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Fc Receptor-Mediated Immune Responses: New Tools But Increased Complexity in HIV Prevention

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Abstract

The modest success of the RV144 HIV vaccine trial in Thailand and the ensuing suggestion that a Fc-receptor-mediated antibody activity might have played a role in the protection observed have intensified investigations on Fc-related immune responses. HIV neutralizing antibodies have been and continue to be the focal point of research into humoral immune protection. However, recent knowledge that their protective efficacy can be augmented by Fc-FcR interactions has increased the complexity of identifying immune correlates of protection. If anything, continued studies of both humoral and cellular immune mechanisms point to the lack of a single protective anti-HIV immune response. Here we focus on humoral immunity, analyzing the role played by Fc receptor-related responses and discussing how new knowledge of their interactions requires further investigation, but may also spur novel vaccination approaches. We initially address classical Fc-receptor mediated anti-viral mechanisms including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell mediated viral inhibition (ADCVI), and antibody-dependent cellular phagocytosis (ADCP), as well as the effector cells that mediate these functions. Next, we summarize key aspects of FcR-Fc interactions that are important for potential control of HIV/SIV such as FcR polymorphisms and post-transcriptional modifications. Finally we discuss less commonly studied non-mechanistic anti-HIV immune functions: antibody avidity and envelope-specific B cell memory. Overall, a spectrum of immune responses, reflecting the immune system's redundancy, will likely be needed to prevent HIV infection and/or disease progression. Aside from elicitation of critical immune mechanisms, a successful vaccine will need to induce mature B cell responses and long-lasting immune memory.

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CONFLICT OF INTEREST

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INTRODUCTION

An oft cited reason for the current lack of a highly successful HIV vaccine is the failure to date of identifying critical immune correlates of protection. It is believed that such information would provide a roadmap for design and development of vaccine components able to elicit appropriate immune responses resulting in potent protective efficacy. A major difficulty in uncovering these key responses is the fact that HIV infection or disease is not cleared by the immune system, leaving naturally infected individuals poor subjects for correlative studies. Although significant advances have been made by studying individuals with “elite control” of HIV infection versus those who progress to AIDS, the complex demographics and characteristics of infected people, together with HIV-induced dysfunction of immune cells, have limited to some extent the knowledge that can be gained from natural infection studies. In that regard, investigations of vaccinated subjects in human clinical trials as well as non-human primates in pre-clinical vaccine studies have made key contributions to elucidating protective responses. In fact over the last decades since the discovery of HIV as the cause of AIDS, numerous protective immune responses have been identified. These encompass elements of both cellular and humoral immunity, including $CD8^+$ T cells, identified as protective against viremia by *in vivo* depletion studies in macaque models [1, 2] and neutralizing antibodies, first identified as protective against acquisition by passive transfer studies in chimpanzees [3, 4], and subsequently by several groups in macaques [5–9]. Here we will limit our discussion to humoral immunity. In the period of time since neutralizing antibodies were found to confer protection, much has been learned concerning their characteristics which are important for protective immunity. However, recently, with the entry of Fc receptor-related responses into the arena of immune correlates of HIV protection, the complexity of uncovering key responses needed for a successful vaccine has greatly increased. Knowledge concerning “mechanistic immune correlates” [10] such as neutralization and antibody-dependent cellular cytotoxicity (ADCC) has greatly expanded, but “nonmechanistic correlates” such as avidity, memory, and even genotype influence protective outcomes and impact clear mechanistic responses. As the focus of this issue is Fc-related immunity, we will first address individual Fc-related non-neutralizing antibody activities, including ADCC, antibody-dependent cell mediated viral inhibition (ADCVI), and antibody-dependent cellular phagocytosis (ADCP). As intracellular inhibition of HIV transcytosis involves the polymeric Ig Receptor (pIgR) [11], we include a brief discussion of this immune response. Subsequently we discuss aspects of Fc-related immunity that have impacted the vaccine field, including effects on protective neutralizing antibodies. As recently summarized by Huber and Trkola, in addition to specificity, antibody isotype, subtype and affinity for Fc receptors and complement factors all modulate immune responses [12]. Finally we summarize elements of immune dysfunction that develop with HIV disease progression and complicate identification of protective immune correlates in

natural HIV infection. The implications of these multiple variables on the path forward to a successful HIV vaccine are discussed.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

Aside from neutralizing antibody, ADCC was one of the earliest antibody functions identified in HIV-infected individuals [13]. The main target antigen was readily identified as the HIV envelope on the surface of cells [14], and the activity was shown to be broadly reactive and to arise before neutralizing antibody [15]. While ADCC was correlated with a better disease outcome following HIV or SIV infection of humans or macaques in some studies [16, 17], this relationship was not always reproduced [18, 19]. This discrepancy was likely attributable in part to the complexity of the assay systems. ADCC activity requires target cells expressing viral antigen, effector cells (generally NK cells) expressing FcR IIIa, and antibody which provides the specificity for antigen recognition. These elements varied between the research groups. For example, target cells used included HIV-infected cell lines or cells coated with inactivated HIV. Moreover, both humoral and cellular defects have been noted to impact ADCC activity over the course of HIV infection [20, 21] introducing further variability into correlative studies. Finally, the Cr^{51}-release assay employed in these studies was cumbersome and lacked the sensitivity of current techniques. Taken together with the continuing focus on elicitation of neutralizing antibody in vaccine design, as well as the potential of protection *via* cellular immunity, these elements led to lack of pursuit of the ADCC mechanism.

Interest in a possible association of ADCC with protective efficacy was rekindled with the observation that vaccine-elicited antibody mediated ADCC activity correlated with reduced viremia following SIV challenge [22]. This study made use of a novel rapid flow-cytometry-based assay which provided increased sensitivity for measurement of target cell killing and greatly facilitated investigations of ADCC activity [23]. Subsequent modifications to the flow-based technique soon emerged including assessments based on hydrolysis of a fluorogenic substrate by granzyme B delivered by effector cells into specific targets [24], and granzyme B expression together with assessment of HIV-infected cell elimination (ICE) [25, 26]. Assessment of NK cell activation by expression of IFN-γ, together with cytotoxic potential by expression of granzyme B, perforin, and CD107a has also been used to effectively map epitopes that are recognized by potentially ADCC-mediating antibodies [27], although direct cell killing is not measured by this technique. Recently, use of an engineered target cell line transduced with a retroviral vector to express luciferase under the control of an LTR promoter and an NK effector cell line expressing macaque FcγRIIIa has facilitated large scale ADCC assays evaluating envelope targets following infection with pseudoviruses [28].

These new methodologies have led to elucidation of the possible role of ADCC in protective efficacy and greater understanding of the overall mechanism. We have studied vaccine-elicited ADCC activity extensively in the SIV/SHIV rhesus macaque model, and have reported a series of significant correlations of ADCC with reduced acute and/or chronic viremia following both SHIV and SIV challenges [22, 29–32]. Moreover, a significant correlation of ADCC activity with delayed SIV acquisition was seen [32]. Similarly, an

association of ADCC with protection using the highly effective SIV Δnef vaccine was recently reported [28]. Importantly, significant correlations with protection in HIV-infected individuals have been observed. Long-term slow progressors have exhibited greater breadth in ADCC responses, including more frequent targeting of epitopes in regulatory and accessory proteins [33]. Additionally, apparent immune escape of specific HIV target epitopes attributed to pressure exerted by ADCC has strengthened a role for ADCC in protection [34]. Antibodies that mediate ADCC were shown to occur frequently in breastmilk, with ADCC activity shown to be significantly higher in breastmilk of women who did not transmit HIV to their infants [35]. However, the observation which has provided the greatest stimulus to further research on ADCC was the finding that ADCC was inversely correlated with infection risk in the RV144 vaccine efficacy trial [36]. In an earlier trial, it was shown that this vaccination protocol elicited ADCC activity in the majority of vaccines [37] and the subsequent association in the RV144 trial with protection was invigorating for those working on FcR-related immune responses, especially as neutralizing antibodies were weak or lacking [38] and the only other clear association was with V1/V2 binding antibody [36].

