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Serum IgA Fc effector functions in infectious disease and cancer

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Abstract

Immunoglobulin A (IgA) is the most abundant antibody isotype present at mucosal surfaces and the second most abundant in human serum. In addition to preventing pathogen entry at mucosal surfaces, IgA can control and eradicate bacterial and viral infections through a variety of antibody-mediated innate effector cell mechanisms. The role of mucosal IgA in infection (e.g. neutralisation) and in inflammatory homeostasis (e.g. allergy and autoimmunity) has been extensively investigated, in contrast serum IgA is comparatively understudied. IgA binding to Fc alpha receptor plays a dual role in the activation and inhibition of innate effector cell functions. Mounting evidence suggests serum IgA induces potent effector functions against various bacterial and some viral infections including *Neisseria meningitidis* and rotavirus. Furthermore, in the era of immunotherapy, serum IgA provides an interesting alternative to classical IgG monoclonal antibodies to treat cancer and infectious pathogens. Here we discuss the role of serum IgA in infectious diseases with reference to bacterial and viral infections and the potential for IgA as a monoclonal antibody therapy.

Abbreviations:

ADCC: Antibody dependent cellular cytotoxicity
dIgA: Dimeric IgA
Fc: Fragment crystallisable region
FcαRI/CD89: Fc alpha receptor I (IgA Fc receptor)/cluster of differentiation 89
HIV: Human immunodeficiency virus
Ig: Immunoglobulin
IgA: Immunoglobulin A
ITAM: Immunoreceptor tyrosine-based activation motif
ITAMi: Immunoreceptor tyrosine-based activation motif inhibitory
NETs: Neutrophil extracellular traps
mAb: Monoclonal antibody
mIgA: Monomeric IgA
pIgA: Polymeric IgA

sIgA: Secretory IgA

Introduction

Immunoglobulins (Ig) are involved in the control and clearance of infectious diseases including viral (e.g. human immunodeficiency virus (HIV)), bacterial (e.g. *Mycobacterium tuberculosis, Neisseria meningitidis)* and parasitic pathogens (e.g. *Plasmodium* spp., *Leishmania* spp.) via various different mechanisms including neutralisation, and fragment crystallisable (Fc) effector functions including antibody-dependent cellular cytotoxicity (ADCC), phagocytosis and complement activation [1]. IgG has been extensively studied and this is highlighted by the dozens of IgG monoclonal antibodies (mAb) approved for therapeutic use by the US FDA [2]. Recently there have been a growing appreciation for other antibody isotypes including IgA as mAb therapeutics for cancer treatment and some viral and bacterial pathogens [3-5]. IgA can neutralise invading pathogens and induce a range of Fc effector functions to control and clear various bacterial (e.g. *Neisseria meningitidis* and *Streptococcus pneumoniae*) and viral infections (e.g. rotavirus and HIV) [4, 6-10]. Furthermore, IgA maintains homeostasis of inflammation at mucosal surfaces and in the blood and tissues [11]. Mucosal IgA is important for first line defence from invading pathogens at mucosal surfaces. However, the role of serum IgA and associated Fc functions in infectious disease is incomplete and understudied. Here we will discuss serum IgA Fc effector functions in the context of control and elimination of invasive pathogens.

IgA structure

The five human antibody isotypes (IgG, IgA, IgE, IgD and IgM) mediate an array of functional activities. IgA is the most abundant antibody at mucosal surfaces, and the second most abundant in serum (~15%; 2-3mg/ml) behind IgG (80%; ~10-20mg/ml) [12]. More IgA is synthesised per day compared to all other antibody isotypes combined (66mg/ml/day) [12], however, rapid catabolism of serum IgA results in a relatively short half-life (4-6 days) [13]. IgA consists of the typical monomeric antibody structure (Fig. 1) with differences in N-linked glycans and disulphide bridge arrangements distinguishing IgA from other antibody isotypes. The Fragment antigen-binding (Fab) region is critical for antigen binding, neutralization and opsonisation; the Fc portion is essential for initiating innate immune effector functions. Two heavy and light chains make up IgA, each folded into various globular domains including four heavy chain domains (VH, Cα1, Cα2 and Cα3) and two light chain domains (VL and CL) (Fig. 1).

Two IgA subclasses, IgA1 and IgA2, have been isolated from humans, gibbons, gorillas and chimpanzees and are distinguished by the length of the hinge region, numerous sequence differences in heavy chain constant regions and glycosylation patterns (Fig. 1) [14]. However, most other non-human primates and mammals including mice, possess one IgA subclass (IgA2 like), with the exception of orangutans which only possess IgA1 [14]. IgA1 adopts a T-shaped formation due to an elongated hinge region including a 16 amino acid insertion (Fig. 1). IgA2 lacks this insertion and adopts a protease resistant closed hinge formation resulting in IgA2's characteristic Y-shape (Fig. 1). Currently only one IgA1 allotype has been identified in humans and two IgA2 allotypes; IgA2m(1) and IgA2m(2) distinguished by the presence or absence of disulphide bridges between the heavy and light chains and different glycosylation patterns [12] with a third possible allotype also described IgA2n [15]. The functional differences of IgA allotypes are yet to be characterised, however it is reasonable to predict that variation in structure of the IgA2m(1) and IgA2m(2) allotypes would influence functional characteristics similar to IgG allotypes [16, 17]. Glycosylation of IgA1 differs from that of IgA2 in that 3-5 *O*-linked oligosaccharides are present in the extended hinge region [18], affecting the hinge structure (Fig. 1) [18]. Furthermore, both IgA subclasses carry N-linked oligosaccharides making up 6-7% molecular mass of IgA1 and 8-10% of IgA2 [19] (Fig. 1). Glycosylation patterns of sIgA can mediate anti-viral activity [20]. Sialic acid on the C-terminal tail (position 459) of sIgA interacts with haemagglutinin (HA) of Influenza A to disrupt cell surface attachment, however the impact of serum IgA glycosylation for other Fc functions is poorly understood [20]. It is interesting to speculate as to the reasons to why evolutionarily humans have maintained both IgA1 and IgA2 subclasses, whilst most other mammals only possess an IgA2-like subclass. We hypothesise that humans may have undergone divergent evolution from other mammals and adapted to the selection pressure on IgA1 by bacterial pathogens through evolution of IgA2. IgA2 is functionally important in mucosa, whilst IgA1 may be important for serum IgA functions (e.g. homeostasis or viral control), as reflected by differential distribution of IgA1 and IgA2. However, functional differences between IgA1 and IgA2 are yet to be fully characterised.

Heterogenous IgA molecular forms occur in humans consisting of monomeric (mIgA), dimeric (dIgA), polymeric (pIgA) and secretory IgA (sIgA) (Fig. 1). These molecular forms, in addition to IgA subclasses are differentially distributed throughout bodily compartments [21]. In serum, IgA is primarily mIgA1 (90%) synthesised in the bone marrow and transported into the blood [21].

Conversely in most mucosal secretions there is a proportional increase in IgA2 due to the protease resistant hinge region. Additionally, mucosal IgA is locally produced as dIgA in organised gut-associated lymphoid tissues (GALT) with site specific homing of IgA2 plasmablasts[22]. dIgA undergoes transcytosis through epithelial cells via polymeric immunoglobulin receptor (pIgR) into the mucosal lumen [21]. Throughout this process pIgR is cleaved resulting in a complex consisting of dIgA and secretory component (SC) which is released as sIgA (Fig. 1) [21]. Interestingly, the heterogenous forms of IgA have various roles in homeostasis and in infection.

