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The Multifaceted Nature of Immunoglobulin A and Its Complex Role in HIV

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Abstract

IgA is the most abundant immunoglobulin in mucosal secretions, and understanding the role of IgA in both protection from HIV acquisition and modulation of HIV disease progression is a field of considerable controversy and renewed research interest. Analysis of the RV144 clinical trial associated plasma HIV envelope-specific monomeric IgA from vaccines with reduced vaccine efficacy. The RV144 trial, however, only assessed for plasma IgA, which was not further subclassed, and the role of mucosal IgA was not addressed as mucosal samples were not collected. On the other hand, several studies have detected envelope-specific IgA in mucosal secretions of highly exposed persistently seronegative cohorts, while recent macaque simian-HIV passive immunization studies have suggested a potentially protective role for mucosal IgA. It is well established that total IgA in serum appears to correlate with HIV disease progression. In contrast, a selective deficit of anti-HIV IgA responses in HIV infection is apparent, with a number of recent studies beginning to elucidate the mechanisms behind these dysfunctional IgA responses. In this review, we highlight the dichotomy that exists in the literature as to whether anti-HIV IgA is protective or harmful to the host. Herein, we emphasize the importance of distinguishing between monomeric, multimeric, and isoforms of IgA and review what is known about the complex and diverse interactions of various molecular forms of IgA with HIV in both the systemic circulation and mucosal compartments.

Keywords: IgA, IgA receptors, secretory IgA (SIgA), HIV, antibody, mucosal

Introduction

Immunoglobulins (Igs), also known as antibodies, are one of the key cornerstones of the adaptive immune system, which over the course of evolution have fine-tuned themselves to protect us from invading pathogens. Their ability to bind antigens with exquisite specificity and act as effective mediators of adaptive immunity is responsible for the efficacy of most protective and therapeutic vaccines that exist to this day.[1] Since the discovery of antibodies, a significant understanding of their functional mechanisms has been uncovered; however, this is far from complete. Of the five immunoglobulin classes (IgM, IgD, IgG, IgE, and IgA), IgA has a key role in protection from mucosal pathogens. The multifaceted nature of IgA has, however, meant that its potential role in protecting the host from HIV acquisition or disease progression has yet to be fully elucidated.

Structure and Subclasses of IgA

IgA is the most abundant antibody located at the most vulnerable interface with the environment, the mucosa. The mucosa presents the greatest site of external antigenic challenge to the host, and it is at this front line that IgA is continuously produced at a rate that far exceeds the synthesis of all other immunoglobulin classes.[2] IgA is also the second most prevalent antibody class in serum, with levels of IgA and IgG representing $\sim$15% and 80% of all antibodies in human serum, respectively.[3] In comparison with other immunoglobulins, IgA is unique, in that it displays significant heterogeneity in its molecular forms (Fig. 1), each with a distinctive distribution adapted to either the systemic or mucosal immune compartments (Table 1). In humans, IgA is subdivided into two subclasses, IgA1 and IgA2, which differ principally in heavy chain pairing and carbohydrate composition.[2]

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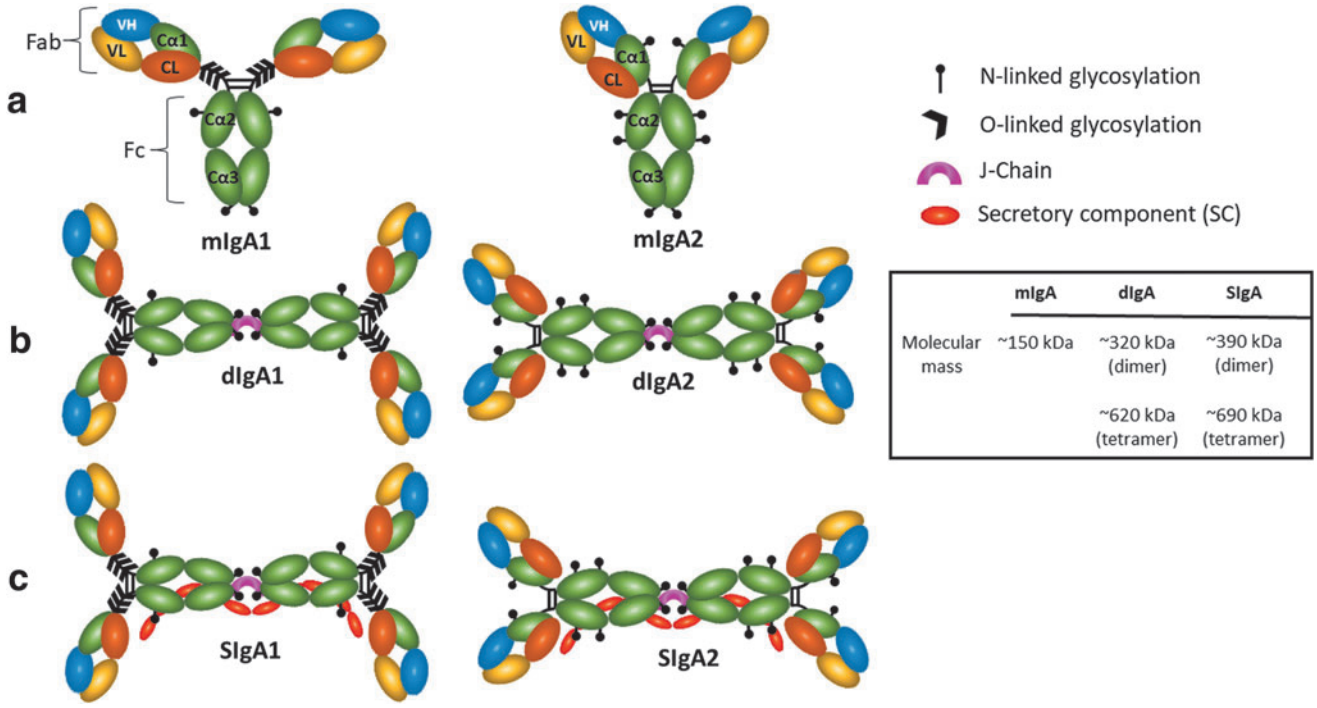