As was previously shown with neutralizing antibody, the most direct evidence for a protective effect of ADCC would be obtained *via* passive antibody transfer resulting in no infection or diminished viremia post viral challenge, but to date this has not been accomplished. Transfer of polyclonal IgG from vaccinated rhesus macaques lacking neutralizing activity against the SIV_{mac251} challenge virus failed to protect newborn rhesus macaques from oral infection when administered subcutaneously prior to SIV exposure [39]. A number of factors may have accounted for the lack of protection in this study including the high challenge dose and route of challenge, insufficient polyclonal antibody transferred, and the low frequency and immaturity of NK cells in neonates (chosen to minimize the amount of IgG needed for the transfer). Further passive transfer studies of non-neutralizing antibodies have not been published. However, transfer into rhesus macaques of the broadly-neutralizing monoclonal antibody, b12, or mutants engineered to prevent complement activation (KA mutant) or to bind complement and Fc receptor poorly (LALA mutant) resulted in diminished protection by the LALA mutant but not the KA mutant in comparison to the parental b12 antibody [40]. Thus, Fc receptor function seems to play a role in protection mediated by the b12 neutralizing antibody. Whether FcR-mediated activity alone can confer protection will need to be assessed by further passive transfer experiments.

The interest stimulated in ADCC activity has led to rapid expansion in knowledge concerning its mechanism of action and overall properties. As mentioned above, while Env was first recognized as the target of antibodies mediating ADCC, other viral gene products have been identified in killing assays, including Nef [41] and Tat [30], and by their activation of NK cells, including Pol [42] and Vpu [34]. Nevertheless, Env remains as the key ADCC target. However, unlike neutralizing antibodies which in general lack breadth and require years before broad reactivity develops, antibodies mediating ADCC often exhibit broad killing across clades as seen by sera of vaccinated non-human primates [43] and sera of HIV-infected individuals [25]. This appears due to the fact that multiple epitopes are suitable for ADCC-mediating antibodies. Polyclonal sera exhibit greater ADCC levels than

monoclonal antibodies, and overall ADCC activity has been correlated with the amount of IgG bound to target cells [25].

While antibodies that mediate ADCC rather than neutralization appear less restricted in epitope recognition leading to effective activity, preferred or immunodominant epitopes have been identified. Non-neutralizing monoclonal antibody A32, isolated from an HIV-infected individual, has been identified as a potent mediator of ADCC activity [44], and the Fab fragment of this antibody blocks a major portion of ADCC activity mediated by plasma of HIV-infected people. Moreover, the majority of RV144 vaccinees tested exhibited ADCC activity, over 90% of which was also blocked by the A32 Fab fragment [45], confirming the immunodominance of this epitope. The A32 epitope is discontinuous within the C1, C4, and C5 envelope domains, and exposed after binding of envelope to cell surface CD4 and co-receptor and initiation of fusion with the cell membrane [46]. The A32 antibody is non-neutralizing, as the fusion process has already begun at the time it is exposed. However, it has been suggested that exposure of the A32 epitope persists for an extended period of time, providing one explanation for its immunodominance [47]. A more extensive investigation of HIV envelope epitopes categorized as cluster A (the gp120 region hidden by gp41 in the Env trimer), cluster B (proximal to the co-receptor binding site) and cluster C (the co-receptor binding site) confirmed that all elicited ADCC activity, with cluster A antibodies which includes A32, being the most potent. Only antibodies in cluster C possessed neutralizing activity as well [48].

Overall the ADCC mechanism has been well explored. As summarized below, it is impacted by Fc genotypes and HIV/SIV infection status. Moreover, it contributes to neutralizing antibody effects *in vivo* and has broadened our understanding of neutralizing antibody responses. The issue of antibodies that mediate both ADCC and neutralization will be discussed below.

ANTIBODY-DEPENDENT CELL-MEDIATED VIRAL INHIBITION

The ADCVI assay was derived from a method used to evaluate the effect of ADCC on measles virus [49], termed antibody-dependent cell-mediated immunity. Forthal and colleagues adapted the method to evaluate inhibition of HIV replication [50], and the activity assessed became known as ADCVI. While the literature sometimes refers to ADCVI and ADCC interchangeably, ADCVI actually represents a broader mechanism, which can reduce HIV replication not only by direct lysis of infected cells in the presence of antibody and effector cells, but also by cytokine secretion and opsonization. Therefore, we address it separately here. As with ADCC, several studies have suggested a role for ADCVI in protection against HIV or SIV infection. Pooled hyperimmune serum from macaques that were initially vaccinated with live-attenuated SIV and exhibited successful viremia control after subsequent SIV_{mac251} exposure with development of high SIV-specific antibody titers was shown to protect neonatal macaques against oral challenge with SIV_{mac251} [51]. This same hyperimmune serum, lacking neutralizing activity, was later shown to have potent ADCVI activity, suggesting that it played a role in preventing infection in the earlier passive transfer study [52]. With regard to protection against HIV, the VAX 004 phase III trial of a bivalent, recombinant gp120 vaccine showed no protective efficacy, however, an inverse

correlation was noted between vaccine-induced antibody and the HIV infection rate [53]. Sera obtained from vaccinees in this study exhibited ADCVI activity inversely correlated with the rate of HIV acquisition [54], again suggesting a protective role for this immune response.

Other investigators have obtained similar correlations with protective efficacy against both SIV [29, 32] and SHIV [30, 31, 55] in non-human primate models. As expected based on the inclusion of ADCC within the ADCVI mechanism, the two activities are often associated, with protective efficacy attributed to one reproduced by the other. In fact the two activities arise within the same time period post infection. Both ADCC and ADCVI activities can be detected within 3 weeks of infection with SIV, associated with the appearance of binding antibody to the viral envelope [56, 57] and long before comparable titers of neutralizing antibody arise. In general, factors which modulate protective effects attributed to ADCC also modulate ADCVI activity.

ANTIBODY-DEPENDENT CELL PHAGOCYTOSIS

Of the several antibody activities that utilize an Fc-related mechanism, ADCP has been the least explored, and little information exists on a possible protective role. This is likely to quickly change, as a simple, high-throughput assay has recently been developed, facilitating assessment of this immune response [58]. ADCP activity is of particular interest as NK cells have been shown to be depleted in the gut mucosa following HIV infection [59, 60]. Thus ADCC activity mediated by NK cells may not be a reliable mucosal protective measure against HIV/SIV infection which preferentially targets the gut. However, as effector cells of ADCP include macrophages which are plentiful in the mucosa, this mechanism may play an important role in controlling or preventing HIV/SIV infection and spread.

ADCP is generally mediated by the expression of FcγRII on the surface of macrophages, immature dendritic cells, and neutrophils [61], leading to uptake of opsonized virus into endosomes and degradation. A recent study showed that compared to uninfected control subjects, HIV-infected individuals exhibited preferential binding to the activating receptor FcγRIIa compared to the inhibitory receptor, FcγRIIb [62]. Moreover, controllers and untreated progressors exhibited greater phagocytic activity, accounted for by the skewed FcγRIIa recognition. FcγR1 can also mediate phagocytosis, but has exhibited an alternative function in enhancing the neutralization potency of MPER antibodies, attributed to a pre-positioning effect [63]. Expression of Fc RI on TZM-bl cells greatly increased the neutralization titers of monoclonal antibodies 4E10 and 2F5. Rather than ADCP, the enhanced neutralization was attributed to the antibody Fc-FcR interaction which positioned the antibody at the viral surface, thus lessening steric hindrance at the virus-cell interface.

Complement receptors can also mediate antibody-dependent phagocytosis. Here our focus is Fc receptor mechanisms, but we can point out that complement activities of monoclonal antibodies have to date shown no association with protection in a passive transfer study [40] and in a human therapy trial [64]. It has been noted, however, that complement-related activities are more effective with polyclonal antibodies compared to monoclonals, perhaps due to reaching a necessary threshold for complement activation [64]. In contrast, ADCP *via*

Fc receptors is effective with both monoclonal and polyclonal antibodies [65, 66]. The degree to which ADCP plays a role in protective efficacy awaits further studies.

TRANSCYTOSIS INHIBITION

HIV/SIV transcytosis through mucosal epithelial barriers is a crucial early step in the establishment of productive infection [67]. This “high jacking” of the epithelial transcellular vesicular pathway is used not only by HIV/SIV, but also by a number of other bacterial [68, 69] and viral [70] pathogens. For HIV infection, where over 90% of global transmission occurs across mucosal surfaces [71, 72], barrier protection through transcytosis inhibition by HIV/SIV-specific antibodies (both neutralizing and nonneutralizing) is an important early immune response. Given that secretory IgA is the most predominant immunoglobulin at mucosal sites [73, 74], special attention has been given to the study of mucosal HIV/SIV-specific IgA antibodies [75, 76]. Despite this, HIV/SIV-specific IgG antibodies have also been studied due to their abundance within secretions of the male and female reproductive tracts [77] and in rectal samples [29].