Historically IgA has been considered a non-inflammatory antibody due to the involvement of sIgA in the down regulation of pro-inflammatory responses to pathogens and food antigens by preventing binding to other Fc receptors, rather than by activating anti-inflammatory pathways such as described below. The role of sIgA as a non-inflammatory antibody is highlighted in secretory IgA deficient (sIgAD) patients where an increased risk of autoimmunity and allergy is observed [23]. Extensive research of mucosal secretions supports the role of sIgA in passive and potentially active immune protection of newborns within colostrum and breast milk IgA [24]. Furthermore, adult sIgA maintains homeostasis of microbiota diversity and growth and contributes to passive immunity from invading pathogens [11]. In comparison, the role of serum IgA (mIgA, dIgA or pIgA) is relatively understudied.

Serum IgA and FcαRI

Recent technological developments have fostered the study of the serum IgA system in greater detail. It is clear that IgA is a poor activator of complement as it lacks a C1q binding site in the Fc region, although activation via the alternative and lectin pathway may be possible [25, 26]. Research over the past two decades shows a dichotomous role of serum IgA in inflammation [27, 28]. On one hand serum IgA can aid in homeostasis and anti-inflammatory responses and on the other hand serum IgA can induce inflammation [27]. Binding of IgA Fc region has been described for two IgA receptors: Fcα/μR (IgA and IgM) and Fc alpha receptor I (FcαRI) [28]. Additional IgA receptors have also been described; however their functions are yet to be characterised [28].

Human FcαRI (CD89) is constitutively expressed on cells of myeloid lineage including monocytes, eosinophils, some macrophages, intestinal dendritic cells, Kupffer cells, and

neutrophils, which are the most abundant cells in blood expressing FcαRI [29]. FcαRI has a ligand binding α chain mapping to chromosome 19 with the genes for NK cell receptors (KIR) and leukocyte Ig-like receptors, unlike IgG (FcγR) and IgE (FcεR) Fc receptors which map to chromosome 1 [30]. FcαRI shares closer homology with KIR and leukocyte Ig-like receptors compared to other Fc receptors (e.g. FcγR) [30]. FcαRI orthologs have been identified in various other mammals including rats, chimpanzees, cattle, horses, macaques and swine; however no known ortholog has been identified for mice [31-34]. Furthermore, in humans there are no reported cases of low or no FcαRI expression on myeloid cells, unlike defects reported in FcγRI which correlate with susceptibility to autoimmunity, chronic inflammation and infection [35], highlighting the potential importance of FcαRI in homeostasis and inflammation in humans. However, it is important to note that IgA deficiencies have been reported in humans, which have been associated with increased susceptibility to infectious diseases and autoimmunity [36].

The FcαRI α chain has two Ig-like-extracellular domains, transmembrane region and a short cytoplasmic tail without any recognised signalling motifs [37]. For signalling to occur FcαRI must associate with immunoreceptor tyrosine-based activation motif (ITAM), which can be phosphorylated to initiate signal transduction. Binding of monomeric serum IgA Cα1 and Cα2 Fc domains to the membrane distal domain of FcαRI occurs in 1:2 stoichiometry (1 IgA: 2 FcαRI) as shown in Figure 2 [37]. In the presence of ITAM, binding of IgA-antigen complex to FcαRI initiates signal cascades, ultimately leading to an inflammatory response (Fig. 2). However, when un-complexed mIgA associates with FcαRI, ITAM inhibitory (ITAMi) signal cascade is initiated resulting in inhibition of cells and associated anti-inflammatory/homeostatic role (Fig. 2) [37]. Furthermore, two FcαRI single nucleotide polymorphisms have been identified in humans: Ser248/Gly248 and Asp92/Asn92. Gly248 FcαRI has been associated with increased proinflammatory potential of serum IgA [36] and Asn92 FcαRI has been associated with increased risk of myocardial infarction [39]. Interestingly sIgA and dIgA bind poorly to FcαRI due to steric hindrance associated with the J chain and SC, however, dIgA has been reported to initiate effector functions via FcαRI against bacteria [37]. A recurrent theme in early literature suggests that serum dIgA and pIgA enhances phagocytosis compared to mIgA, even with steric hindrance [40, 41]. This may occur through FcαRI binding of dIgA at alternative binding sites, increased stability of IgA *in*

vitro and a greater capacity for antigen binding due to increased valency and avidity compared to mIgA (Table 1) [37].

Anti-tumour role of IgA

A small number of research groups have recently focused on IgA and FcαRI engagement to treat cancer [44]. Whilst most research has focused on IgG in mAb therapy due to potent anti-tumour mechanisms including complement activation and NK cell mediated ADCC; IgA appears to be potent in the recruitment and activation of neutrophils via the FcαRI to kill tumours, providing an attractive target for mAb anti-tumour therapy [45]. Several neutrophil IgA-mediated anti-tumour functions have been described *in vitro,* such as ADCC, phagocytosis, immune cell recruitment, release of cytotoxic molecules and induction of necrosis [44, 46]. Target-specific IgA mAbs enable formation of an immunological synapse by bringing neutrophils and target tumour cells together to enhance killing (Fig. 3). Recently, IgA mAbs targeting tumour cells such as HER2 (mammary carcinoma) and CD20 (B cell lymphoma) have shown promising anti-tumour effects [46]. Interestingly, the use of FcαRI transgenic mouse models has shown that IgA2 anti-EGFR antibodies can induce tumour cell killing, most likely mediated by macrophages [3]. However, more *in vivo* work is needed to dissect the contribution of FcαRI expressing effector cells in tumour killing. While there are several properties of IgA that make it advantageous as an anti-tumour mAb, IgG remains the antibody isotype of choice when it comes to mAb development as outlined in Table 1 Reviewed by [46]. Moreover, there is great debate in the field as to how effective IgA will be as a mAb therapy as high concentrations of serum IgA can be extremely detrimental as observed in the case of IgA nephropathy [47]. Furthermore, technologies available for the expression and purification of IgA (especially dimeric/polymeric forms) are comparatively more complicated than IgG [48]. However, modifications to IgA mAb can improve half-life and stability [49, 50]. Combinations of IgG and IgA mAbs can enhance tumour killing and work on "cross type antibodies" such as IgGA and tandem antibodies combine the best of both IgG (complement binding) and IgA (cytotoxicity/phagocytosis) anti-tumour effects [51].

