Published in final edited form as:

Curr Opin HIV AIDS. 2017 May ; 12(3): 222–228. doi:10.1097/COH.0000000000000369.

Antibody-mediated immune exclusion of HIV

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Abstract

Purpose of review—Although approximately 90% of all HIV transmissions in humans occur through mucosal contact, the induction of mucosal anti-HIV immune responses has remained understudied. Here we summarize data demonstrating the powerful protection that is achievable at mucosal frontlines through virus-specific mucosal IgA alone or combined with IgG.

Recent findings—Passive immunization with different monoclonal antibody (mAb) subclasses but identical epitope specificity (the conserved V3-loop crown of HIV gp120) has revealed that the dimeric IgA1 (dIgA1) form with its open hinge can prevent SHIV acquisition in rhesus macaques at a higher rate than dIgA2. Both dIgAs neutralized the challenge SHIV equally well. Protection was linked to better virion capture and inhibition of cell-free virus transcytosis by dIgA1. Synergistic interactions at the mucosal level between the IgG1 and dIgA2 versions of this mAb yielded complete protection. Active vaccine strategies designed to induce mucosal IgA and systemic/mucosal IgG have given promising data.

Summary—This review seeks to highlight the importance of mucosal IgAs in preventing virus acquisition. Passive immunization gave proof-of-concept for immune exclusion by mucosally administered monoclonal dIgAs. Unanswered questions remain regarding the interplay between mucosal IgA and other host immune defenses, including their induction with active immunization.

Keywords

Immune exclusion; mucosal antibodies; dimeric and secretory IgA; virion capture and transcytosis; SHIV/primate models

INTRODUCTION

Immune exclusion: definition

Immune exclusion at mucosal barriers was first identified in 1975 by passive immunization as an IgA-based mechanism to exclude antigen from the systemic circulation [1].

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Conflicts of Interest

There are no conflicts of interest.

Intratracheally administered myeloma IgA with known specificity significantly decreased the uptake of radiolabeled antigen from the trachea into the systemic circulation. These data conclusively linked IgA to antigen retention in the mucosal lumen. Subsequent work by different groups has identified antibody (Ab)-mediated immune exclusion as an important mechanism to protect the host against various mucosal pathogens (reviewed in [2*–4*]).

Structure and function of IgA

Humans and great apes have two subclasses of IgA, IgA1 and IgA2 [5], which differ predominately in the shape of the hinge region. In IgA1, the hinge is 19 amino acids (aa) long [6, 7] and contains several O-linked oligosaccharides [8, 9], giving it a T-shaped appearance. In contrast, the IgA2 hinge is Y-shaped, only six aa long [6], and lacks glycosylation [10]. As a consequence, the distance between Fab fragments is 16 nm in IgA1 [11–13] and only 10 nm in IgA2 [12–14]. Rhesus macaques (RMs) have only one IgA2-like form with a short hinge [6, 7, 15, 16].

IgAs exist in different forms, including monomers, dimers linked at the constant region by a joining (J chain), and secretory IgA (SIgA). The latter contains the secretory component (SC) and can exist in higher-order multimers. Dimeric SIgA represents the predominant IgA form in mucosal fluids (reviewed in [4*]).

Generation of SIgA

SIgA production starts in the lamina propria by local plasma cells that secrete dimeric IgA (dIgA) containing J chains. In contrast, serum IgA is produced by bone marrow plasma cells. After secretion from subepithelial plasma cells, dIgAs associate through the J chain with polymeric immunoglobulin receptors (pIgRs) [17] expressed on the basolateral surface of epithelial cells (Figure 1). The dIgA1-pIgR complexes undergo endocytosis, transport across epithelial cells inside transcytotic vesicles, and release into mucosal fluids at the apical side, a step during which proteases digest pIgR into SC and receptor remnants. Following this cleavage, SIgA is released into mucosal fluids either as dimers or larger multimers that interact with mucus.