There are several mechanisms by which transcytosis inhibition can occur. One involves the pIgR which complexed with pIgA as it traverses the epithelial cell from the basolateral surface binds HIV as it is transcytosed through the cell and redirects the virus back to the apical side where it is cleared [78]. This mechanism is called intracellular neutralization, and one would expect that it occurs regularly due to the abundant pIgA produced mucosally. However, the extent to which it occurs *in vivo* is not known, nor are factors which influence binding of pIgA to its receptor, or the potential impact of this mechanism on protection in natural infection or following vaccination. A functional assay that evaluates transcytosis inhibition across epithelial cells by IgG as well as IgA and sIgA has been used in characterizing mucosal antibodies, although the inhibitory mechanism does not involve the pIgR [79]. As we believe mucosal IgA is critically important in protection against HIV, we include a brief discussion of transcytosis inhibition here as it relates to protective efficacy.

In 1999, Hocini and Bomsel demonstrated that both secretory IgA and IgG purified from colostrum of HIV-seropositive women were capable of inhibiting the spread of HIV across mucosal sites through transcytosis of endosome-internalized viral particles using an *in vitro* transwell system [80]. A year later, Devito *et al.* demonstrated that HIV-specific IgA purified from cervicovaginal and salivary fluids was capable of significantly inhibiting HIV transcytosis as well [76]. Our group has previously shown that mucosal immunization with replication-competent adenoviral HIV or SIV recombinants followed by intramuscular boosting with Env proteins significantly decreases acute [31] and chronic [29] SHIV or SIV viremia. In both cases viral-specific IgG and IgA nonneutralizing antibodies that mediated transcytosis inhibition were detected in plasma and rectal secretions, and the inhibition correlated with the decreased viremia. In a separate study, intramuscular and intranasal immunization with virosomes grafted with HIV-1 gp41-subunit antigens was shown to elicit strong protection from $SHIV_{SF162P3}$ vaginal challenges. Four of five vaccinated macaques remained virus-negative, even after 13 vaginal challenges [81]. Although no neutralizing activity could be detected in plasma, transcytosis inhibiting gp41-specific vaginal IgA antibodies and neutralizing and/or ADCC-mediating vaginal IgG antibodies were found in

all protected animals. Protection from acquisition was strongly associated with the presence of these antibodies [81]. Recently, vaccination of healthy human subjects with virosomes harboring surface HIV-1 gp41-derived P1 lipidated peptides has been shown to elicit rectal and vaginal P1-specific IgG non-neutralizing antibodies in 100% of subjects that received a high dose of the vaccine. Unlike IgG, P1-specific IgA was more difficult to induce and was only detected in a portion of vaccinated subjects [82]. Even though vaginal secretions of these vaccinated subjects lacked neutralizing activity, they had detectable HIV-1-specific transcytosis inhibitory activity, which correlated with the presence of P1-specific IgG antibodies [82]. Clearly mucosal antibody elicitation, function, and mechanisms of protection are areas ripe for study and exploitation in the HIV vaccine field. Key questions include the extent to which IgG and IgA transudated from peripheral blood mediates transcytosis inhibition compared to locally produced mucosal sIgA. Further, mechanisms of transcytosis inhibition mediated by IgG and IgA need to be elucidated.

EFFECTOR CELLS

A discussion of Fc-related immune responses would not be complete without providing information on the cells which express the receptors and mediate the functions summarized above. We have already mentioned macrophages, dendritic cells, and neutrophils, as mediators of ADCP. Here we will discuss NK cells as important mediators of ADCC and also γδT cells which are abundant in the mucosa and able to mediate a spectrum of immune functions.

Natural Killer Cells

NK cells are innate lymphocytes that provide a first line of defense against pathogen-infected and neoplastic cells. Due to their rapid response potential and widespread circulatory and tissue distribution, NK cells are key components of the immune system that bridge innate and adaptive immunity [83]. NK cells are specialized in detection and elimination of pathogen-infected and neoplastic cells through cytotoxic function [84] and can also play immunomodulatory roles through the production of inflammatory (IFN-γ, TNF-α) and regulatory (IL-10) cytokines and chemokines [85]. Unlike T cells, NK cells are not capable of antigen-specific receptor somatic recombination, and therefore rely on the surface recognition of MHC class I, class I-like molecules, and other ligands, by germline-encoded activating and inhibitory NK cell receptors (NKRs) to induce or arrest their cytotoxic activity against target cells [86, 87]. Human NK cells are commonly characterized as $CD3^{-}CD56^{dim/bright}$ cells, whereas the majority of circulatory rhesus macaque NK cells are $CD3^{-}CD8\alpha^{+}CD159a^{+}$ [88–90]. A small but functional subset able to mediate direct lysis of cells lacking MHC class I expression as well as antibody-dependent killing are $CD3^{-}CD8\alpha^{-}CD16^{+}CD56^{+}$ [91]. Macaque circulatory NK cells ($CD3^{-}CD8\alpha^{+}CD159a^{+}$) can be subdivided in three subpopulations based on their CD16 (FcγRIIIa) and CD56 expression patterns: $CD16^{+}CD56^{-/dim}$ cells represent ~85% of circulatory NK cells and are mostly cytotoxic effectors, whereas $CD16^{-}CD56^{+}$ cells are the major cytokine-producing cells and account for ~5% of circulatory NK cells. A third subset, which does not have a human counterpart, exists within macaque NK cells. These double negative (DN, $CD16^{-}CD56^{-}$) NK cells are thought to have both cytolytic and cytokine-producing potential and account for

up to 10% of circulatory NK cells [89, 90]. In rhesus macaques, tissue-resident NK cells ($CD3^{-}CD8\alpha^{+}CD159a^{+}$) can be divided into 4 subpopulations based on their surface expression of CD16 and CD56. The proportional distribution of these 4 subsets varies greatly depending on the tissue being studied [89, 90, 92]. Recently, a new subset of innate lymphocytes that express some NK cell markers and produce the cytokine IL-22 (NK22 cells) has been identified in different species [92, 93]. A recent study by Reeves *et al.* delineated rhesus macaque mucosal NK cells as two mutually exclusive subsets based on their expression of CD159a (NKG2A) or CD336 (NKp44) [92]. $CD336^{+}$ NK cells were restricted to mucosal tissues, were non-cytotoxic and only produced IL-22 and IL-17, making them resemble lymphoid tissue inducing cells [92, 94]. Both $CD159a^{+}$ and $CD366^{+}$ NK cell subpopulations are functionally perturbed during chronic SIV infection.

There is strong evidence that NK cells can contribute to protection against both initial HIV infection and disease progression [95]. It has been previously shown that certain NKR phenotypes are associated with protection against HIV infection [96], and non-pathogenic HIV infections are associated with higher levels of NK cell cytotoxicity [50]. Moreover, vaccine-elicited non-neutralizing anti-envelope antibodies contribute to protection against intrarectal SIV and intravenous $SHIV_{89.6P}$ challenges through cell-mediated activities that include ADCC and ADCVI, both of which are strongly associated with NK cell function [31, 97, 98]. Reports on NK function during HIV or SIV infection vary however, making a clear association with protection difficult. For example, during SIV infection, circulating CD16+ NK cells were reported to be expanded in number and exhibited increased markers of cytotoxicity [89], suggesting an increased potential for ADCC activity. In contrast, ADCC activity in HIV-infected individuals was reported to be inhibited, and linked to low CD16 expression [99]. As discussed below, a multitude of pathogenic effects on immune cells make immune correlates difficult to identify during HIV/SIV infection.

In recent years, murine NK cells have been shown to possess memory-like functions, previously thought to be a property only of immune cells capable of receptor somatic recombination [100–103]. Canonical NK cell memory has been difficult to prove in human and non-human primate models, given the technical difficulties associated with the rarity of these cells in blood and the limited amount of tissues available for research. Despite this, studies using human PBMCs have reported that an antigen-specific IL-2-dependent co-operation exists between CD4 T cells and NK cells [104, 105]. Using immunization strategies specific for *P. falciparum* and rabies virus, several groups have shown that following stimulation, antigen-specific IL-2 production by memory $CD4^{+}$ T cells is directly correlated with, and necessary for, NK cell activation measured as IFN-γ production and CD107a expression [106–108]. Using a similar *in vitro* strategy, our group reported SIV-specific T cell-dependent NK cell effector responses in a group of SIV-infected controller macaques able to maintain low levels of chronic viremia These IL-2-dependent co-operative responses were exhibited by all NK cell subsets in the circulation and in various tissues [90]. This co-operative response introduces an additional variable in assessing humoral immune correlates. The presence of immune T cells secreting IL-2 and most likely additional cytokines that influence NK activation will alter the function of NK effector cells *in vivo* and possibly also in assay systems *in vitro* that use PBMC from virus-positive donors as effector cells.