Bacteria

Invasive bacterial infections can cause severe disease such as sepsis and meningitis. Early research from the 1970's through to the early 2000's highlights the role of serum IgA in the second (serum)

and potentially third line (liver) of defence from bacteria that enter the blood and tissues. Killing of various bacterial species including *Streptococcus pneumoniae*, *Bordetella pertussis*, *Escherichia coli*, *Staphylococcus aureus* and *Neisseria meningitidis* was associated with IgA mediated intracellular killing via phagocytosis as highlighted in various vaccine studies (Fig. 3) [40, 41]. Johnson *et al*. [41] observed an initial capsule specific serum pIgA response in both natural infection (1 month) and in immunisation (1-3 months). Janoff *et al*. [40] later reported killing of *S. pneumoniae* via phagocytosis using human polymorphonuclear leukocytes (PMN) and HL-60's mediated through binding of capsule specific serum pIgA to FcαRI. Interestingly, phagocytosis of *S. pneumoniae* in this study also required complement as shown by inhibition of FcαRI and CD35/CD11b, where killing was reduced by 50%. Thus, killing of the *S. pneumoniae* in the blood involves a combination of serum pIgA/FcαRI and complement [40]. Anti-bacterial phagocytosis mediated by serum IgA/FcαRI has been observed against *B. pertussis* in FcαRI transgenic mice using IgA-coated *B. pertussis* with human PMN leading to enhanced bacterial clearance in the lungs [6]. The phagocytic role of serum IgA in other bacterial species is more controversial, like that of *Neisseria* spp., the causative agents of gonorrhoea (*N. gonorrhoeae*) and meningitis (*N. meningitidis*) [7]. Some studies have reported IgA opsonised bacteria being phagocytosed, whilst others fail to observe such a phenomenon [7, 56, 57]. Under "normal" conditions (not vaccine studies) serum IgA often fail to induce phagocytosis of *Neisseria* spp. and we now understand that this is due to secretion of anti-IgA molecules discussed below (see section Anti-IgA Mechanisms) [56]. Furthermore, the role of IgA in the third line of defence was demonstrated in an *in vivo* study using Kupffer cells of the liver which naturally express FcαRI. van Egmond *et al*. [58] observed efficient removal of serum IgA-opsonized *E. coli* from portal circulation mediated by interaction between serum IgA (mIgA, dIgA and pIgA) and FcαRI. It is evident from existing research that serum IgA and FcαRI have the potential to initiate phagocytosis of IgA opsonised bacteria.

Serum IgA can induce additional effector functions such as ADCC and powerful neutrophil effector functions, although limited literature describes such processes in bacterial infection (Fig. 3) [37]. ADCC has been observed to occur using vaccine-induced sIgA and serum IgA against various bacterial species including *Salmonella enterica* serotype typhi [59]. Interestingly, other structures such as neutrophil extracellular traps (NETs) may also be key to IgA/FcαRI role in bacterial infection. NETs are web-like structures extruded by neutrophils trapping and killing

pathogens [60]. NET formation can occur in two forms; rapid formation within minutes independent of reactive oxygen species (ROS) or slow formation over several hours dependent on generation of ROS, resulting in cell membrane rupture and cell death, commonly referred to as NETosis [61]. Recently Aleyd *et al*. [60] observed *S. aureus* opsonised with IgA resulted in NETosis via the FcαRI, compared to non-IgA opsonised bacteria which did not. The study of serum IgA in vaccine settings has highlighted the potential of IgA Fc effector function in bacterial clearance. However, in natural infection, as briefly mentioned above regarding *Neisseria* spp., bacteria can overcome the anti-bacterial Fc effector functions of serum IgA [56, 62].

Anti-IgA mechanisms

Evolution of anti-IgA bacterial mechanisms are a unique feature of many pathogenic bacteria highlighting the importance of IgA in the control and clearance of invasive bacterial diseases including *N. meningitidis*, *Haemophilus influenzae* and group A and B Streptococci. Two such mechanisms include IgA proteases and IgA binding proteins (Fig. 3). Interestingly such anti-IgA mechanisms are yet to be reported for viruses, although some viruses have evolved to secrete FcγR blocking proteins [63]. This suggests that IgG mediated Fc functions may evolutionarily be more efficient at viral control than IgA mediated Fc mechanisms. Furthermore, evolution of alternative mechanisms, such as B cell dysfunction in HIV, ultimately disrupts antibody maturation as a whole, including the function of IgA [64].

Bacterial mechanisms

IgA Proteases

IgA1 proteases are secreted by many bacterial pathogens including *N. meningitidis*, *Haemophilus influenzae*, and *S. pneumoniae* to aid invasion into tissues and potentially the blood leading to septicaemia and bacterial meningitis. These enzymes cleave the exposed hinge region of IgA1 at various different sites including specific Pro-Ser or Pro-Thr peptide bond [62]. Furthermore, cleaved IgA1 may compete for functional antibodies via binding of the Fab region to antigen preventing binding of intact antibodies [62]. These proteins have arisen through convergent evolution and are associated with virulence [65]. Closely related strains of these bacteria lacking IgA1 proteases are non-virulent [65]. Interestingly some bacteria including *Pseudomonas aeruginosa* secrete broad spectrum proteases that can cleave IgA1 and IgA2. Although, IgA2 possesses a closed and more

protected hinge region, bacteria such as *Clostridium ramosum* and *Pasteurella multocida* secrete proteases that cleave IgA2m(1) and IgA2m(2) respectively [66, 67].

IgA binding proteins

Another evasion mechanism present in bacteria are IgA binding proteins expressed by many strains of group A and B Streptococci. Streptococcus group A possess Arp4 and Sir22 (M peptide family) IgA binding proteins associated with virulence and group B Streptococcus has an unrelated β protein [68]. These proteins interact with the Fc inter-domain region between the Cα2 and Cα3 domains, competing for FcαRI binding and inhibits IgA Fc functions in natural infections [68]. An IgA binding protein has also been identified in pathogenic *E. coli* (EsiB) which impairs neutrophil activation via IgA [57]. Vaccination and mAb therapy aiming to increase serum IgA levels may overwhelm bacterial evasion mechanisms and thus induce effective clearance of bacteria via IgA/FcαRI activation. However, prolonged elevation of IgA levels may be detrimental in the long-term [42] (see section The future of IgA in infectious disease mAb therapy).

Viruses

Although research into the role of serum IgA in viral infections is less comprehensive compared to bacterial infection, the potential, for serum IgA to mediate protection is highlighted in rotavirus and human immunodeficiency virus (HIV) infections. In various rotavirus vaccine trials, serum IgA has been established as a correlate of protection for vaccine efficacy in a systemic review of anti-rotavirus serum IgA titres of Rotarix [RV1] and RotaTeq [RV5] vaccines [69]. Patel *et al.* [69] proposed that serum IgA titres >90 post vaccination showed a significant increase in efficacy of the vaccines. However, in a study with children from the United States showed greater IgA titres (>200) correlated with protection from natural infection [70]. As for the mechanism of protection, it has been hypothesised the serum and/or sIgA may neutralise rotavirus [71]. However, work using IgA mAb's directed against the intermediate capsid protein VP6 of rotavirus in mice did not neutralise the virus, but inhibition of viral transcription in epithelial cells was observed [4]. The role of serum IgA Fc functions in rotavirus protection and clearance is yet to be reported.

HIV

The protective potential of serum IgA has been suggested in elite controllers (ECs) (individuals whom spontaneously control HIV-1 viremia) where higher titres of HIV-1-specific serum IgA have been observed compared to HIV-1 progressors [72]. *In vitro* studies have demonstrated that monoclonal IgA has the capacity to activate antibody functions against HIV-1 antigens (ADCC and phagocytosis) [10, 73]. Furthermore, mucosal IgA (secretory IgA) may prevent HIV-1 infection via immune exclusion/opsonisation as observed in highly exposed seronegative individuals (HESN) and various non-human primate vaccine trials (Fig. 3) [74].