FIG. 1. **(a)** Human IgA1 and IgA2 molecules as monomeric (mIgA), **(b)** dimeric (dIgA1 and dIgA2), and **(c)** secretory forms (SIgA1 and SIgA2), with estimated molecular weight for each of the molecular forms. Color images are available online at www.liebertpub.com/aid

In serum, IgA derives from the bone marrow and is mainly (~90%) monomeric IgA1 (mIgA1).[4] In contrast, dimeric IgA (dIgA) locally produced by plasma cells within the lamina propria predominates at mucosal surfaces, with a proportional increase of IgA2 to IgA1 observed due to increased resistance of the hinge region of IgA2 to bacterial *proteases.*[5] The ratio of IgA1 to IgA2 varies in different mucosal secretions, with proportional increases of IgA2 observed particularly in rectal fluids, and female secretions[4] as presented in Table 1. Unlike IgG, IgA can polymerize through the linkage of multiple monomer units, predominantly dimers, to form dIgA using a 16-kDa joining chain (J chain)[6,7] (Fig. 1). IgA is further observed as a more specialized molecular form of dIgA, adapted for transport through the mucosal epithelium into secretions by the polymeric immunoglobulin receptor (pIgR).[8] This IgA, known as secretory IgA (SIgA), differs from dIgA by the presence of the 75-kDa secretory component, a proteolytic cleavage product of the pIgR, which is wrapped around the heavy chains and J chain of IgA monomers[7] (Fig. 1).

IgA Receptors

As with other immunoglobulins, the Fc region of IgA provides a crucial link between the humoral and cellular arms of the immune system. This interaction is mediated principally through the IgA-specific Fc receptor αRI (FcαRI), also known as CD89.[9] It is noted that other IgA receptors, although less characterized, have been described, which include CD71,[10] DC-SIGN/SIGNR1,[11] asialoglycoprotein receptor,[12] Fca/lR,[13] and FcRL4,[14] as well as mucins, which bind SIgA as it diffuses through mucus to coat and trap microorganisms.[15,16]

CD89 (FcαRI) is principally expressed on neutrophils, monocytes, dendritic cells, and eosinophils. On neutrophils,

TABLE 1. REPORTED IGA LEVELS (μG/ML) AND KNOWN ESTIMATED PERCENTAGES OF VARIOUS IGA MOLECULAR SUBCLASSES AND MOLECULAR FORMS IN HUMAN SERUM AND MUCOSAL SECRETIONS

	IgA total (μg/mL)	*IgA1 (%)*	*IgA2 (%)*	*SIgA (%)*	*mIgA (%)*	*pIgA (%)*	*References*
Serum	500–3,500	85	11–23	0.53–1	90	10	4,139,140
Tears	80–400	59	41		5	95	4,141
Saliva	99–206	63	37	56	4	96	139,142
Colostrum	12,340–53,800	40–77	23–59	95	5	95	143,144
Seminal plasma	11–36						145,146
Cervicovaginal fluid	21–118	50	50	15	20	80	139,147,148
Endocervical secretions	279			46			139
Intestinal fluid	166	70	30				142
Rectal fluid	3,624	30	70				149
Urine	0.1–1				10	90	150

mIgA: monomeric IgA, pIgA: polymeric IgA, SIgA: secretory IgA.

monocytes, and dendritic cells, CD89 has an estimated molecular mass of 55–75 kDa,[9] whereas eosinophil CD89 is ~70–100 kDa due to more extensive glycosylation.[17] CD89 is a low-to-moderate affinity receptor for IgA.[18] Monomeric IgA (mIgA) binds to CD89 only transiently, whereas polymeric IgA (pIgA) and IgA immune complexes (ICs) bind more avidly.[19] The initial association of mIgA and pIgA with CD89 is similar; however, mIgA dissociates more rapidly than pIgA.[19] Given that plasma IgA is predominantly mIgA, mIgA has been suggested to outcompete pIgA for binding to CD89, thus preventing receptor aggregation and consequent cellular activation.[19] Unlike FcγRs and FcϵRI, which bind with a 1:1 stoichiometry, two CD89 molecules have been shown to bind one IgA Fc region.[20]