γδ T Cells

These cells make a number of unique contributions to immunologic functions, recently summarized by Vantourout and Hayday [109]. Importantly, while γδT cells make up a small percentage of peripheral blood T cells, they are present at high frequencies in the gut [110], where they could potentially play a major role in protection against HIV/SIV. Here we will limit our discussion to their antibody interactions, which represent a small fraction of their overall impact on the immune system.

γδ T cells belong to the innate immune system, display rapid, antigen-triggered responses, and lack classical MHC restriction [111]. They respond to non-peptide phosphoantigens and display cytotoxicity against cells infected with a variety of pathogens. They have been reported to be high in HIV elite controllers [112]. The Vδ1 subset is prevalent in tissues, including the gut, while the Vδ2 subset is more prevalent in peripheral blood, making up 1 to 10% of lymphocytes in humans [111]. In AIDS, a loss of Vδ2 cells in the blood occurs, leading to an inversion in the normal Vδ2/Vδ1 ratio [113]. Vδ2 cells can mediate a number of effector functions, including secretion of Th1 cytokines, induction of DC maturation and activation, recruitment of phagocytes, and mediation of cytotoxic activity. With regard to the latter, human Vδ2 cells, like NK cells, exhibit 2 phenotypes: one which produces large amounts of cytokines but little perforin, and the other which expresses low levels of cytokine, but high levels of perforin and FcγRIIIa (CD16) [114, 115], the receptor closely associated with ADCC activity. Peripheral blood Vδ2 cells can be expanded with zoledronate and IL-2, and are able to mediate ADCC [116]. In addition, $CD16^{+}$ γδ T cells produce IFN-γ when incubated with IgG-opsonized human cytomegalovirus [117], suggesting an additional potentially protective mechanism of these cells. Finally, bloodγδ T cells have been reported to have phagocytic activity [118], taking up opsonized material *via* expressed FcRγIIIa and also presenting processed antigen on MHC class II. The significance of this activity with regard to control of HIV/SIV or other infectious agents remains to be explored. Further, the overall contribution of γδ T cells to protective efficacy has not been explored due in part to the difficulty of obtaining cells from the mucosal compartment. This is an area of investigation where use of non-human primate models could make important contributions.

FC RECEPTORS

It is beyond the scope of this chapter to exhaustively review Fc Receptors. Several excellent reviews are already in the literature concerning cellular receptors for IgG [119–121] and IgA [122, 123]. However, as FcR-Fc interactions are essential for the various activities we have discussed here, we will mention a few points of particular relevance to potential control of HIV/SIV including FcR polymorphisms, interaction with IgA, and glycosylation of the Fc component of immunoglobulins.

Fcγ Receptors

In humans, cellular receptors for IgG include the inhibitory receptor, FcγRIIb, and five other activating receptors, FcγRI, FCγRIIa, FcγRIIc, FcγRIIIa, and FcγRIIIb. The latter lacks a signaling function. FcγRI is a high affinity receptor, whereas the others exhibit low to

medium affinity for the IgG Fc component. Polymorphisms in FcγRIIa and FcδRIIIa in particular have been associated with infectious disease risk. A point mutation (G to A) in FcγRIIa results in an arginine (R) to histidine (H) substitution at amino acid position 131. The histidine allotype results in a higher binding efficiency for human IgG2 and IgG3 compared to the arginine allotype and more efficient phagocytosis of IgG2-opsonized particles [124]. Similarly, a T to G substitution in the FcγRIIIa gene results in a valine (V) to phenylalanine (F) change at amino acid position 158. The homozygous VV phenotype exhibits greater affinity for IgG1, IgG3, and IgG4, and enhanced NK activity [124]. These polymorphisms with clear functional consequences have been seen to impact infectious diseases such as placental malaria (increased risk in HH phenotypes) [125] and cryptococcosis (RR and VV phenotypes with greater risk) [126]. Relevant to our discussion here is the association of the low affinity Fc RIIIa FF phenotype with limited development of Kaposi's sarcoma (KS) and human herpesvirus-8 (HHV-8) seropositivity [127]. In contrast, the heterozygous phenotype, VF, was associated with KS development and appearance of HHV-8 antibodies, suggesting that the higher affinity receptor facilitated uptake of HHV-8 into susceptible cells or provided greater immune activation. KS is a common malignancy occurring in HIV-infected individuals, associated with human herpesvirus-8 infection. In the same vein, The FcγRIIa HH high affinity phenotype was associated with greater susceptibility to perinatal HIV infection in infants [128]. This result may reflect the fact that FcγRIIa binds IgG2, which is inefficiently transferred across the placenta in contrast to IgG1, IgG3, and IgG4. Low levels of IgG2 bound to the receptor may lead to low levels of opsonization and infection of macrophages rather than endocytosis and viral clearance.

In 2007, Forthal *et al.* reported that HIV-infected men with the low affinity homozygous Fc RIIaγR/R phenotype exhibited a faster rate of CD4+ T cell decline then heterozygous men or men with the H/H phenotype [129]. However, progression to AIDS, as defined by acquisition of an opportunistic infection was not affected, because the high affinity H/H phenotype was associated with increased risk of *Pneumocystis jiroveci* pneumonia. These conflicting outcomes might be explained by diminished clearance of opsonized HIV in the RR individuals, whereas enhanced phagocytosis of opsonized *P. jiroveci* in the HH individuals again may have led to increased immune activation as well as facilitated infection of susceptible cell types. These findings while illustrating the clear effects that FcγRIIa phenotype has on immune response, also underscores the complexity of the interacting variables.

This same study also confirmed the previous finding of an effect of FcγRIIIa on KS acquisition [129]. Moreover, examination of HIV suppressors versus progressors showed the homozygous high affinity FcγRIIIa phenotype (VV) was associated with the HIV progressors [130]. No associations were seen for the FcγRIIa phenotype. A further study of vaccinees and placebo recipients in the Vax004 phase III clinical trial which assessed a recombinant gp120 vaccine showed no associations between infection rate and FcγRIIa phenotype. However, the FcγRIIIa VV phenotype showed a significant association with infection rate among low but not high-risk vaccinees [131]. Moreover, low-risk vaccinees with the VV phenotype had a greater infection rate than low-risk VV placebo recipients, suggesting that the vaccine may have increased the probability of becoming infected. This latter finding is especially troubling with regard to vaccine development. FcγRIIIa is present

on monocyte/macrophages, dendritic cells, NK cells and some γδ T cells and mediates ADCC, a suspected immune correlate in the RV144 trial. The greater infection rate seen in the Vax004 vaccinees occurred early after enrollment when vaccine-elicited antibody levels had not yet reached their peak suggesting the quality of antibody response may have contributed to the outcome. Antibody-dependent enhancement of viral infections is a well-known phenomenon [132] and should be carefully investigated with regard to on-going vaccine development. Improved Fc-FcR interactions are beneficial for therapeutic antibodies in the cancer arena, but whether enhanced binding affinity of HIV antibodies will improve protection or enhance infection needs to be carefully explored.