However, the role of serum IgA in HIV-1 infection is controversial. This was highlighted by the protective RV144 human HIV-1 vaccine trial (31.2%) where HIV-1-specific IgG was associated with ADCC and protection from HIV-1 infection in vaccinated individuals [75]. However, RV144-induced serum IgA was associated with reduced ADCC and vaccine efficacy, as a result of IgA epitope competition with protective HIV-1-specific IgG for the same binding site on HIV envelope proteins [52]. Interestingly, low titres of HIV-1 specific antibodies are produced especially during chronic infection [74]. This suggests that the probability of HIV-specific IgA complexed with HIV-1 binding to FcαRI, initiating ITAM signalling and associated effector cell functions may be very low. Furthermore, un-complexed serum mIgA may initiate ITAMi signalling via FcαRI, dampening inflammatory cellular effector functions. Thus, polarising the immune response to an anti-inflammatory response and hindering viral clearance (Fig. 3).

The future of IgA in infectious disease mAb therapy

In the era of mAb therapy, IgA may provide a viable alternative to IgG mAb for various bacterial and viral diseases including *Mycobacterium tuberculosis* the causative agent of tuberculosis. In 2011, FcαRI transgenic mice showed protection against Tuberculosis after being given a novel human IgA (monomeric IgA1) monoclonal antibody as part of passive immunotherapy [5]. Balu *et al.* [5] hypothesised that binding of mIgA complexed with *M. tuberculosis* to FcαRI-positive alveolar macrophages and/or neutrophils activated anti-bacterial activity of the infected cells. As knowledge of chimeric IgA mAb design for cancer therapy increases, researchers can begin tailoring of mAbs for bacterial clearance such as increasing resistance to bacterial proteases and IgA binding proteins and enhancing activation of potent IgA Fc effector functions.

Limitations of IgA in infectious diseases and mAb therapy

Although mice models have been extensively used in the study of serum IgA and mAb therapy, there are several substantial differences in the IgA systems between humans and mice summarised in Table 2. Although recombinant FcαRI mice models have been created, translation of serum IgA research in transgenic mice infection to human infections should be interpreted carefully.

IgA autoantibodies have been reported as the mediator for several diseases including IgA nephropathy (elevated IgA levels), rheumatoid arthritis, coeliac disease and various IgA associated skin diseases reviewed in [44]. In many of these cases, elevated IgA levels coincide with increased IgA autoantibodies, resulting in high levels of inflammation including excessive activation of neutrophils. This suggests that prolonged elevation of serum IgA, especially IgA targeting self-antigens, can have dire consequences. Therefore, extreme care should be taken when developing IgA mAbs and a personalised medicine approach may need to be considered based on basal serum IgA levels and IgA autoantibody levels to maintain a healthy balance between inflammation and anti-inflammatory mechanisms.

Future Directions and Conclusions

Creating a balance between inflammatory response to clear infection whilst not inducing an over inflammatory environment is crucial to effective serum IgA response to pathogenic infections. However, further research into FcαRI signalling pathways (ITAM and ITAMi) is critical to understand this balance. Uncovering how these pathways moderate inflammation, downregulates the overall activation of effector cells and discovering if this is associated with persistence of infection will give researchers insight into the importance of serum IgA in infection. Furthermore, the role of IgA/FcαRI in infectious disease appears to vary between pathogens (bacterial or viral) and between species (e.g. HIV and rotavirus.). Thus, IgA/FcαRI level of activation and/or inhibition should be characterised independently for each pathogen to confirm the respective roles of IgA function for specific infections. Renewed research will provide valuable insights as to the therapeutic potential of serum IgA.

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References:

1. Arnold KB, Chung AW. Prospects from systems serology research. *Immunology* 2018; **3**: 279-289.
2. Singh S, Kumar NK, Dwiwedi P, *et al*. Monoclonal antibodies: a review. *Curr Clin Pharmacol* 2018; **2**: 85-99.
3. Boross P, Lohse S, Nederend M, *et al.* IgA EGFR antibodies mediate tumour killing *in vivo*. *EMBO Mol Med* 2013; **8**: 1213-1226.
4. Feng N, Lawton JA, Gilbert J, *et al.* Inhibition of rotavirus replication by a non-neutralizing, rotavirus VP6–specific IgA mAb. *J Clin Invest* 2002; **9**: 1203-1213.
5. Balu S, Reljic R, Lewis MJ, *et al.* A novel human IgA monoclonal antibody protects against Tuberculosis. *J Immunol* 2011; **5**: 3113-3119.
6. Hellwig SM, van Spriel AB, Schellekens JF, Mooi FR, van de Winkel JG. Immunoglobulin A-mediated protection against *Bordetella pertussis* infection. *Infect Immun* 2001; **8**: 4846-4850.
7. Lowell G, Smith L, Griffiss JM, Brandt B. IgA-dependent, monocyte-mediated, antibacterial activity. *J Expl Med* 1980; **2**: 452-457.
8. Griffiss JM, Broud DD, Bertram MA. Bactericidal activity of meningococcal antisera: blocking by IgA of lytic antibody in human convalescent sera. *J Immunol* 1975; **6**: 1779-1784.
9. Vidarsson G, van der Pol W-L, van den Elsen JMH, *et al*. Activity of human IgG and IgA subclasses in immune defense against *Neisseria meningitidis* Serogroup B. *J Immunol* 2001; **10**: 6250-6256.
10. Duchemin M, Khamassi M, Xu L, Tudor D, Bomsel M. IgA targeting human immunodeficiency virus-1 envelope gp41 triggers antibody-dependent cellular cytotoxicity cross-clade and cooperates with gp41-specific IgG to increase cell lysis. *Front Immunol* 2018; **9**: 244.
11. Corthésy B. Multi-faceted functions of secretory IgA at mucosal surfaces. *Front Immunol* 2013; **4**: 185.
12. Kerr M. The structure and function of human IgA. *Biochem J* 1990; **2**: 285.
13. Morell A, Skvaril F, Noseda G, Brandun S. Metabolic properties of human IgA subclasses. *Clin Exp Immunol* 1973; **4**: 521.