FcαRI (CD89): A Bifunctional Regulator of IgA Responses

In the last few decades, numerous studies have found that IgA is capable of downregulating immune responses in the absence of antigen, suggesting an anti-inflammatory inhibitory role for the CD89 receptor.[21–24] The molecular mechanisms behind this were largely unknown until the ability of CD89 to mediate inhibition through the ITAM of its associated Fc$R\gamma$ chain was discovered.[21,25] The CD89 receptor is now well known to trigger both inhibitory and activating pathways, and it is the balance between these that is considered imperative for the role of IgA in maintaining homeostasis in both systemic and mucosal compartments.[21,22]

Several studies have since demonstrated the paradoxical nature of mIgA.[21,26,27] On one hand, in the absence of antigen, nonspecific serum mIgA can interact with the associated Fc$R\gamma$ chain of CD89 to initiate a cascade of anti-inflammatory effects through potent inhibitory ITAM signaling.[25] Such effects include the inhibition of IgG-mediated functions, including phagocytosis, Ab-dependent cell-mediated cytotoxicity (ADCC), and oxidative burst activity.[22,23,28–30] On the other hand, multimeric CD89 cross-linking in the presence of antigen by mIgA has been demonstrated to induce the opposite effect through a signaling pathway that induces cellular activation,[25] including ADCC, phagocytosis, respiratory burst, and release of various cytokines and inflammatory mediators.[31,32] IgA ICs have also been found to trigger neutrophil activation more effectively than IgG ICs.[33] Furthermore, a number of studies have manipulated the IgA-CD89 interaction as a therapeutic target against tumor cells in cancer.[34] This has been demonstrated with the use of antitumor IgA mAbs[35] and with bispecific antibodies to engage potent neutrophil-mediated effector functions such as ADCC, which trigger nonapoptotic tumor cell death.[36,37] CD89 thus serves as a key regulator between the anti- and proinflammatory immune responses of IgA.

Consistent with the previously described anti-inflammatory functions of IgA, many IgA-deficient individuals have an increased incidence of autoimmune disease, recurrent mucosal infections, and an increased incidence of allergy,[38] although others do not seem to suffer from any significant negative health effects.[39] Interestingly, despite the lack of IgA, CD89 remains expressed on cells in these individuals.[40] It is therefore thought that in the absence of mIgA interactions with CD89, anti-inflammatory IgA immune modulation does not occur, thus favoring autoimmunity in the majority of IgA-deficient individuals.[41] In contrast, an increase of serum IgA levels and IgA ICs is associated with HIV and several other inflammatory diseases such as IgA nephropathy (IgAN), Sjögren's syndrome, and celiac disease.[42–45] The IgA Fc receptor CD89 has been proposed to be important for clearance of these potentially detrimental IgA ICs from the circulation, and decreased CD89 expression levels and glycosylation differences altering CD89 binding have been observed in some of these diseases.[42,46]

Mucosal IgA

The mucosal immune system comprises specialized structures and sites that contain a complex assortment of cells that undergo constant activation, terminal differentiation, and effector functions.[2,47] In healthy individuals, this system both protects against invasion by potential pathogens and permits tolerance against commensal bacteria and harmless environmental and dietary antigens.[47] IgA is primarily found in secretions that bathe mucosa as SIgA and is predominantly derived from local synthesis and is mainly dimeric, with subclass proportions and molecular forms varying with the mucosal site as shown in Table 1. SIgA plays a fundamental role in this homeostatic process known as immune exclusion by continuously defending mucosal portals from pathogens by entrapping them in mucus through adhesion to mucin glycoproteins, thereby hindering their access to epithelial receptors.[47] The ability of IgA to polymerize and bind to pIgR at the basolateral aspect of epithelial cells lends IgA many of its distinct functional capabilities. Mucosal IgA has also been shown to neutralize and aggregate virions and bacteria into large IgA-pathogen complexes for clearance through peristalsis.[48] Although much is known about SIgA in humans and some studies in mice,[49] a distinct lack of knowledge regarding mucosal IgA responses in nonhuman primates (NHPs) remains.

IgA in HIV: Dysfunctional IgA Responses?