Fcα Receptors

HIV is a mucosal infection, yet little is known about functional interactions of IgA with receptors at mucosal surfaces of HIV infected or vaccinated individuals. There are several excellent reviews on IgA receptors [122], FcαRI [133], and IgA function [123]. The main IgA myeloid receptor is FcαRI (CD89), which mediates such responses as respiratory burst, degranulation, and phagocytosis by granulocytes, monocytes and macrophages [122]. Monomeric IgA interaction with Fc RI blocks activation by other receptors, while multimeric Fc RI crosslinking leads to phagocytosis, antigen presentation, ADCC, and release of cytokines [133]. However there are other IgA receptors in addition to FcαR1 including the polymeric Ig receptor (pIgR) discussed above, and others that have yet to be identified [123]. The pIgR is important for transporting IgA into mucosal secretions, but also can clear immune complexes from the lamina propria [134] or from epithelial cells as discussed for HIV transcytosis inhibition. While serum IgA has been reported to bind FcαRI, providing phagocytosis of opsonized bacteria, secretory IgA did not mediate phagocytosis [135]. However, human NK cells have been shown to bind sIgA by FcαR, different from FcαRI (CD89), providing a mechanism for mediating signal transduction and cell killing [136]. Moreover, binding of IgA to intestinal epithelial cells occurs *via* a receptor distinct from pIgR and CD89 [137]. This receptor binds monomeric IgA but not secretory IgA, as the presence of the secretory component and/or the J chain prevents binding. However sIgA binds to eosinophils by an as yet uncharacterized receptor whereas monomeric IgA does not [138]. SIgA can also interact with dendritic cells, not *via* CD89, but by carbohydrate-recognizing receptors such as the mannose receptor [139]. This mechanism has been suggested as a means to modulate mucosal immune responses. As mucosal immunity moves into the forefront of HIV vaccine research, identification and full characterization of these various IgA receptors will become critical to our understanding of the role of IgA antibodies in protective immunity. Of special interest is the correlation of high serum IgA antibodies with increased risk of infection in the RV144 vaccine trial [36]. However, at the same time, viral envelope specific mucosal IgA antibodies have correlated with delayed acquisition in an SIV macaque model [32]. Understanding these conflicting observations will help elucidate mechanisms of protective immunity.

Fc Glycosylation

Immunoglobulins are glycosylated proteins (for review see [140]). The attached glycans perform several critical functions including maintenance of structure, solubility and conformation, facilitation of subcellular transport, secretion and clearance, and maintenance

of effector functions by control of binding of Fc to Fc receptors [140]. Much attention has been given to the fact that the presence or absence of fucose impacts the binding of IgG1 to FcγRIIIa [141, 142] especially in the therapeutic cancer field and more recently with regard to HIV protection. In the absence of fucose, binding affinity of Fc-FcγRIIIa is enhanced. Fucose removal from human IgG has resulted in enhanced ADCC activity [143]. However, this enhancement depends on the type of effector cell expressing FcγRIIIa. Lack of fucose on IgG led to enhanced ADCC activity by mononuclear cells (T cells, NK and monocytes) while neutrophils exhibited better cell killing when the antibody was highly fucosylated [144].

The impact of glycosylation on antibodies in the HIV field is exemplified by studies in which the neutralizing monoclonal antibody 2G12 was engineered to lack fucose. The non-fucoslyated variants exhibited enhanced binding to FcγRIIIa and mediated higher ADCVI activity against HIV and SHIV isolates [145]. As discussed above, ADCVI encompasses several activities including ADCC, chemokine secretion, and depending on the effector cells, phagocytosis. Thus it is hoped that elicitation of non-fucosylated antibodies by vaccination will generate greater protective efficacy. A caveat, however, is that while a similar non-fucosylated broadly neutralizing antibody, b12 exhibited enhanced ADCVI and ADCC activity *in vitro*, *in vivo* low-dose repetitive SHIV challenge studies in macaques did not reveal any greater protection using the non-fucosylated variant compared to the wild type antibody [146]. As Fc-receptor-mediated activity was previously shown to influence b12-mediated protection [40], these results suggest an alternative Fc mechanism other than ADCC might be involved in protection. It is also possible that wild type b12 activity was already optimal *in vivo*, so that enhancement by defucosylation was not possible.

A recent publication reports that the degree of fucosylation is not the only variable that impacts Fc-FcR interaction and hence immune function. HIV infected individuals exhibiting better control of HIV exhibited an antibody profile shifted towards agalactosylated forms overall, with HIV specific antibodies displaying an even greater proportion of agalactosylated, asialyated, and afucosylated glycans [147]. The glycoforms had enhanced ADCVI activity along with enhanced Fc-receptor binding, and were in concert with the overall glycosyltransferase profile of B cells in the subjects.

Given the varied outcomes reported for FcR polymorphisms with regard to protection against HIV or enhancement of infection, it will be necessary to confirm whether an enhanced antibody activity seen *in vitro* translates to improved protection *in vivo*. Additional research is needed to sort out the sometimes subtle effects of Fc glycosylation on antibody function. Analysis and control of Fc glycosylation is currently a very active area of research, with a potential for high impact on protection against HIV infection and/or disease progression.

Fc Receptor Polymorphism and Post-Transcriptional Modifications in Non-Human Primates

In view of the important contributions of non-human primates to elucidation of protective immune mechanisms, it is surprising how little is known about effects of Fc receptor polymorphisms and post-transcriptional Fc modifications on antibody function in these

species, especially macaques. Literature regarding genetic differences is very limited, and to the best of our knowledge no studies on Fc post-transcriptional glycosylation in macaques have been conducted. The IgG Fc receptor genes CD16 (FcγRIII), CD32 (FcγRII) and CD64 (FcγRI) have been characterized for rhesus macaques and found to be 91.7%, 88% and 94% homologous to their human counterparts, respectively [148, 149]. Interestingly, a high level of intra-species polymorphism seems to be present in macaque FcγR sequences. Among nine macaques examined, five CD32 and three CD64 and CD16 different allelic sequences differing by at least one amino acid were detected [148]. Despite this, few investigators have examined the functional effects of such polymorphisms. In one study that associated a clear biologic effect with FcγRIII polymorphisms, anti-CD20 (rituximab) was administered to SIV-infected rhesus macaques to deplete B cells in order to determine the contribution of antibodies on control of SIV replication. Three macaques (group A) failed to make an antibody response and became rapid progressors, while three others (group B) developed protective antibody responses and controlled viremia. Notably, group A and B macaques displayed distinct allelic variants of FcγRIII [150]. Whether the genetic polymorphisms contributed to the difference in response observed requires further study.

Despite the high percentage of amino acid sequence similarity between human and macaque genes, several differences have been observed in the cell-type expression patterns and number of Fcγ isoforms observed when comparing human and macaques [149]. For example, while CD16 is expressed on human lymphocytes, monocytes, and neutrophils, such expression was not observed on macaque neutrophils. Although the genetic variation in these receptors has been largely elucidated in macaques, further functional characterization studies will be necessary to elucidate the specific role of these FcγR variations in macaque-specific Fc-related immune responses.

NON-MECHANISTIC IMMUNE CORRELATES OF PROTECTION

In addition to specific features of Fc and FcR molecules that modulate interactions and subsequent immune responses, other characteristics of humoral immune responses observed in vaccine studies have been correlated with protective efficacy, although precise mechanisms have not been elucidated. These properties include B cell memory and antibody avidity.

Envelope-specific B Cell Memory

Induction of long-lasting plasma cells that produce viral-specific antibody, in particular broadly-neutralizing anti-HIV/SIV antibodies, is critical for protection from HIV acquisition [151]. To date, HIV envelope vaccine candidates have elicited antibodies with protective activities, but the immune response is generally short-lived, as seen in the RV144 clinical vaccine trial [152]. In natural HIV infection, B cell dysfunction is a general occurrence [153] but this occurs prior to $CD4^+$ T cell loss, suggesting that HIV might possess a component that impairs B cell responses [154]. It is known that the HIV envelope can have deleterious effects on T cells [155], but whether it also has an adverse effect on B cell maturation is not known. This question can be addressed in part through vaccine trials.

Prior to the generation of long-lived plasma cells [156], the B cell maturation process leads to memory B cells and plasma blasts which appear in the bone marrow as well as the circulation. Phenotypic identification of these cell types has allowed detailed study of their contribution to protection in pre-clinical vaccine studies. Flow cytometry techniques using CD21 and CD27 markers have identified memory B cell subpopulations in both humans [157] and rhesus macaques [158]. In humans CD27 is the classical marker for memory B cells, however, in macaques $CD27^{-}$ cells have also been characterized as spontaneous antibody secreting cells (ASC) [159]. Plasma cell/plasma blast populations are most easily identified by direct assessment of ASC by ELISpot assay. Using these methods, our laboratory has previously shown that vaccine-elicited Env-specific ASCs in rhesus macaques are correlated prior to challenge with non-neutralizing antibody-mediated functional responses including ADCC, ADCVI and transcytosis inhibition. Furthermore, when Env-specific ASC were evaluated post-challenge, their magnitude was directly correlated with a reduction in chronic viremia [160]. The results link protection with the quality of the functional humoral immune response. Interestingly, it seems that the quality and longevity of ASCs induced through vaccination is greatly dependent on the vaccine type, as macaque immunization with soluble Env trimers generated short-lived, albeit robust, peripheral B cell responses [161]. Induction of memory B cells is also dependent on the state of the resident B cells. Therapeutic Env vaccination during antiretroviral therapy (ART) in SIV chronically-infected macaques was shown to transiently increase ASC in PBMC and bone marrow cells, but levels returned to pre-treatment levels upon ART cessation and viral rebound [159]. This outcome suggested that during SIV chronic infection B cells are capable of responding to antigen stimulation but are not capable of generating long term memory, most likely related to B cell dysfunction attributable to SIV infection [162, 163].