14. Kawamura S, Saitou N, Ueda S. Concerted evolution of the primate immunoglobulin α-gene through gene conversion. *J Biol Chem* 1992; **11**: 7359-7367.
15. Chintalacharuvu KR, Raines M. and Morrison SL. Divergence of human α-chain constant region gene sequences. A novel recombinant α 2 gene. *J Immunol* 1994; **11**: 5299-5304.
16. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* 2014; **5**: 520.
17. Damelang T, Rogerson SJ, Kent SJ, Chung AW. Role of IgG3 in infectious diseases. *Trends Immunol* 2019; **3**: 197-211.
18. Royle L, Roos A, Harvey DJ, *et al.* Secretory IgA N-and O-glycans provide a link between the innate and adaptive immune systems. *J Biol Chem* 2003; **22**: 20140-53.
19. Tomana M, Niedermeier W, Mestecky J, Skvaril F. The differences in carbohydrate composition between the subclasses of IgA immunoglobulins. *Immunochemistry* 1976; **4**: 325-328.
20. Maurer MA, Meyer L, Bianchi M, *et al*. Glycosylation of human IgA directly inhibits Influenza A and other sialic-acid-binding viruses. *Cell Rep* 2018; **1**: 90-99.
21. van Egmond M, Damen CA, van Spriel AB, Vidarsson G, van Garderen E, van de Winkel JG. IgA and the IgA Fc receptor. *Trends Immunol* 2001; 4: 205-211.
22. Pakkanen SH, Kantele JM, Moldoveanu Z, *et al*. Expression of homing receptors on IgA1 and IgA2 plasmablasts in blood reflects differential distribution of IgA1 and IgA2 in various body fluids. *Clin Vaccine Immunol* 2010; **3**: 393-401.
23. Erkoçoğlu M, Metin A, Kaya A, *et al*. Allergic and autoimmune disorders in families with selective IgA deficiency. *Turk J Med Sci* 2017; **2**: 592-598.
24. Hanson LÅ, Korotkova M. The role of breastfeeding in prevention of neonatal infection. *Semin Neonatal Med* 2002; **4**: 275-281.
25. Roos A, Bouwman LH, van Gijlswijk-Janssen DJ, Faber-Krol MC, Stahl GL, Daha MR. Human IgA activates the complement system via the mannan-binding lectin pathway. *J Immunol* 2001; **5**: 2861-2868.
26. Chuang PD, Morrison SL. Elimination of N-linked glycosylation sites from the human IgA1 constant region: effects on structure and function. *J Immunol* 1997; **2**: 724-32.
27. Monteiro RC. The role of IgA and IgA Fc receptors as anti-inflammatory agents. *J Clin Immunol* 2010; **1**: 61-64.
28. Monteiro RC, van De Winkel JG. IgA Fc receptors. *Annu Rev Immunol* 2003; **1**: 177-204.

29. Hamre R, Farstad I, Brandtzaeg P, Morton H. Expression and modulation of the human immunoglobulin A Fc receptor (CD89) and the FcR γ chain on myeloid cells in blood and tissue. *Scand J Immunol* 2003; **6**: 506-516.
30. Kremer E, Kalatzis V, Baker E, Callen D, Sutherland G, Maliszewski C. The gene for the human IgA Fc receptor maps to 19q13. 4. *Hum Genet* 1992; **1**: 107-108.
31. Morton HC, Pleass RJ, Storset AK, Brandtzaeg P, Woof JM. Cloning and characterization of equine CD89 and identification of the CD89 gene in chimpanzees and rhesus macaques. *Immunology* 2005; **1**: 74-84.
32. Morton HC, Pleass RJ, Storset AK, *et al*. Cloning and characterization of an immunoglobulin A Fc receptor from cattle. *Immunology* 2004; **2**: 204-211.
33. Maruoka T, Nagata T, Kasahara M. Identification of the rat IgA Fc receptor encoded in the leukocyte receptor complex. *Immunogenetics* 2004; **10**: 712-716.
34. Chen Y, Liu Y, Zhang G, *et al*. Cloning and characterization of the IgA Fc receptor from swine. *J Microbiol Biotechnol* 2016; **12**: 2192-2198.
35. Takai T. Roles of Fc receptors in autoimmunity. *Nat Rev Immunol* 2002; **8**: 580.
36. Vo Ngoc DL, Krist L, van Overveld FJ, Rijkers GT. The long and winding road to IgA deficiency: causes and consequences. *Expert Rev Clin Immunol* 2017; **4**: 371-382.
37. Breedveld A, van Egmond M. IgA and FcαRI: Pathological roles and therapeutic opportunities. *Front Immunol* 2019;**10**: 553.
36. Wu J, Ji C, Xie F, Langefeld CD, *et al*. FcαRI (CD89) alleles determine the proinflammatory potential of serum IgA. *J immunol* 2007; **6**: 3973-3982.
39. Iakoubova OA, Tong CH, Chokkalingam AP, *et al*. Asp92Asn polymorphism in the myeloid IgA Fc receptor is associated with myocardial infarction in two disparate populations: CARE and WOSCOPS. *Arterioscler Thromb Vasc Biol* 2006; **12**: 2763-2768.
40. Janoff EN, Fasching C, Orenstein JM, Rubins JB, Opstad NL, Dalmasso AP. Killing of *Streptococcus pneumoniae* by capsular polysaccharide–specific polymeric IgA, complement, and phagocytes. *J Clin Invest* 1999; **8**: 1139-1147.
41. Johnson S, Opstad NL, Douglas J, Janoff EN. Prolonged and preferential production of polymeric immunoglobulin A in response to *Streptococcus pneumoniae* capsular polysaccharides. *Infect Immun* 1996; **10**: 4339-4344.

42. Mkaddem SB, Rossato E, Heming N, Monteiro RC. Anti-inflammatory role of the IgA Fc receptor (CD89): from autoimmunity to therapeutic perspectives. *Autoimmun Rev* 2013; **6**: 666-669.
43. Mkaddem SB, Murua A, Flament H, *et al*. Lyn and Fyn function as molecular switches that control immunoreceptors to direct homeostasis or inflammation. *Nat Commun* 2017; **1**: 246.
44. Heineke MH, van Egmond M. Immunoglobulin A: magic bullet or Trojan horse? *Eur J Clin Invest* 2017; **2**: 184-192.
45. Dechant M, Valerius T. IgA antibodies for cancer therapy. *Crit Rev Oncol Hematol* 2001; **1-2**: 69-77.
46. van Egmond M, Bakema JE. Neutrophils as effector cells for antibody-based immunotherapy of cancer. *Semin Cancer Biol* 2013; **3**: 190-199.
47. Burton DR, Desrosiers RC, Doms RW, *et al*. HIV vaccine design and the neutralizing antibody problem. *Nat Immunol* 2004; **3**: 233.
48. Bakema JE, van Egmond M. July. Immunoglobulin A: A next generation of therapeutic antibodies?. *MAbs* 2011; **4**: 352-361.
49. Lohse S, Meyer S, Meulenbroek LA, *et al*. An anti-EGFR IgA that displays improved pharmacokinetics and myeloid effector cell engagement *in vivo*. *Cancer Res* 2016; **2**: 403-417.
50. Meyer S, Nederend M, Jansen JM, *et al*. Improved *in vivo* anti-tumor effects of IgA-Her2 antibodies through half-life extension and serum exposure enhancement by FcRn targeting. *MAbs* 2016; **1**: 87-98.
51. Kelton W, Mehta N, Charab W, *et al*. IgGA: a "cross-isotype" engineered human Fc antibody domain that displays both IgG-like and IgA-like effector functions.*Chem Biol* 2014; **12**: 1603-1609.
52. Tomaras GD, Ferrari G, Shen X, *et al*. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. *Proc Natl Acad Sci U S A* 2013; **22**: 9019-90124.
53. van Egmond M, van Spriel AB, Vermeulen H, Huls G, van Garderen E, van de Winkel JG. Enhancement of polymorphonuclear cell-mediated tumor cell killing on simultaneous engagement of FcγRI (CD64) and FcαRI (CD89). *Cancer Res* 2001; **10**: 4055-4060.