HIV enters the host through the mucosa most commonly through infected mucosal fluids such as semen and cervicovaginal fluid during sexual intercourse and less frequently through breast milk from mother to child. Upon breaking through the mucosal epithelium, the virus has been shown to rapidly establish a localized founder population of infected cells.[50] The localized founder population of infected cells rapidly expands during the first week of infection and then goes on to create a viral pool that propagates systemically in secondary lymphoid organs.[50] Subsequent HIV disease progression is associated with severely compromised production of total mucosal IgA, resulting in compromised mucosal barrier integrity.[50] Loss of the mucosal barrier due to the damage inflicted upon the mucosal B cell compartment likely contributes to microbial translocation that is associated with severe immune activation, which in turn further enhances virus replication in mucosal tissues and disease progression.[50–52]

Although often transient in nature,[53] HIV-specific mucosal IgA antibodies can be detected in mucosal secretions such as saliva,[54,55] cervicovaginal secretions,[53,56] seminal plasma,[53] colostrum, intestinal fluids, bronchoalveolar lavage fluids, and tears[57] of infected persons, although at lower concentrations than anti-HIV IgG.[53,54,58] Unsurprisingly, there is a distinct lack of correlation between serum total IgA (mIgA derived from bone marrow plasma cells) and mucosal IgA concentrations (derived from mucosal plasma cells), highlighting the compartmentalization between systemic and mucosal immunity.[59]

A variety of common aberrancies of the B lymphocyte compartment of the immune system have been well documented in HIV-infected individuals,[60] including elevated total serum IgA levels during early HIV infection.[61] Elevated total serum IgA levels are such a hallmark of HIV infection that they have even been proposed as a potential marker of disease progression.[62,63] The exact mechanisms behind this increase in total serum IgA in HIV-infected individuals are unknown; however, it has been hypothesized by several studies that this is likely due to nonspecific polyclonal B cell activation.[60,64] When the composition of IgA in HIV individuals has been dissected, increased production of mIgA1 and not pIgA1 has been found to be responsible for the marked increase in total serum IgA.[61] The elevation in serum of SIgA in HIV infection has also been observed during chronic infection, and is most notable in patients with the lowest blood $CD4^+$ cell counts.[65]

A number of studies have also observed CD89 expression to be significantly decreased on monocytes and neutrophils in patients with diseases such as IgAN[26] and ankylosing spondylitis,[66] with CD89 levels observed to be inversely correlated with serum IgA. This decrease in CD89 expression has also been found in HIV-infected individuals.[43] The authors of these studies go on to hypothesize that impaired CD89 expression may contribute to a defective clearance of total serum IgA and IgA immune complexes. They suggest that this contributes to elevated total serum IgA levels during HIV infection and that CD89 expression may be a potential marker for disease progression.

Despite an elevation in total serum IgA, anti-HIV IgA levels, which tend to be directed primarily to envelope antigens, can be 100-fold lower in comparison with anti-HIV IgG levels.[53] While HIV-specific IgG responses tend to increase and remain stable following acute infection, this contrasts with anti-HIV IgA responses, which have a short half-life ($\sim$2.7 days) and tend to peak and then subsequently decline during acute infection.[53] Paradoxically, individuals with elevated total IgA typically have the poorest anti-HIV IgA and IgG responses.[67] This distinction in kinetics between anti-HIV IgG and anti-HIV IgA responses highlights the importance of sample collection timing, especially when anti-HIV IgA responses tend to be examined.

The recurring low frequency of anti-HIV IgA in comparison with IgG may be reflective of HIV *nef* that can interfere with CD40 ligand-mediated signaling and thus inhibit immunoglobulin class switching.[68] Similarly, it has been hypothesized that depletion of $CD4^+$ helper cells during chronic HIV infection may also limit the maturation and class switching of B cells to IgA.[51] Indeed, a significant increase of IgM levels and a decrease of IgA/IgM ratios to antigens derived from intestinal microbiota have been observed in mucosal intestinal secretions of HIV-infected individuals, indicative of reduced B cell class switch recombination.[69] This is, however, in contrast to observations of other mucosally acquired viral infections such as influenza[70] and Epstein–Barr virus (EBV), which induce comparatively higher IgA responses in serum and mucosal secretions than HIV. Furthermore, in the case of EBV, anti-EBV IgA levels are observed to increase in patients with HIV.[71]

HIV also preferentially infects activated HIV-infected $CD4^+$ T cells,[72] thus it has been hypothesized that this may contribute to the decrease in $CD4^+$ T cell help and class switching of anti-HIV IgA, while having less of an effect upon humoral responses to other infectious diseases.[51,73] As HIV also preferentially infects activated $CD4^+$ T cells, dysfunctional class switch recombination may be targeted to specific activated antigen-specific T cells. The precise mechanisms behind why this skewed IgA/IgM isotype ratio occurs in HIV infection for certain microbial antigens, but not others, warrant further investigation.

