IgA1 did not provide protection against lethal parasite infection, as did mouse IgG2b containing the identical V region, presumably because human IgA1 fails to interact with mouse Fc receptor(s). In humans, Fc $\alpha$ RI is expressed on monocytes and neutrophils, potent effector cells in merozoite killing, as well as on Kupffer and dendritic cells, which are involved in MSP1<sub>19</sub> antigen presentation. Stimulation of Fc $\alpha$ RI on human

monocytes inhibits tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 synthesis, which when elevated has shown to correlate with poor prognosis in malaria. In addition, high titers of *Plasmodium*-specific IgA in serum (Biswas et al., 1995) and breast milk (Leke et al., 1992) have been found in humans living in endemic areas, suggesting that IgA plays a crucial role in immunity against malaria and that recombinant IgA might be an effective therapeutic agent against malaria.

A chimeric murine–human IgA1 against a major ragweed pollen antigen (Sun et al., 1995) was shown to prevent increased airway hyperresponsiveness and lung eosinophilia in sensitized mice (Schwarze et al., 1998).

#### 15.5.4. *SIgA as a Mucosal Vaccine Delivery System*

Studies suggest that SIgA can also serve as a mucosal vaccine delivery system. An eight-amino-acid sequence in the loop connecting the E and F  $\beta$ -strands of domain I of rabbit pIgR/SC (residues 79–86) was replaced with a nine-amino-acid linear epitope from *S. flexneri* invasin B to engineer an “antigenized” SC. Although this mutation resulted in a significantly lower rate of secretion and, when expressed on MDCK cells, failed to transport dimeric IgA, the mutated SC was able to associate with murine dimeric IgA. When the antigenized SIgA together with cholera toxin as adjuvant was orally administered to mice, it was able to elicit production of invasin B- and rabbit SC-specific antibodies (Corthesy et al., 1996). In addition, when the *H. pylori* GroES chaperonin was fused at the C-terminus of human SC to serve as a mucosal vaccine carrier, the antigenized SC was shown to associate with polymeric and dimeric IgA isolated from murine hybridoma HNK20 (Favre et al., 2003).

#### 15.5.5. *Cancer Therapy*

Recombinant IgAs have shown promise as anticancer therapeutics. Recombinant human IgA1 produced in BHK cells specific for Ep-CAM, a tumor-associated antigen, was shown to mediate the killing of tumor cells by unstimulated PMNs. It also mediated phagocytosis of tumor cells by macrophages, although not as efficiently as human IgG1 containing the same V regions (Huls et al., 1999a). In addition, chimeric murine–human IgA1 and IgA2 against the HLA class II molecule expressed on B-cell lymphomas were effective in directing the killing of a variety of target cell lines by PMNs. Target cell lysis was inhibited by anti-Fc $\alpha$ RI, indicating that Fc $\alpha$ RI is the receptor responsible for antitumor activity (Dechant et al., 2002). Neutrophils can exert a potent cytolytic capacity against a variety of tumor cells in the presence of antitumor antibody. Immature neutrophils mobilized from the bone marrow by granulocyte colony-stimulating factor treatment have been shown to efficiently trigger tumor cell lysis via Fc $\alpha$ RI, but not through the Fc $\gamma$ R (Otten et al., 2005). Carcinoembryonic antigen (CEA) is expressed on the apical side of carcinoma cells (Buchecker et al., 1989; Burtin et al., 1973), and it is thought that much

of the radiolabeled anti-CEA IgG antibody used for detection (Baum et al., 1994) or therapy of colon carcinomas (Breitz et al., 1992) is unable to reach it due to its particular histological localization. Because chimeric IgA2m(2) against CEA can be transcytosed by the pIgR, it might be a more effective agent (Terskikh et al., 1994). A novel mouse–human chimeric dimeric IgA specific for the human transferrin receptor (hTfR) has been shown to inhibit proliferation of and induce apoptosis in myeloma cell lines ARH-77 and IM-9 (Chintalacharuvu et al., unpublished results; Prost et al., 1998; Shinohara et al., 2000).

## 15.6. Concluding Remarks

Immunoglobulin A mediates immune protection at mucosal surfaces, the major portal of entry for many pathogens, and therefore holds great potential as a therapeutic and prophylactic agent. One of the challenges in producing therapeutic IgAs is to ensure that this complex recombinant protein has the desired functional properties. Advances in molecular biology techniques have allowed for rapid cloning, expression, and characterization of antibodies, resulting in a greater understanding of protein assembly and secretion pathways, ligand–receptor interactions, the role of carbohydrates in glycoprotein function, and antibody effector functions. The challenge is to now use the resulting information to produce effective IgA-based therapeutic molecules.

Posttranslational modifications are important for correct antibody function. An important posttranslational modification is glycosylation, and consistent and correct glycosylation is especially important for producing functional IgA and SIgA in which the H-chain, J-chain, and SC all have significant amounts of surface-exposed carbohydrates. Murine myelomas and CHO cells have been the expression system of choice for the production of antibodies. However, these expression systems do not add carbohydrates identical to those found on human IgA. The use of human cell lines for expression might help to resolve this problem. Transgenic animals are an attractive expression system, especially for the production of SIgA in milk (Houdebine, 2000). However, the choice of species becomes important because there are species-specific differences in glycan addition. However, these differences might be less of an issue when the recombinant protein will be used for topical administration.

The recombinant antibody industry is in its infancy and shows great promise for rapid and extensive growth. It is expected that IgA will take its place alongside IgG as an important therapeutic molecule. However, for this to take place, certain issues and problems must be addressed. These include the issue of the cost of these complex biologics that require the use of expensive media along with the high costs associated with the purification process. The availability and cost of bovine serum in many growth media are issues that can be addressed by the use of serum-free growth media. Batch-to-batch variation and heteroge-



neity in the final product are also important considerations (Stoll et al., 1996). The design of novel bioreactor configurations for large-scale culture in suspension (McKinney et al., 1995; Sauer et al., 2000), careful monitoring and control of culture conditions (Monica et al., 1993; Nyberg et al., 1999; Schneider et al., 1996), and engineering of the production cell lines to improve glycosylation and assembly might solve some of these problems (Davies et al., 2001; Weikert et al., 1999).

Recombinant IgAs have been shown to be effective against pathogens such as bacteria and viruses. In addition, IgA might also be protective against cancer cells. Although mice do not express an Fc $\alpha$ RI homologue, the use of human Fc $\alpha$ RI transgenic mice (van Egmond et al., 1999) might facilitate the study of the role of IgA in immune protection in various disease models. IgA is important in mucosal secretions and SC has an important role in anchoring SIgA to the mucus; however, most clinical trials have been done using oral or nasal administration of pIgA lacking SC (Zeitlin et al., 1999). With all things considered, we can expect recombinant IgA and SIgA to assume an ever increasing role in the clinic.

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