54. Worley MJ, Fei K, Lopez-Denman AJ, Kelleher AD, Kent SJ, Chung AW. Neutrophils mediate HIV-specific antibody-dependent phagocytosis and ADCC. *J Immunol Methods* 2018;**457**:41-52.
55. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci Transl Med* 2014; **228**:228ra38-ra38.
56. Mulks MH, Plaut AG, Feldman HA, Frangione B. IgA proteases of two distinct specificities are released by *Neisseria meningitidis*. *J Exp Med* 1980; **5**: 1442-1447.
57. Pastorello I, Paccani SR, Rosini R, *et al*. EsiB, a novel pathogenic *Escherichia coli* secretory immunoglobulin A-binding protein impairing neutrophil activation. *MBio* 2013; **4**: e00206-13.
58. van Egmond M, van Garderen E, van Spriel AB, *et al*. FcαRI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity. *Nat Med* 2000; **6**: 680.
59. Tagliabue A, Villa L, De Magistris MT, *et al*. IgA-driven T cell-mediated anti-bacterial immunity in man after live oral Ty 21a vaccine. *J Immunol* 1986; **5**: 1504-1510.
60. Aleyd E, van Hout MW, Ganzevles SH, *et al*. IgA enhances NETosis and release of neutrophil extracellular traps by polymorphonuclear cells via Fcα receptor I. *J Immunol* 2014; **5**: 2374-2383.
61. Yipp BG, Kubes P. NETosis: how vital is it? *Blood* 2013; **16**: 2784-94.
62. Mistry D, Stockley RA. IgA1 protease. *Int J Biochem Cell Biol* 2006; **8**: 1244-1248.
63. Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. *Trends Microbiol* 2000; **9**: 410-418.
64. Moir S, Fauci AS. Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease. *J Allergy Clin Immunol* 2008; **1**: 12-19.
65. Polissi A, Pontiggia A, Feger G, *et al*. Large-scale identification of virulence genes from *Streptococcus pneumoniae*. *Infect Imun* 1998; **12**: 5620-5629.
66. Pouedras P, Andre P, Donnio P, Avril J. Cleavage of immunoglobulin A1, A2 and G by proteases from clinical isolates of *Pasteurella multocida*. *J Med Microbio* 1992; **2**: 128-132.
67. Senda S, Fujiyama Y, Ushijima T, *et al*. *Clostridium ramosum*, an IgA protease-producing species and its ecology in the human intestinal tract. *Microbio Immunol* 1985; **11**: 1019-1028.

68. Pleass RJ, Areschoug T, Lindahl G, Woof JM. Streptococcal IgA-binding proteins bind in the Cα2-Cα3 interdomain region and inhibit binding of IgA to human CD89. *J Biol Chem* 2001; 11: 8197-8204.
69. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. *J Infect Dis* 2013; **2**: 284-294.
70. O'Ryan ML, Matson DO, Estes MK, Pickering LK. Anti-rotavirus G type-specific and isotype-specific antibodies in children with natural rotavirus infections. *J Infect Dis* 1994; **3**: 504-511.
71. Jiang B, Gentsch JR, Glass RI. The role of serum antibodies in the protection against rotavirus disease: an overview. *Clin Infect Dis* 2002; **10**: 1351-1361.
72. Nabi R, Moldoveanu Z, Wei Q, *et al*. Differences in serum IgA responses to HIV-1 gp41 in elite controllers compared to viral suppressors on highly active antiretroviral therapy. *PloS one* 2017; **7**: e0180245.
73. Wills S, Hwang K-K, Liu P, *et al*. HIV-1-specific IgA monoclonal antibodies from an HIV-1 vaccinee mediate galactosylceramide blocking and phagocytosis. *J Virol* 2018; **7**: e01552-17.
74. Lopez E, Shattock RJ, Kent SJ, Chung AW. The multifaceted nature of immunoglobulin a and its complex role in HIV. *AIDS Res Hum Retroviruses* 2018; **9**: 727-738.
75. Haynes BF, Gilbert PB, McElrath MJ, *et al*. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 2012; **14**: 1275-1286.
76. Shimizu Y, Honda S, Yotsumoto K, *et al*. Fc(α)/μ receptor is a single gene-family member closely related to polymeric immunoglobulin receptor encoded on Chromosome 1. *Immunogenetics* 2001; **8**: 709-711.
77. Woof JM, Kerr MA. IgA function--variations on a theme. *Immunology* 2004; 2: 175-177.
78. Wines BD, Ramsland PA, Trist HM, *et al*. Interaction of human, rat, and mouse immunoglobulin A (IgA) with Staphylococcal superantigen-like 7 (SSL7) decoy protein and leukocyte IgA receptor. *J Biol Chem* 2011; **38**: 33118-33124.
79. Rifai A, Fadden K, Morrison SL, Chintalacharuvu KR. The N-glycans determine the differential blood clearance and hepatic uptake of human immunoglobulin (Ig)A1 and IgA2 isotypes. *J Exp Med* 2000; **12**: 2171-2182.

80. Blaese RM, Strober W, Levy AL, Waldmann TA. Hypercatabolism of IgG, IgA, IgM, and albumin in the Wiskott-Aldrich syndrome. A unique disorder of serum protein metabolism. *J Clin Invest* 1971; **11**: 2331-2338.
81. Bournazos S, Ravetch JV. Diversification of IgG effector functions. *Int Immunol* 2017; **7**: 303-310.
82. Monteiro RC, Ben Mkaddem S, Benhamou M. Understanding Fc receptor involvement in inflammatory diseases: from mechanisms to new therapeutic tools. *Front Immunol* 2019; **10**: 811.

Tables

Table 1. Antibody properties of IgG1, IgA1, IgA2 and dIgA/pIgA in terms of effector function and viability as therapeutic mAb (summarised from [12, 13, 16, 49, 52-55, 81-82]).

Property	**IgG1**	**Serum IgA**		
		IgA1	**IgA2**	**dIgA/pIgA**
Half-life	~21 days (FcRn recycling)	5.9 days	4.5 days	*
Valency/avidity	+	+	+	+++
Expression/purification	+++	+/++		+
Neutralising/opsonisation capacity	+++	+		++
Neutrophil activation	+++	+++		++
NK cell mediated ADCC	+++	-		-
Myeloid cell mediated ADCC and phagocytosis	+++	++		++
Anti-inflammatory role	+ (FcγRIIb)	+++ (FcαRI)		++ (FcαRI)
Complement activation	+++ (all pathways)	+ (potentially alternative and lectin pathways)		
Therapeutic antibody	+++	++		

potential		
Diseases/conditions of interest	Various infectious diseases and some cancers	Some cancers, autoimmunity/allergy and some infectious diseases