IMMUNE EXCLUSION OF SHIV BY MONOCLONAL ANTIBODIES

We sought to test whether monoclonal antibodies (mAbs) with identical epitope specificity but antibody (Ab) backbones from different immunoglobulin (Ig) subclasses would differ in their ability to prevent mucosal SHIV acquisition in rhesus macaques (RMs). We administered dIgA1, dIgA2, and IgG1 versions of the human mAb HGN194 by passive intrarectal (i.r.) immunization [21**]. HGN194 recognizes a conserved epitope in the V3-loop crown of HIV-1 gp120 [22] and neutralized all HIV-1 tier 1 strains tested as well as some tier 2 viruses. Thirty minutes after topical i.r. administration of the three HGN194 isoforms, all RMs were challenged i.r. with the tier 1 R5 clade C SHIV, SHIV-1157ipEL-p [23, 24]. Surprisingly, dIgA1 protected 83% of the RMs, whereas dIgA2 protected only 17% ($P=0.045$); the IgG1 version gave 33% protection (Table 1). All three HGN194 isoforms neutralized the challenge virus equally well. Protection was significantly linked to virion

capture and inhibition of transcytosis of cell-free virus across an epithelial monolayer in vitro.

This study provided the first proof-of-concept that dIgA can protect against mucosal virus challenge, especially the dIgA1 version with its open, flexible hinge. We postulate that the dIgA structure favors virion aggregation, thereby blocking transcytosis in vitro [21**]. The data imply that dIgA1-virion immune complexes may be bigger than those generated with dIgA2 and IgG1. Direct comparisons of the immune complex sizes have not been published. However, Stieh et al. have demonstrated that multimeric mAbs have the capacity to aggregate HIV-1 virions [25].

Our data also imply that the selective induction of anti-HIV-1 mucosal SIgA1 is a desirable vaccine strategy. However, inducing *only* mucosal IgA responses will leave the host defenseless should the mucosal barrier be breached.

NEUTRALIZING IgA1 IN THE FORESKIN OF UNCIRCUMCISED MEN: ASSOCIATION WITH LOWER RISK OF HIV ACQUISITION

Hirbod et al. [26**] sought to establish a link between HIV acquisition, mucosal IgA and other parameters in a blinded study using foreskin swabs collected in a randomized Ugandan trial of male circumcision for HIV prevention. The study's aim was to determine correlates of HIV acquisition in foreskin using a case-control design. Jacalin column chromatography was used to isolate IgA from swabs, a method that predominately yields IgA1 (jacalin specifically binds to IgA1 hinge O-linked oligosaccharides [27–29]). The presence of IgA neutralizing capacity in foreskin samples was associated with an odds ratio (OR) of 0.31 for HIV acquisition in these uncircumcised men at initial enrolment and 0.21 at the last visit when cases were still seronegative – a result that was strikingly similar to data obtained in high-risk Kenyan women, where the OR of HIV infection among study subjects with neutralizing IgA in cervical/vaginal secretions was found to be 0.31 [30]. Together, these data imply a protective effect of mucosal IgA against sexual HIV transmission.

DEFENSE-IN-DEPTH BETWEEN SYSTEMIC IgG1 AND MUCOSAL dIgA2

To test whether systemic IgG1 could modulate the protection provided by mucosal dIgA2, we administered HGN194 IgG i.v. either alone or combined with i.r. HGN194 dIgA2. We deliberately administered the IgG1 form at a suboptimal dose [31**]. We enrolled SHIV-exposed but persistently aviremic RMs that had been protected previously with passively administered HGN194 mAb isotypes. Our primate experiment was also designed to test whether monoclonal IgA could block protection mediated by IgG1 with the same epitope specificity, as has been implied by data from the RV144 trial [32]. Vaccine-induced IgG1 with Ab-mediated cellular cytotoxicity (ADCC) activity had been linked to lower risks of HIV acquisition among vaccinees compared to individuals given placebo; however, plasma IgA with anti-HIV Env specificity was described to counteract the benefits of such IgG1 effector functions [33, 34].