IgA in NHP Models of HIV Infection

Several key advances in HIV/AIDS research have been made possible by the use of NHP models of simian immunodeficiency virus (SIV). SIV infection of macaques leads to a disease that is similar in many respects to AIDS in humans.[74] It should be noted that there are numerous models of pathogenic SIV infection based on the macaque species (e.g., rhesus, cynomolgus, and pigtail). The virus can be further diversified and studied to mimic human infection through the use of simian–human immunodeficiency virus (SHIV), a chimeric virus constructed by replacing the envelope gene region of SIV with HIV; this results in the expression of HIV *env* as well as three other HIV genes: *vpu, tat,* and *rev* in the context of an SIV backbone.[75]

Regarding research relevant to the study of IgA, there are, however, significant differences between humans and NHPs despite their evolutionary links. Unlike the great apes, which encode IgA1 as well as IgA2, macaques only encode the IgA2-like subclass.[76] Macaque CD89 has been found to be highly homologous to that of humans and shares similar expression patterns on different cell types.[77] While macaque CD89 has also been shown to bind human IgA1 and IgA2,[78] how human IgA interacts with macaque and other NHP CD89 or Fc receptors and their downstream activation capacity are poorly understood. Furthermore, immunoglobulin Cα allelic polymorphisms have been found to be extremely high in rhesus macaques, making it necessary to take into consideration differences in IgA structure between animals, which may result in varying levels of protection.[79]

Further characterization of CD89, IgA, and its subclasses is therefore required in NHPs, and interpretation of NHP studies, which use passive or topical transfer of humanized IgA-based therapeutics, needs to consider these potential caveats. Great apes, most notably chimpanzees, would therefore be a very useful model to study the heterogeneous IgA subclasses; however, since the 1990s, chimpanzees are rarely used due to ethical and logistic considerations.[74,80] Nevertheless, the use of the macaque model in HIV research has greatly illuminated the HIV field, particularly passive and active immunization studies relating to mucosal IgA, as discussed in later sections below.

Passive Transfer of Anti-HIV IgA and Vaccine Studies Conducted in Naive NHP Hosts

Recent studies of the SHIV/rhesus macaque model using a variety of immunogens, such as subunit vaccines, and whole inactivated virus have demonstrated some promise that mucosal IgA can be harnessed to assist in protective immunity,[81–83] providing a degree of protection against mucosal and intravenous challenges. One study using an HIV gp41 subunit virosome delivery found that a combined regime of intramuscular and mucosal vaccination was the best strategy for

generating anti-HIV IgA in serum and in vaginal fluids.[84] These protected animals had gp41-specific IgAs in vaginal secretions that could block HIV transcytosis *in vitro*.[84] Reflecting well-known observations that higher total IgG levels are observed in vaginal secretions compared with gut mucosal fluids as observed in humans,[58] anti-HIV IgA responses were less pronounced than IgG in vaginal mucosal secretions. Controversially, epitope specificity differed between systemic and mucosal antibody responses.[84]

Another study investigated the influence of mucosal administration of human monoclonal neutralizing antibody isotype (IgG1, dIgA1, or dIgA2) in seronegative rhesus macaques.[83] These findings were quite dramatic, in that 100% of animals were protected from the mucosal SHIV challenge when IgG1-neutralizing mAb was administered at a low dose intravenously (i.v), followed by the dimeric IgA2 isotype intrarectally. In contrast, no animals administered the IgG1 mAb i.v alone were protected following challenge, and only 17% were protected following the IgA2 mAb given intrarectally. Eighty-three percent of animals were, however, protected with dIgA1 alone. This dIgA1 mAb was also found to block transcytosis of virus across an epithelial monolayer *in vitro* and captured twice as much virus than dIgA2 *in vitro*.[83] These findings suggest that the advantage of the longer, more flexible dIgA1 hinge region versus the more restricted hinge region of dIgA2 (Fig. 1) may confer additional viral binding ability to the dIgA1 isotype mAb. Furthermore, results of this study suggest that efficient dIgA responses might work synergistically with plasma IgG responses in preventing virus acquisition. This suggestion is also highlighted by a recent study, which found that IgA and IgG 2F5 and 10E8 mAb isotypes work together to enhance cell lysis by ADCC.[85] While these findings are promising and suggest that inducing mucosal IgA by vaccination could work to synergize with IgG antibody responses, there are many challenges to recapitulating such protective responses through vaccination, where induced antibody responses are polyclonal and typically far lower than those achieved by passive application of mAbs.

Evidence of a Potential Protective Role of Mucosal IgA in Humans

Neutralization and prevention of HIV acquisition

A protective role for mucosal IgA is often inferred from studies of individuals referred to as highly exposed to HIV, but IgG seronegative (HESN) individuals, despite repeated sexual exposure to seropositive partners. Many of these studies report detection of anti-HIV IgAs in serum,[86] genitourinary fluids,[86–88] and saliva[89] of these individuals who are often from studies examining sex worker cohorts. Some of these studies have correlated resistance with HIV with high levels of IgA against the envelope protein in vaginal secretions and saliva of these HESN individuals.[90,91] These mucosal anti-HIV IgAs have since been shown to block infection of peripheral blood mononuclear cells by HIV *in vitro*. Two studies have shown that mucosal and plasma IgAs specific for HIV antigens from HESN subjects can neutralize HIV infection of various cell types *in vitro* and prevent adherence of virus to epithelial cells that may be required for HIV transcytosis.[90,92] Some of these IgAs have been found to selectively target conserved regions of gp41,[92,93] making them capable of cross-clade neutralization.[89] Other studies, including blinded multisite analysis, looked for, but were unable to detect, anti-HIV IgA mucosal responses in HESN cohorts.[94–97]