- None
+ Weak
++ Moderate
+++ Strong
* contrasting literature reported

Table 2. Characteristics of human and mouse serum IgA systems

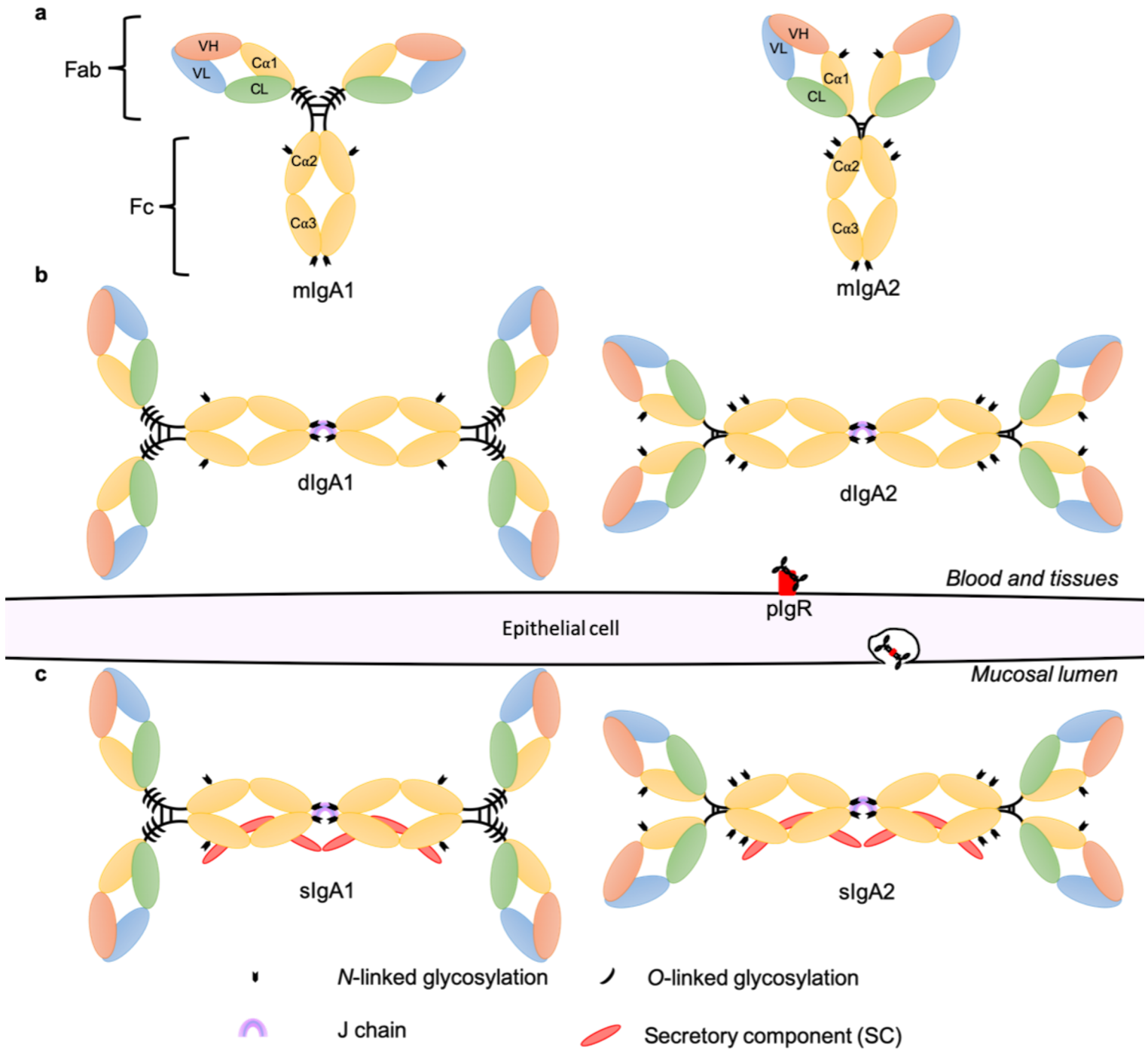
	Human	**Mouse**
Isotypes	Two (IgA1 and IgA2) [21]	One [21]
Major molecular form of serum IgA	Monomeric (IgAI) [21]	Dimeric Reviewed by [21]
Presence of Fcα/μR (CD351)	Yes Reviewed by [28]	Yes [76]
Presence of FcαRI (CD89)	Yes Reviewed by [28]	No [77]
Ability to bind bacterial IgA binding proteins	Yes [78]	No [78]
Human IgA half-life	4-6 days [79]	10-14 hours [80]