To test the interaction between HGN194 IgG1 given intravenously (i.v.) with mucosally administered HGN194 dIgA2, we enrolled three RM groups. Group 1 received only i.v. HGN194 IgG1 24 h before i.r. SHIV challenge at a suboptimal dose. Group 2 received the same i.v. HGN194 IgG1 dose but at 30 min before SHIV challenge, the animals also received i.r. HGN194 dIgA2. Controls were left untreated.

The results were surprising. None of the RMs given only i.v. HGN194 IgG1 were protected, whereas all animals given the combination remained virus-free (Table 1). These data imply that combining suboptimal Ab defenses in the mucosal and systemic compartments yields synergistic protection. The mechanisms involved remain to be deciphered and likely involve Ig-mucus interactions in mucosal fluids [35]. Systemically administered IgG1 is known to distribute into tissue and mucosal fluids over time – thus, HGN194 IgG1 as well as dIgA2 were present in the mucosal compartment at the time of i.r. virus challenge.

The high level of protection in our passive immunization study with i.v. IgG1 + i.r. dIgA2 also implies that *active* vaccine regimens should focus on inducing not only mucosal Ab responses, but systemic IgG as well – in analogy to defense-in-depth, a well-known military strategy that employs defensive fall-back positions well behind the fortified frontline. Should the latter be breached, a fully armed second line of defense will be ready.

EXPLOITING HIV IMMUNE EXCLUSION CLINICALLY

The promising primate model data imply that trapping HIV virions in mucosal lumina through immune complex formation can be a powerful preventative strategy as shown for other pathogens [36]; reviewed in [3]. Immune exclusion of HIV can be achieved either through passive administration of anti-HIV mAbs alone or in combination or through induction of mucosal antibody defenses by active immunization. Passive immunization implies topical administration of preformed mAbs as anti-HIV gels/microbicides (Reviewed in [37]). Our passive immunization data with different HGN194 mAb subclasses imply that dIgA1 or SIgA1 mAbs might be the most potent. The dIgA1 success was most likely due to dIgA1 structure with its open, flexible hinge and quadruvalency. The longer distance between antigen-combining sites in dIgA1 versus dIgA2, coupled with reproducibly increased virion capture by dIgA1, implies that dIgA1 is better suited to efficiently trap virus particles in large aggregates compared to other Ig subclasses.

Topical application of recombinant mAbs

Generating dIgA1 or SIgA1 mAb versions for mucosal application is technically challenging as mAb production is labor-intensive and thus costly. Novel production platforms are being explored, including generating SIgA1 in plants. Paul et al.[38] have produced a recombinant human SIgA1 version of the mAb 2G12 in plants; such 2G12 SIgA1 was highly active in terms of HIV neutralization, HIV Env binding, and virion aggregation. Other platforms to produce recombinant mAbs are also under investigation, including several strains of yeast and filamentous fungi (reviewed in [39]).

Systemic administration of dIgA to lactating RMs and transport into milk

The group of Permar transplanted the Ig heavy and light chain variable regions of b12, a broadly neutralizing anti-CD4 binding site mAb, onto the constant regions of the RM κ light chain, the RM IgG1 heavy chain or the RM IgA heavy chain sequences, thus creating RM b12 isoforms [40]. Of note, RM DNA – unlike human DNA – only encodes one IgA isotype, a molecule that resembles the human IgA2 with its short, Y-shaped hinge region (reviewed in [4*]). The various RM b12 versions were administered i.v., which yielded high plasma concentrations in all monkeys, except that monomeric IgA was rapidly cleared from the circulation [40]. Peak concentrations of RM IgG1 b12 in saliva, rectal and vaginal secretions were higher than those for dIgA except in milk, where the b12 concentration was significantly higher after dIgA administration compared to IgG1 or monomeric IgA. Plasma samples of all passively immunized RMs showed neutralization. In contrast, only milk samples of RMs passively infused with the dIgA moderately neutralized the test virus. The authors also found specific SIgA in mucosal fluids after i.v. dIgA infusion and concluded that passive immunization with dIgA will be more effective than with IgG1 to neutralize virus in breastmilk samples [40].