Antibody Avidity

Antibody avidity is defined as the sum of strength of the bonds formed between an antibody and the antigen-binding sites found in its target [164]. More than ten years ago it was identified as one of a number of complex properties of a mature antibody response associated with protection in macaques infected with attenuated SIV [165] and in horses infected with the lentivirus equine infectious anemia virus [166]. More recently, several pre-clinical non-human primate studies have shown correlations between the avidity of non-neutralizing antibodies and protection [31, 167–170]. Barnett *et al.* showed that anti-Env non-neutralizing antibodies with high avidity indexes were induced by a prime-boost approach consisting of alphavirus replicon particles encoding SF162 gp140 V2, followed by intramuscular trimeric Env protein in MF59 adjuvant. The avidity of such antibodies present before challenge was correlated with protection from an intrarectal challenge with $SHIV_{SF162P4}$ [167]. In two other independent studies also using prime-boost strategies, high avidity anti-Env antibodies were elicited by either DNA/MVA [168], or replication-competent adenovirus priming and Env protein boosting [31]. The avidity indices correlated with protection from SIVsmE660 and $SHIV_{89.6P}$, respectively. Interestingly, the study by Lai *et al.* showed that co-expression of GM-CSF in the DNA prime further enhanced antibody avidity [168], in concert with reports that optimized adjuvant selection can greatly impact antibody affinity and avidity maturation [171, 172]. Moreover, it has previously been demonstrated that avidity maturation is a process dependent on both time and antigenic load.

The study by Xiao *et al*. nicely exemplifies the latter by showing that although all groups of adenovirus immunized animals displayed variable levels of avidity indexes, the highest values were observed in macaques that were further boosted by immunization with $HIV_{89.6P}$ gp140 protein [31]. Similarly, in the clinical setting, the HIV-specific antibody avidity index can be used to differentiate between recent and long-standing HIV infections [173].

More recently, greater attention has been given to non-neutralizing antibodies and their associated characteristics after IgG binding antibodies that targeted the V1V2 region of HIV Env [36, 174] were correlated with the limited protection achieved in the RV144 trial [152]. A recent vaccine trial in non-human primates designed to mimic the RV144 human trial found that protection from SIV_{mac251} acquisition was observed in animals whose non-neutralizing antibodies had high avidity to gp120 and recognized the variable envelope V1/V2 region [170].

COMPLEXITY OF IMMUNE CORRELATE IDENTIFICATION

Neutralizing and Non-Neutralizing Antibodies

Research into Fc receptor-related immune responses has blurred the distinction of neutralizing antibodies as completely separable from non-neutralizing antibodies. In fact it was recognized early on that a monoclonal neutralizing antibody could also mediate ADCC [175], although the significance of this in terms of protective capability was not recognized. The benefit of Fc-FcγR-interactions in enhancing inhibition of HIV infection by neutralizing antibodies *in vitro* has been reported based on studies using polyclonal IgG from HIV-infected individuals [176]. The passive transfer study of the broadly neutralizing b12 monoclonal antibody showing that a variant engineered to lack both complement and Fc-related activities but not complement activity alone decreased protective efficacy against a SHIV challenge clearly demonstrated the contribution of Fc-related activity to protection [40]. Similarly, a study comparing b12, the weakly neutralizing related b6 monoclonal, and F240, a non-neutralizing monoclonal recognizing a cluster 1 epitope in gp41, showed protection elicited by b12 but not b6 against a SHIV challenge [177]. However, F240 exhibited partial protection, perhaps related to its better ability to capture infectious virions *in vitro*, although a protective mechanism has not been clearly identified. A further argument for the contribution of Fc-related activities to protection mediated by a monoclonal neutralizing antibody comes from the study of the broadly neutralizing monoclonal 2G12, where a low serum neutralizing titer was shown able to provide partial protection against a SHIV intravaginal challenge [178]. This contrasted to the high serum neutralizing titer exhibited by monoclonal b12 needed to protect against viral challenge. One explanation for the 2G12 outcome is enhanced Fc-related activity. It is also the case that neutralizing antibodies do not always exhibit Fc-related activity such as ADCC. Using a highly sensitive ADCC assay based on an engineered NK effector cell line expressing CD16 and a $CD4^+$ target cell line, plasma samples from both HIV-infected individuals and SIV-infected rhesus macaques that neutralized virus did not always exhibit ADCC activity, suggesting perhaps differences in antibody subtype and Fc characteristics that influence Fc-mediated interactions as discussed above [179].

Effects of HIV/SIV Pathogenesis on Fc-Related Activities

Distinguishing immune responses correlated with protective efficacy in natural infection can be complicated by viral effects. For example, in a study comparing elite controllers and viremic individuals, neutralizing antibody titers were equivalent or lower in the controller compared to the viremics, perhaps reflecting the lower viral load in the controllers and lack of antigenic stimulation [180]. In this study, ADCC activity was seen to be significantly higher in the controllers than in the viremics, although in other investigations, Fc-related activity has been shown to be impaired. Early on, macrophage phagocytosis, ADCC, and cell-medited cytotoxicity (in which antibodies arm NK cells directly) were all seen to be impaired during HIV infection, attributed to both humoral and cellular defects [181, 182]. FcR-mediated phagocytosis is impaired in HIV infection associated with increased levels of cyclic adenosine monophosphate (cAMP) [183]. A cAMP inhibitor, IFN-γ, and an inhibitor of cAMP-dependent protein kinase A were all able to restore phagocytic activity. FcγRIIa expression, important for phagocytic activity, is reduced on monocytes and myeloid dendritic cells in chronic HIV infection [184]. ADCC activity has also been shown to decline with progressive HIV infection, attributed to decreased expression of FcγRIIIa expression on NK cells resulting from increased expression of matrix metalloproteinases (MMP) [185]. This finding is consistent with the report of increased proportions of $CD56^{neg}$ cells along with decreased proportions of $CD56^{dim}/CD16^{pos}$ cells in chronically HIV-infected individuals [186] together with decreased CD107a mobilization [187]. The use of MMP inhibitors has been shown to result in both increased CD16 expression together with increased ADCC activity [185]. The changes seen in NK cells following SIV infection are similar to those described during chronic HIV infection [188], further supporting the macaque non-human primate model for vaccine development and correlates analysis.

It is clear that HIV/SIV pathology impairs immune responses that otherwise might be protective if these viruses did not affect immune effector cells. Therefore, identification of protective immune correlates in infected patient or macaque cohorts may provide skewed results. Clearer outcomes might be obtained from pre-clinical and clinical vaccine trials where immune responses elicited in healthy subjects can be assessed with regard to protective outcome. Moreover, the overlap of Fc-related activities with neutralizing antibody responses have clouded what previously was a clear immune correlate of protection. Regardless of whether these activities are protective on their own, their contribution to protection afforded by neutralizing antibody must continually be assessed.

CONCLUSION

Overall, the recognition of the contributions of Fc-related activities to protection has provided more weapons in the arsenal against HIV/SIV acquisition and disease progression. All are viable candidates for inclusion in an effective vaccine. With regard to induction of humoral immunity, we would recommend focusing vaccine design not only on neutralizing antibody, a clear mechanistic correlate, but also on induction of potent humoral immune memory and antibody avidity, and B-cell maturation. In view of the conflicting results of Fc-FcR binding affinities and protection versus enhanced infection, studies on the affinity of antibody Fc components for FcγRIIa and FcγRIIIa receptors must continue. A clear

understanding of the mechanisms that control these affinities and the processes that lead to development of particular antibody glycosylation patterns will greatly facilitate vaccine design. However, assumptions that higher affinity interactions as seen in the cancer therapeutic field will translate into greater protective efficacy *in vivo* must be confirmed in appropriate studies.