The observation that resistance against HIV infection can occur in the absence of specific antibodies against HIV raises significant questions over the inferred protective role for HIV envelope-specific IgA in resistance against HIV infection in exposed uninfected individuals. Indeed, there remains much contention as to whether the association of mucosal anti-HIV IgA and HESN status is merely a correlate of exposure or a mechanism of protection. Levels of cervical mucosal IgA have been correlated with the frequency of repeated exposure in the absence of any correlation with resistance.[97] Furthermore, the recurrent presence of neutralizing anti-HIV IgA in these cohort studies[87,88,97,98] could possibly be linked to prevention of persistent systemic HIV infection. The reduced risk of infection in these individuals is, however, most likely dependent on multiple interactive factors, and understanding the mechanisms of protection among these populations will likely be key to designing effective vaccine strategies.

Serum Anti-HIV IgA: Negative Consequences

Serum IgG has historically been the principal focus of most studies characterizing the functional properties of HIV-specific antibodies, chiefly because of the quantity produced and the ease of collecting blood, as opposed to mucosal samples. The last decade has directed increased attention to anti-HIV IgA responses, particularly vaccine-elicited IgA responses. This interest has largely been driven by findings from the immune-correlates analysis of the pivotal RV144 ALVAC/AIDSVAX HIV vaccine trial, which found that high levels of envelope-specific monomeric plasma IgA correlate with reduced vaccine efficacy, whereas IgG against HIV envelope variable regions 1 and 2 correlated with reduced risk of infection.[99] It should also be noted that mucosal anti-HIV IgA levels and functions were not evaluated as correlates of infection risk as mucosal samples were not collected as part of the RV144 efficacy trial. RV144 follow-up studies, RV305 and RV306,[100] where mucosal secretions were collected and are still to be assessed, may be able to provide information regarding the mucosal IgA immune response.

There has also been an incorrect inference that plasma IgA in the RV144 trial suggests vaccine-induced enhancement of HIV acquisition risk,[101] and it must be emphasized that IgA was only associated with reduced vaccine efficacy.[99,102] It is also important to highlight that the RV144 plasma IgA findings may be a surrogate marker of other mechanisms that may modulate vaccine efficacy.[103] Subsequent studies have since isolated HIV-specific memory B cells from RV144 vaccines to generate monoclonal antibodies, of which two were originally isolated as IgA isotypes *in vivo*. Studies have demonstrated that these monoclonal IgAs can compete with monoclonal IgG epitopes of same specificities as well as against polyclonal purified IgG from plasma from RV144 vaccines, thereby attenuating the protective IgG ADCC responses in these RV144 vaccines.[104] Moreover, these Env-specific mIgA antibodies were also shown to inhibit NK cell killing of HIV-infected $CD4^+$ T cells coated with RV144-induced IgG antibodies.[104] It is important to note that IgA epitope competition has only been demonstrated using monoclonal antibodies. The potential inhibitory consequences of epitope competition are therefore yet to be explored in a more

biologically relevant polyclonal context or by assessing Fc effector responses using effector cells that also express CD89 or other IgA receptors.[85]

Outside the context of HIV, it is interesting to note that antigen-specific IgA antibodies have also been shown to block IgG and IgM-mediated protective functions, specifically complement-dependent phagocytosis by neutrophils only when CD89 expression has been downregulated.[28,43,105] Evidence of a similar suppressive effect occurring in highly viremic subjects has also been found.[106] Depletion of total IgA from plasma significantly enhanced the magnitude of ADCC.[106] Additionally, HIV-specific IgG levels and IgG/IgA ratios, but not IgA levels, correlated with ADCC responses.[106,107] Furthermore, in a separate study comparing HIV-discordant couples, IgG/IgA ratios also correlated with ADCC activity and increased $CD4^+$ T cell counts in chronically infected HIV individuals who did not transmit HIV, while elevated levels of IgA and the lowest IgG/IgA ratios were observed within chronic HIV transmitters.[108,109] This finding adds further evidence that IgA may interfere with ADCC activity and that the magnitude of ADCC may be related to the balance between inhibitory IgA and levels of IgG subclasses, which induce more potent anti-HIV ADCC responses, such as IgG3 and IgG1.[104,106,110–113]

In contrast, elite controllers (ECs) are distinct from HIV viremic individuals, and total IgA depletion has been found to have no significant effect on functional Ab responses despite high HIV Env IgA levels in the plasma of these individuals and an absence of viral stimulation.[106] These findings suggest that the sustained HIV stimulation that occurs in progressors and not ECs may promote alterations in the biophysical and functional nature of IgA and/or IgA receptors such as CD89. The notion for a potential role for IgA receptors is supported by a study that found serum IgA antibodies from HIV-infected individuals, but not seronegative HIV controls, enhanced HIV infection of monocytes and intestinal mononuclear cells.[114]