Active immunization to generate Ab-mediated mucosal defenses

Topical administration of anti-HIV microbicides has been fraught with compliance issues and has not yielded reliable protection in clinical trials [41–42]. Undoubtedly, topical formulations of recombinant anti-HIV mAbs may encounter similar obstacles. Therefore, induction of long-lasting mucosal Ab responses through active immunization is preferable.

Remarkable results for vaccine-induced IgA-mediated protection have been published by Bomsel et al. [43] who immunized Chinese-origin RMs with two populations of virosomes displaying different gp41 antigens on their surface. Virosomes have been used in humans and have an excellent safety profile with self-adjuvant characteristics. The virosomes were administered either four times intramuscularly (i.m.) or twice i.m. followed by two intranasal (i.n.) immunizations. Control RMs received only empty virosomes. All RMs were challenged via repeated intravaginal low-dose SHIV challenges; after 7 virus inoculations, the virus dose was increased by 50%. Remarkably, all five animals vaccinated through the i.m./i.n. route were protected from persistent systemic infection after all challenges with the upfront heterologous SHIV_{SF162P3}, a tier 2 R5 SHIV; none of these five animals seroconverted to SIV p27 Gag. Protection was linked to gp41-specific vaginal IgA that inhibited cell-free virus transcytosis in vitro. Vaginal IgGs with neutralizing and/or ADCC effector function were also linked to protection. None of the serum Ab responses correlated with protection.

In a follow-up study using Indian-origin RMs and larger groups of animals, the i.m./i.n. gp41 virosome vaccine strategy was retested. The two-component gp41-virosomes were 87% effective in delaying the time to persistent infection in vaccinees versus controls during the first seven intravaginal SHIV_{SF162P3} challenges. When the virus dose was increased by 50% as in the original Chinese RM study, vaccine protection was overcome and most vaccinees became viremic [44]. Of note, the initial SHIV challenge dose against which 87% protection was observed represented ~70,000 times the average human HIV inoculum

passed from an HIV-infected man to an uninfected woman [45]. This repeat study showed that important aspects of gp41-virosome-induced vaccine protection were reproducible even in genetically distinct RM subspecies; immune parameters possibly linked to protection are under study.

A recent study sought to induce anti-HIV clade C Env-specific Abs in lactating RMs. Animals previously vaccinated with MVA prime/i.n. boost regimen were boosted i.n./i.m. with HIV clade C gp120 using the Toll-like receptor (TLR) 7/8 agonist, R848, as adjuvant [46**]. Animals boosted i.n. received a third immunization at week 50 following the initial MVA priming [46**]. Surprisingly, breastmilk IgA responses were higher than plasma Ab responses. Immunizing lactating women with an IgA-inducing vaccination strategy may be a novel approach to prevent HIV transmission through breastfeeding.

Another recent study describes a vaccine efficacy study performed in neonatal RMs against oral SIV challenge [47]. Neonatal RM infants were vaccinated orally with live attenuated *Mycobacterium tuberculosis* strains expressing either SIV Env or Gag. This priming was followed by systemic boosts with heterologous MVA-SIV Env/Gag/Pol. After oral SIV challenge, all infants became viremic. However, a subset of vaccinees had significantly lower peak viremia that was inversely associated with SIV Env-specific IgA responses in saliva and intestinal secretions as well as higher avidity SIV Env-specific plasma IgG. While mucosal SIV immune exclusion and thus prevention of virus acquisition were not achieved, these data point to the importance of simultaneously inducing mucosal IgA and systemic IgG.

CONCLUSION

Pathogen-specific mucosal IgAs protect the host in several model systems [21**, 48–50]. The role of mucosal anti-HIV IgA in humans is less well studied, although data from the Ugandan trial in uncircumcised men and high-risk Kenyan women linked mucosally produced neutralizing IgA to lower risks of HIV acquisition. Passive topical immunization in SHIV-challenged macaques gave direct evidence for dIgA-mediated prevention of mucosal virus transmission. Mucosally administered monoclonal dIgA1 completely protected most animals in contrast to the dIgA2 isoform; better protection was linked to better virion capture and prevention of transcytosis [21**]. Active immunization of RMs with gp41-virosomes likewise linked prevention of persistent viremia to the induction of mucosal IgAs that blocked virus transcytosis in vitro [43].