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REFERENCES

[1]. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999; 283: 857–60. [PubMed: 9933172]

[2]. Jin X, Bauer DE, Tuttleton SE, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med 1999; 189: 991–8. [PubMed: 10075982]

[3]. Prince AM, Reesink H, Pasqual D, et al. Prevention of HIV infection by passive immunization with HIV immunoglobulin. AIDS Res Hum Retroviruses 1991; 7: 971–3. [PubMed: 1812946]

[4]. Emini EA, Schleif WA, Nunberg JH, et al. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature 1992; 355: 728–30. [PubMed: 1741059]

[5]. Shibata R, Igarashi T, Haigwood N, et al. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med 1999; 5: 204–10. [PubMed: 9930869]

[6]. Mascola JR, Lewis MG, Stiegler G, et al. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. J Virol 1999; 73: 4009–18. [PubMed: 10196297]

[7]. Baba TW, Liska V, Hofmann-Lehmann R, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med 2000; 6: 200–6. [PubMed: 10655110]

[8]. Mascola JR, Stiegler G, VanCott TC, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000; 6: 207–10. [PubMed: 10655111]

[9]. Parren PW, Marx PA, Hessell AJ, et al. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization *in vitro*. J Virol 2001; 75: 8340–7. [PubMed: 11483779]

[10]. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis 2012; 54: 1615–7. [PubMed: 22437237]

[11]. Bomsel M, Heyman M, Hocini H, et al. Intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. Immunity 1998; 9: 277–87. [PubMed: 9729048]

[12]. Huber M, Trkola A. Humoral immunity to HIV-1: neutralization and beyond. J Intern Med 2007; 262: 2–25. [PubMed: 17598811]

[13]. Lyerly HK, Reed DL, Matthews TJ, et al. Anti-GP 120 antibodies from HIV seropositive individuals mediate broadly reactive anti-HIV ADCC. AIDS Res Hum Retroviruses 1987; 3: 409–22. [PubMed: 2833917]

[14]. Koup RA, Sullivan JL, Levine PH, et al. Antigenic specificity of antibody-dependent cell-mediated cytotoxicity directed against human immunodeficiency virus in antibody-positive sera. J Virol 1989; 63: 584–90. [PubMed: 2536094]

[15]. Koup RA, Pikora CA, Mazzara G, Panicali D, Sullivan JL. Broadly reactive antibody-dependent cellular cytotoxic response to HIV-1 envelope glycoproteins precedes broad neutralizing response in human infection. Viral Immunol 1991; 4: 215–23. [PubMed: 1726398]

[16]. Baum LL, Cassutt KJ, Knigge K, et al. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. J Immunol 1996; 157: 2168–73. [PubMed: 8757343]

[17]. Banks ND, Kinsey N, Clements J, Hildreth JE. Sustained antibody-dependent cell-mediated cytotoxicity (ADCC) in SIV-infected macaques correlates with delayed progression to AIDS. AIDS Res Hum Retroviruses 2002; 18: 1197–205. [PubMed: 12487826]

[18]. Ojo-Amaize E, Nishanian PG, Heitjan DF, et al. Serum and effector-cell antibody-dependent cellular cytotoxicity (ADCC) activity remains high during human immunodeficiency virus (HIV) disease progression. J Clin Immunol 1989; 9: 454–61. [PubMed: 2576558]

[19]. Dalgleish A, Sinclair A, Steel M, Beatson D, Ludlam C, Habeshaw J. Failure of ADCC to predict HIV-associated disease progression or outcome in a haemophiliac cohort. Clin Exp Immunol 1990; 81: 5–10. [PubMed: 2379323]

[20]. Tyler DS, Stanley SD, Nastala CA, et al. Alterations in antibody-dependent cellular cytotoxicity during the course of HIV-1 infection. Humoral and cellular defects. J Immunol 1990; 144: 3375–84. [PubMed: 2329275]

[21]. Ahmad A, Morisset R, Thomas R, Menezes J. Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. J Acquir Immune Defic Syndr 1994; 7: 428–37. [PubMed: 7908983]

[22]. Gómez-Román VR, Patterson LJ, Venzon D, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol 2005; 174: 2185–9. [PubMed: 15699150]

[23]. Gómez-Román VR, Florese RH, Patterson LJ, et al. A simplified method for the rapid fluorometric assessment of antibody-dependent cell-mediated cytotoxicity. J Immunol Methods 2006; 308: 53–67. [PubMed: 16343526]

[24]. Pollara J, Hart L, Brewer F, et al. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. Cytometry A 2011; 79: 603–12. [PubMed: 21735545]

[25]. Smalls-Mantey A, Doria-Rose N, Klein R, et al. Antibody-dependent cellular cytotoxicity against primary HIV-infected CD4+ T cells is directly associated with the magnitude of surface IgG binding. J Virol 2012; 86: 8672–80. [PubMed: 22674985]

[26]. Migueles SA, Osborne CM, Royce C, et al. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. Immunity 2008; 29: 1009–21. [PubMed: 19062316]

[27]. Stratov I, Chung A, Kent SJ. Robust NK cell-mediated human immunodeficiency virus (HIV)-specific antibody-dependent responses in HIV-infected subjects. J Virol 2008; 82: 5450–9. [PubMed: 18353957]

[28]. Alpert MD, Harvey JD, Lauer WA, et al. ADCC develops over time during persistent infection with live-attenuated SIV and is associated with complete protection against SIV(mac)251 challenge. PLoS Pathog 2012; 8: e1002890. [PubMed: 22927823]

[29]. Hidajat R, Xiao P, Zhou Q, et al. Correlation of vaccine-elicited systemic and mucosal nonneutralizing antibody activities with reduced acute viremia following intrarectal simian immunodeficiency virus SIVmac251 challenge of rhesus macaques. J Virol 2009; 83: 791–801. [PubMed: 18971271]

[30]. Florese RH, Demberg T, Xiao P, et al. Contribution of nonneutralizing vaccine-elicited antibody activities to improved protective efficacy in rhesus macaques immunized with Tat/Env compared with multigenic vaccines. J Immunol 2009; 182: 3718–27. [PubMed: 19265150]

[31]. Xiao P, Zhao J, Patterson LJ, et al. Multiple vaccine-elicited nonneutralizing antienvelope antibody activities contribute to protective efficacy by reducing both acute and chronic viremia following simian/human immunodeficiency virus SHIV89.6P challenge in rhesus macaques. J Virol 2010; 84: 7161–73. [PubMed: 20444898]

[32]. Xiao P, Patterson LJ, Kuate S, et al. Replicating adenovirus-simian immunodeficiency virus (SIV) recombinant priming and envelope protein boosting elicits localized, mucosal IgA

immunity in rhesus macaques correlated with delayed acquisition following a repeated low-dose rectal SIV(mac251) challenge. J Virol 2012; 86: 4644–57. [PubMed: 22345466]

[33]. Wren LH, Chung AW, Isitman G, et al. Specific antibody-dependent cellular cytotoxicity responses associated with slow progression of HIV infection. Immunology 2013; 138: 116–23. [PubMed: 23173935]

[34]. Chung AW, Isitman G, Navis M, et al. Immune escape from HIV-specific antibody-dependent cellular cytotoxicity (ADCC) pressure. Proc Natl Acad Sci USA 2011; 108: 7505–10. [PubMed: 21502492]

[35]. Mabuka J, Nduati R, Odem-Davis K, Peterson D, Overbaugh J. HIV-specific antibodies capable of ADCC are common in breastmilk and are associated with reduced risk of transmission in women with high viral loads. PLoS Pathog 2012; 8: e1002739. [PubMed: 22719248]

[36]. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 2012; 366: 1275–86. [PubMed: 22475592]

[37]. Karnasuta C, Paris RM, Cox JH, et al. Antibody-dependent cell-mediated cytotoxic responses in participants enrolled in a phase I/II ALVAC-HIV/AIDSVAX B/E prime-boost HIV-1 vaccine trial in Thailand. Vaccine 2005; 23: 2522–9. [PubMed: 15752839]

[38]. Montefiori DC, Karnasuta C, Huang Y, et al. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. J Infect Dis 2012; 206: 431–41. [PubMed: 22634875]

[39]. Florese RH, Van Rompay KK, Aldrich K, et al. Evaluation of passively transferred, nonneutralizing antibody-dependent cellular cytotoxicity-mediating IgG in protection of neonatal rhesus macaques against oral SIVmac251 challenge. J Immunol 2006; 177: 4028–36. [PubMed: 16951366]