Serum Anti-HIV IgA: Evidence for a Protective Role

Despite the studies discussed above, which cast a cloud over the protective effect of serum IgAs in HIV, a number of potentially protective qualities, such as ADCC,[115] neutralization,[116] and phagocytosis,[117,118] have been attributed to serum anti-HIV IgA.[73,117] In one such recent study, two HIV envelope IgA mAbs were isolated and characterized from peripheral blood memory B cells from an RV144-vaccinated individual and were found to induce viral phagocytosis and block gp140 binding to the alternative HIV receptor galactosylceramide.[117] In another recent study, targeting of HIV with envelope-specific gp41 IgA mAbs engaged CD89 on HIV-infected monocytes to trigger cell lysis by ADCC.[85] The ability of dimeric and polymeric serum IgA, but not mIgA, to efficiently aggregate HIV into discreet complexes has also been demonstrated with the polyvalency provided by these isoforms associated with superior functionality.[119] Furthermore, ECs have been shown to demonstrate a wider breath of HIV protein recognition and higher avidity serum IgA antibodies to gp41.[73]

Surprisingly, ECs have been found to have stronger Env C1-specific IgA responses compared with noncontrollers in this recent study, which opposes the finding that IgA-C1 responses in RV144 vaccines are associated with risk. The authors go on to suggest that the specificities of anti-gp120 IgA and IgG antibodies in ECs may differ from progressors, potentially explaining the lack of interference in ADCC function rather than lower concentrations or avidity of these IgA-binding antibodies. Moreover, in a different study, serum IgA of HIV-exposed uninfected individuals was found to inhibit HIV through recognition of a region within the alpha-helix of gp41,[93] once again highlighting the potentially protective role for anti-HIV IgA.

Technical Issues Measuring Anti-HIV IgA

From various studies that have examined antigen-specific responses in mucosal secretions of HIV-infected humans, chimpanzees,[120] or SHIV/SIV macaques, a recurring theme is that anti-HIV IgA responses are modest at best in comparison with IgG responses in serum and in the mucosa.[67] Despite this commonality, significant contention exists in the field surrounding quantitation and detection of anti-HIV IgA, particularly in mucosal secretions.[67,121] Conflicting frequencies observed in different studies are most likely to be the result of more than just one factor, and there is no doubt that the heterogeneity in total and anti-HIV-specific IgA recovered from mucosal secretions is largely dependent on genetic and environmental factors as well as the mode and frequency of HIV exposure.

The high variability of published findings regarding IgA quantitation is likely also influenced by the lack of standardized assays to measure HIV-binding mucosal IgA antibodies and the significant variation in mucosal sampling and purification techniques.[122–124] Immunoassays to detect IgA binding to monomeric envelope proteins may not reliably capture functional antibodies targeted to the native HIV virus envelope. Furthermore, lack of antibody HIV-inhibitory activity could indicate that other HIV-independent factors may be involved in host protection. This was demonstrated by inhibition of viral transport across endometrial and intestinal epithelial cells by CCR5-specific IgA antibodies isolated from serum and mucosal secretions of HIV seronegative and long-term nonprogressors.[125]

With regard to quantitation of molecular forms of IgA, these have been shown to be differentially recognized in immunoassays by antibodies toward the human α-chain, which may present an issue with interpreting IgA levels in mucosal secretions where proportions of molecular forms and subclasses of IgA vary considerably.[126,127] This is compounded by limited availability of pure IgA for each of the molecular forms, which can be used as standards in immunoassays, especially dIgA and SIgA isoforms. Furthermore, determination of IgA subclasses has historically been hindered by the lack of specific reagents as antibodies specific for IgA1 and IgA2 have often been difficult to prepare due to relatively minor structural differences between subclasses, which can show significant cross-reactivity.[128]

Determination of molecular forms of IgA may be further complicated as mIgA may be complexed into multimeric forms by aggregation or IC formation, making it difficult to distinguish true polymeric (J chain) IgA. Currently, there is no standardized assay to quantitate pIgA, and immunoassays based on detection of the J chain can be problematic as the J chain is also shared by IgM, and in serum, methods in which the first step specifically captures both monomeric and polymeric forms can lead to underestimation of the polymeric form due to saturation of the capture antibody by mIgA.[129]

An additional factor complicating antibody recovery and evaluation in mucosal secretions is the high presence of bacterial contamination and endogenous lytic enzymes. IgA isotypes such as mIgA might therefore be underrepresented in some cases in comparison with isotypes such as SIgA and IgA2, which are more resistant to bacterial degradation.[130] The time between sample collection and analysis should therefore be minimized, and care should be taken in storing and transporting samples to avoid antibody degradation. Methods used to isolate IgA are also important technical issues that need attention, and much of the literature does not make a distinction between the molecular forms of IgA or use sensitive and specific isolation methods to recover and differentiate the IgA subclasses. Finally, IgA concentrations have been shown to fluctuate with circadian rhythms, particularly in saliva,[131] and female genital secretions are strongly influenced by menstrual cycle hormonal levels,[132] further adding to the long list of technical considerations for evaluating IgA antibodies, particularly in mucosal secretions.