In contrast to the promising results regarding IgA action in mucosal compartments, RV144 trial data paint a negative picture of plasma anti-HIV Env IgAs that were described to block IgG-mediated ADCC directed against shared HIV Env targets [34]. While passive and active immunization studies in macaques gave proof-of-concept that SHIV acquisition can be prevented by mucosal IgA, it is still unclear whether anti-HIV IgA responses overall will be beneficial or detrimental to the host [4*, 51]. We propose that the most important aspect for IgA responses will be location, location, location. At the mucosal barrier, immune exclusion is a potent protective mechanism.

Acknowledgments

We thank Juan Esquivel for his assistance in the preparation of this manuscript.

Financial support and sponsorship

This work was supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (P01 AI048240 to R.M.R and S.K.L; R01 AI100703 to R.M.R), and The Bill and Melinda Gates Foundation (OPP1111654 to R.M.R).

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KEY POINTS

- Immune exclusion through multimeric mucosal IgA is a potent protective mechanism – in contrast to data from the RV144 clinical trial that imply a negative effect of plasma anti-HIV Env IgA
- Protection against mucosal SHIV challenge by monoclonal dimeric IgA1 (dIgA1) administered mucosally was significantly more potent than by dIgA2 with the same epitope specificity
- dIgA and IgG1 versions with the same epitope specificity act synergistically at the mucosal level – the molecular mechanisms of this interaction remain to be determined
- Vaccine-induced mucosal IgA has been linked to significant protection against mucosal SHIV transmission in macaques

FIGURE 1.

IgA forms and their role in immune exclusion. (a) Monomeric IgA1; (b) monomeric IgA2. VH, variable heavy chain; Ca2, and Ca3, constant regions of the Ig heavy alpha chain; VL, variable light chain; CL, constant region of the light chain (either κ or λ). (c) Dimeric IgA1 (dIgA1). Two monomers are linked through their Ca3 moieties by the Joining chain (J chain). (d) Secretory IgA1 (SIgA1). The secretory component (SC) wraps around Ca3 regions and the J chain (in blue). SC is derived from the polymeric Ig receptor (pIgR) through proteolytic cleavage (e). In (c) and (d), dimeric and secretory versions of IgA1 are shown; similar forms of IgA2 exist (reviewed in [4*]). (e) Local generation of SIgA. Plasma cells in the lamina propria secrete dIgAs (either dIgA1 or dIgA2 in humans). dIgAs then associate with pIgRs (blue, Step ①), traverse epithelial cells inside transcytotic vesicles (Step ②), and are released at the apical side after proteolytic cleavage of pIgR resulting in remnants on epithelial cells (Step ③) and SC wrapped around dIgA molecules to yield SIgAs. Of note, pIgR without cargo can also undergo proteolytic cleavage at the luminal side, resulting in free SC molecules in mucosal fluids [18–20] (reviewed in [4]). (f) Passive

immunization: Protection by exogenous monoclonal dIgA through immune exclusion. Monoclonal dIgA is topically applied in the mucosal lumen (shown in ochre). SHIV virions are captured and retained in the lumen through the formation of large antigen–dIgA complexes. This results in immune exclusion of virions from the systemic compartment and prevention of SHIV acquisition (yellow arrow; red X).

[The Figure has been adapted from reference 4. Retrovirology is published Open Access.]

Table 1
Protection by passive immunization with topically and/or systemically administered mAbs

Study	HGN194 Ig subclass	Route	Dose time	Dose	% Protected RM
1 [21]	IgG1	i.r.	-30 min	1.25 mg	33
	dlgA1	i.r.	-30 min	1.25 mg	83
	dlgA2	i.r.	-30 min	1.25 mg	17
2 [31]	IgG1	i.v.	-24 h	1.45 mg/kg	0
	IgG1 + dlgA2	i.v.	-24 h	1.45 mg/kg	100
		i.r.	-30 min	1.25 mg	