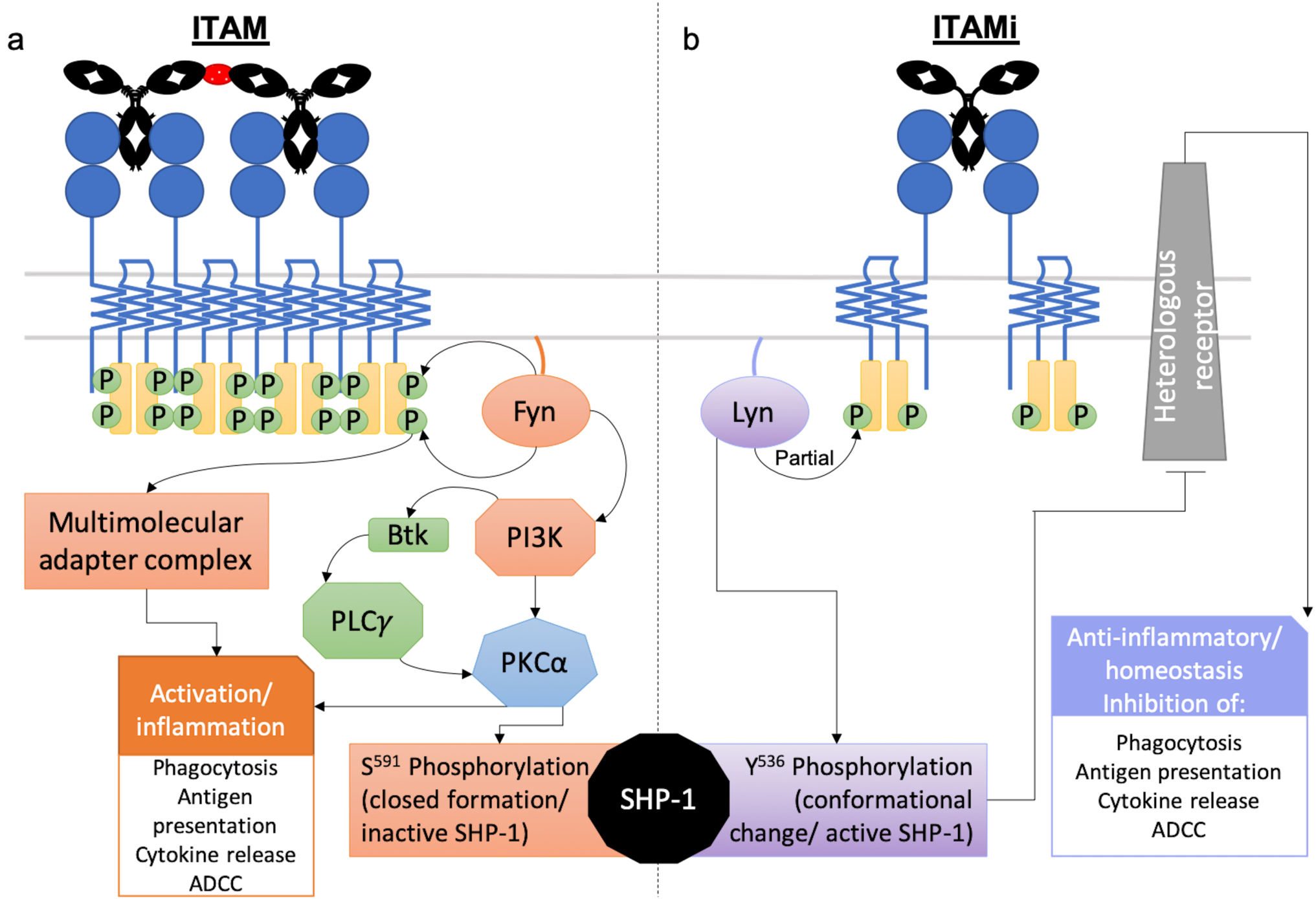
Figure legends

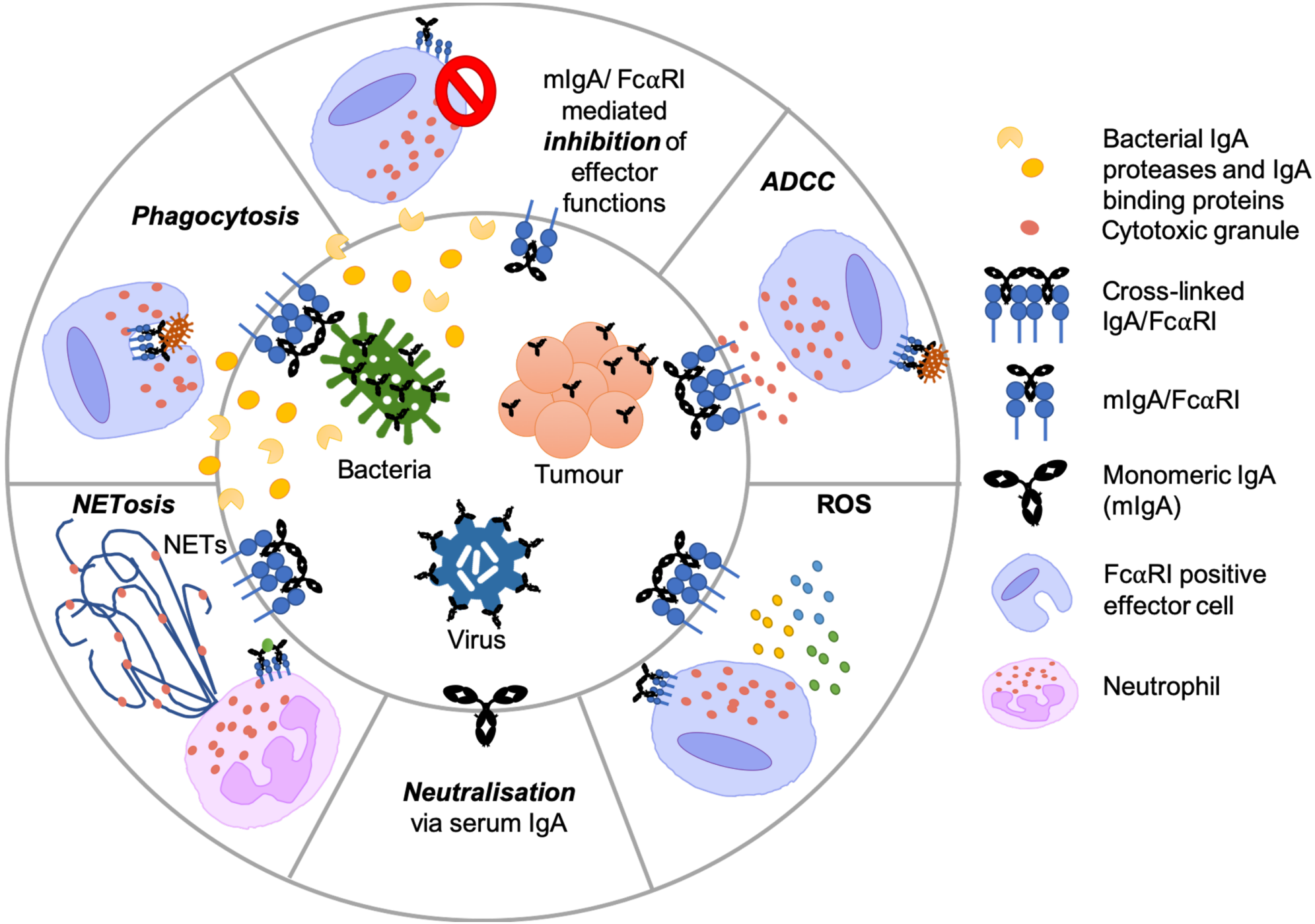
Figure 1. Schematic diagram of IgA subclasses; IgA1 and IgA2, glycosylation patterns and their respective heterogenous molecular forms. In blood and tissue compartments **(a)** monomeric IgA (mIgA) and to a lesser extent **(b)** dimeric IgA (dIgA) (two IgA monomer Fc portions connected via a joining (J) chain) are present. dIgA is secreted through epithelial cells via the polymeric immunoglobulin receptor (pIgR) into the mucosal lumen with secretory component (SC) to form **(c)** secretory IgA (sIgA).

Figure 2. Initiation of IgA/FcαRI ITAM and ITAMi signal cascades and resulting Fc effector functions reviewed by [42]**. (a)** IgA-antigen complex cross-linking of FcαRI initiates phosphorylation of ITAM with Fyn [43] followed by generation of (1) multimolecular adapter complex (Cbl, SLP-76, Grb2, CrkL, Shc, Sos, SHIP) and/or (2) phosphoinositide 3-kinase (PI3K) which phosphorylates Btk and activates protein kinase C (PKCα). PKCα ultimately leads to activation/inflammatory effector functions and inactivation of SHP-1 via S^{591} phosphorylation. **(b)** Un-complexed mIgA binding to FcαRI initiates partial phosphorylation of ITAM by Lyn, leading to ITAMi signalling. Lyn also phosphorylates SHP-1 at Y^{536} triggering a conformational change which activates SHP-1 leading to inhibition of heterogenous receptors causing the cell to enter a resting state and take on homeostatic (anti-inflammatory) functions [43].

Figure 3. Serum IgA effector functions dependent and independent (neutralisation) of FcαRI against bacteria, viruses and tumour cells and IgA counter measures enabling persistence of infection. Crosslinking of FcαRI with IgA results in FcαRI dependent effector functions via ITAM signalling (ADCC, phagocytosis, NETosis and ROS). Binding of mIgA to FcαRI leads to ITAMi and resulting effector cell inhibition aiding in persistence of infection/cancer. Release of anti-IgA molecules by bacteria reduces bacterial clearance via IgA.


a
Fab
VH
VL
Cα1
CL
Fc
Cα2
Cα3
mIgA1
mIgA2
b
dIgA1
dIgA2
Blood and tissues
pIgR
Epithelial cell
Mucosal lumen
c
sIgA1
sIgA2
N-linked glycosylation
O-linked glycosylation
J chain
Secretory component (SC)


a
ITAM
P
Fyn
Multimolecular adapter complex
Btk
PI3K
PLCγ
PKCα
Activation/ inflammation
Phagocytosis
Antigen presentation
Cytokine release
ADCC
S591 Phosphorylation (closed formation/ inactive SHP-1)
SHP-1
b
ITAMi
Lyn
Partial
Heterologous receptor
Y536 Phosphorylation (conformational change/ active SHP-1)
Anti-inflammatory/ homeostasis
Inhibition of:
Phagocytosis
Antigen presentation
Cytokine release
ADCC


mIgA/ FcαRI mediated *inhibition* of effector functions
ADCC
Phagocytosis
Bacteria
Tumour
NETosis
NETs
ROS
Virus
Neutralisation via serum IgA
Bacterial IgA proteases and IgA binding proteins
Cytotoxic granule
Cross-linked IgA/FcαRI
mIgA/FcαRI
Monomeric IgA (mIgA)
FcαRI positive effector cell
Neutrophil