Despite the broad technical issues that may account for conflicting findings in the literature, some studies have proposed that there may be selective defects in the production of anti-HIV IgA after establishment of HIV infection.[68,73] This has been suggested by studies that have found readily detectable levels of influenza-specific IgA antibodies, but not anti-HIV IgA antibodies, in various mucosal secretions from HIV-infected individuals using various methodologies.[133] However, as mentioned earlier, while HIV is acquired at mucosal sites, it is largely a systemic infection that induces a dominant IgG response[67] and only transient IgA responses with a short half-life.[53] In contrast, the influenza virus predominantly infects and remains at mucosal surfaces, which may explain why influenza-specific IgA mucosal antibodies are more readily detectable in comparison with anti-HIV IgA.[67,133] This paucity in anti-HIV IgA has been observed in several other studies, where IgA responses to other antigens such as EBV, herpesviruses, and hepatitis virus B have been found to be elevated or unaltered in HIV-infected individuals,[71,134,135] although in-depth comparisons between these other antigens and HIV using multiple mucosal secretions have yet to be conducted.

Future Directions

Despite the potential importance of IgA in host defense, the roles that various molecular forms and isotypes of IgA (IgA1, IgA2, monomeric, dimeric, polymeric, and SIgA) play in prevention of HIV transmission are not yet well defined. Moreover, the facts that not all IgAs are created equal and that each molecular form has a disparate role and interaction with the CD89 receptor are becoming increasingly apparent. Recent studies in SHIV-challenged macaques passively immunized with the broadly neutralizing HIV-specific dIgA1 mAb isotype have demonstrated superior protection, virion capture, and prevention of transcytosis compared with IgG or dIgA2,[83] while a separate study observed protection by passive immunization of macaques with an IgG1 mAb and dIgA2 of the same specificity.[82] Mapping out the respective qualities and functional capacities of various IgA isoforms as well as their interactions with CD89 and other IgA receptors may therefore prove critical for development of passive antibody transfer studies and a future HIV vaccine. There is also a growing need to determine the mechanisms that lead to long-lived, effective, mucosal antibody responses. Evidence that SHIV can be prevented by mucosal IgAs in macaques highlights the fact that it would be advantageous for an effective HIV vaccine to induce robust, long-term, mucosal immune responses as well as systemic responses.

The recent immune-correlates analysis of the RV144 vaccine trial raised the hypothesis that plasma mIgA may mitigate otherwise protective IgG responses.[104] Determining the mechanism behind the inhibitory IgA effect or identifying whether IgA is merely a surrogate marker for other immunomodulatory mechanisms is an important question to address. Several groups, especially in autoimmunity research, have demonstrated that nonspecific mIgA[25,136] and targeting of FcαRI (CD89) by anti-CD89 Fab induce a potent and long-lasting inhibitory signal. Whether the anti-inflammatory property of mIgA may hinder Fc-mediated IgG responses to HIV, particularly in individuals with elevated serum mIgA, is a future avenue to explore. Furthermore, recent studies[137,138] have revealed the importance of the microbiome and its contribution to HIV susceptibility, thus it will be interesting to examine how differences in the microbiome and microbiota-specific IgA may influence inflammation, anti-HIV IgA levels, and disease progression in infected individuals.

Conclusions

The respective contribution of anti-HIV IgA and whether it is beneficial or detrimental to the host are highly complex areas of research. The answer to this question is likely dependent on several factors, such as whether anti-HIV IgA is being elicited in immunocompromised HIV-infected individuals, or upon vaccination of naïve individuals, as well as the respective systemic or mucosal immune compartments being surveyed, and IgA isoforms induced.[53] Although plasma mIgA induced by RV144 vaccination was found to be associated with reduced vaccine efficacy, further consideration of underlying mechanisms of this association is necessary. At the same time, results obtained from passive immunization of macaques with recombinant monoclonal antibodies show promise in suggesting a potentially protective capacity of monoclonal neutralizing antibodies of the IgA isotype. Furthermore, *in vivo* studies have demonstrated the ability of HIV-specific dIgA antibodies, particularly dIgA1 administered mucosally, to protect against mucosal SHIV transmission. In addition, dIgA1 has also been shown to prevent transcytosis *in vitro*.

Mucosal IgA responses in HIV-infected individuals often show great heterogenicity, which may not only be largely dependent on genetic factors and the mode and frequency of HIV exposure but also may reflect diverse technical considerations necessary for evaluating an immunoglobulin, which exists in multiple molecular forms in mucosal secretions and plasma. Moreover, anti-HIV IgA is short-lived and low in titer, adding to the challenge of studying anti-HIV IgA responses. In summary, the inherent multifaceted nature of IgA is evident, such that the role of IgA in HIV deserves further consideration and remains to be fully elucidated.

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