[40]. Hessell AJ, Hangartner L, Hunter M, et al. Fc receptor but not complement binding is important in antibody protection against HIV. Nature 2007; 449: 101–4. [PubMed: 17805298]

[41]. Yamada T, Watanabe N, Nakamura T, Iwamoto A. Antibody-dependent cellular cytotoxicity *via* humoral immune epitope of Nef protein expressed on cell surface. J Immunol 2004; 172: 2401–6. [PubMed: 14764710]

[42]. Isitman G, Chung AW, Navis M, Kent SJ, Stratov I. Pol as a target for antibody dependent cellular cytotoxicity responses in HIV-1 infection. Virology 2011; 412: 110–6. [PubMed: 21269655]

[43]. Gómez-Román VR, Florese RH, Peng B, et al. An adenovirus-based HIV subtype B prime/boost vaccine regimen elicits antibodies mediating broad antibody-dependent cellular cytotoxicity against non-subtype B HIV strains. J Acquir Immune Defic Syndr 2006; 43: 270–7. [PubMed: 16940858]

[44]. Ferrari G, Pollara J, Kozink D, et al. An HIV-1 gp120 envelope human monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. J Virol 2011; 85: 7029–36. [PubMed: 21543485]

[45]. Bonsignori M, Pollara J, Moody MA, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol 2012; 86: 11521–32. [PubMed: 22896626]

[46]. Finnegan CM, Berg W, Lewis GK, DeVico AL. Antigenic properties of the human immunodeficiency virus envelope during cell-cell fusion. J Virol 2001; 75: 11096–105. [PubMed: 11602749]

[47]. DeVico AL. CD4-induced epitopes in the HIV envelope glycoprotein, gp120. Curr HIV Res 2007; 5: 561–71. [PubMed: 18045112]

[48]. Guan Y, Pazgier M, Sajadi MM, et al. Diverse specificity and effector function among human antibodies to HIV-1 envelope glycoprotein epitopes exposed by CD4 binding. Proc Natl Acad Sci USA 2013; 110: E69–78. [PubMed: 23237851]

[49]. Forthal DN, Landucci G. *In vitro* reduction of virus infectivity by antibody-dependent cell-mediated immunity. J Immunol Methods 1998; 220:129–38. [PubMed: 9839934]

[50]. Forthal DN, Landucci G, Keenan B. Relationship between antibody-dependent cellular cytotoxicity, plasma HIV type 1 RNA, and $CD4^+$ lymphocyte count. AIDS Res Hum Retroviruses 2001; 17: 553–61. [PubMed: 11350669]

[51]. Van Rompay KKA, Verardi CJ, Dillard-Telm S, et al. Passive Immunization of Newborn Rhesus Macaques Prevents Oral Simian Immunodeficiency Virus Infection. J Infect Dis 1998; 177: 1247–59. [PubMed: 9593009]

[52]. Forthal DN, Landucci G, Cole KS, Marthas M, Becerra JC, Van Rompay K. Rhesus macaque polyclonal and monoclonal antibodies inhibit simian immunodeficiency virus in the presence of human or autologous rhesus effector cells. J Virol 2006; 80: 9217–25. [PubMed: 16940533]

[53]. Gilbert PB, Peterson ML, Follmann D, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis 2005; 191: 666–77. [PubMed: 15688279]

[54]. Forthal DN, Gilbert PB Landucci G, Phan T. Recombinant gp120 Vaccine-Induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. J Immunol 2007; 178: 6596–603. [PubMed: 17475891]

[55]. Bialuk I, Whitney S, Andresen V, et al. Vaccine induced antibodies to the first variable loop of human immunodeficiency virus type 1 gp120, mediate antibody-dependent virus inhibition in macaques. Vaccine 2011; 30: 78–94. [PubMed: 22037204]

[56]. Asmal M, Sun Y, Lane S, et al. Antibody-dependent cell-mediated viral inhibition emerges after simian immunodeficiency virus SIVmac251 infection of rhesus monkeys coincident with gp140-binding antibodies and is effective against neutralization-resistant viruses. J Virol 2011; 85: 5465–75. [PubMed: 21450829]

[57]. Sun Y, Asmal M, Lane S, et al. Antibody-dependent cell-mediated cytotoxicity in simian immunodeficiency virus-infected rhesus monkeys. J Virol 2011; 85: 6906–12. [PubMed: 21593181]

[58]. Ackerman ME, Moldt B, Wyatt RT, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. J Immunol Methods 2011; 366: 8–19. [PubMed: 21192942]

[59]. Mela CM, Steel A, Lindsay J, Gazzard BG, Gotch FM, Goodier MR. Depletion of natural killer cells in the colonic lamina propria of viraemic HIV-1-infected individuals. AIDS 2007; 21: 2177–82. [PubMed: 18090044]

[60]. Sips M, Sciaranghella G, Diefenbach T, et al. Altered distribution of mucosal NK cells during HIV infection. Mucosal Immunol 2012; 5: 30–40. [PubMed: 21993602]

[61]. Holl V, Peressin M, Moog C. Antibody-mediated Fcγ receptor-based mechanisms of HIV inhibition: recent findings and new vaccination strategies. Viruses 2009; 1: 1265–94. [PubMed: 21994593]

[62]. Ackerman ME, Dugast AS, McAndrew EG, et al. Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for Fc R2a and Fc R2b. J Virol 2013; 87(10): 5468–76. [PubMed: 23468489]

[63]. Perez LG, Zolla-Pazner S, Montefiori DC. Antibody-dependent, Fc RI-mediated neutralization of HIV-1 in TZM-bl cells occurs independently of phagocytosis. J Virol 2013; 87: 5287–90. [PubMed: 23408628]

[64]. Huber M, von Wyl V, Ammann CG, et al. Potent human immunodeficiency virus-neutralizing and complement lysis activities of antibodies are not obligatorily linked. J Virol 2008; 82: 3834–42. [PubMed: 18234794]

[65]. Holl V, Peressin M, Schmidt S, et al. Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. Blood 2006; 107: 4466–74. [PubMed: 16469871]

[66]. Holl V, Peressin M, Decoville T, et al. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. J Virol 2006; 80: 6177–81. [PubMed: 16731957]

[67]. Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nat Med 1997; 3: 42–7. [PubMed: 8986739]

[68]. Kochut A, Dersch P. Bacterial invasion factors: Tools for crossing biological barriers and drug delivery? Eur J Pharm Biopharm 2012; 84(2): 242–50. [PubMed: 23207324]

[69]. Nikitas G, Deschamps C, Disson O, Niault T, Cossart P, Lecuit M. Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. J Exp Med 2011; 208: 2263–77. [PubMed: 21967767]

[70]. Martin-Latil S, Gnadig NF, Mallet A, et al. Transcytosis of HTLV-1 across a tight human epithelial barrier and infection of subepithelial dendritic cells. Blood 2012; 120: 572–80. [PubMed: 22589473]

[71]. Denton PW, Garcia JV. Mucosal HIV-1 transmission and prevention strategies in BLT humanized mice. Trends Microbiol 2012; 20: 268–74. [PubMed: 22503637]

[72]. Tebit DM, Ndembi N, Weinberg A, et al. Mucosal transmission of human immunodeficiency virus. Curr HIV Res 2012; 10: 3–8. [PubMed: 22264040]

[73]. Fagarasan S, Honjo T. Intestinal IgA synthesis: regulation of front-line body defences. Nat Rev Immunol 2003; 3: 63–72. [PubMed: 12511876]

[74]. Macpherson AJ, Slack E. The functional interactions of commensal bacteria with intestinal secretory IgA. Curr Opin Gastroenterol 2007; 23: 673–8. [PubMed: 17906446]

[75]. Jain S, Rosenthal KL. The gp41 epitope, QARVLAVERY, is highly conserved and a potent inducer of IgA that neutralizes HIV-1 and inhibits viral transcytosis. Mucosal Immunol 2011; 4: 539–53. [PubMed: 21525865]

[76]. Devito C, Broliden K, Kaul R, et al. Mucosal and plasma IgA from HIV-1-exposed uninfected individuals inhibit HIV-1 transcytosis across human epithelial cells. J Immunol 2000; 165: 5170–6. [PubMed: 11046049]

[77]. Mestecky J, Moldoveanu Z, Smith PD, Hel Z, Alexander RC. Mucosal immunology of the genital and gastrointestinal tracts and HIV-1 infection. J Reprod Immunol 2009; 83: 196–200. [PubMed: 19853927]

[78]. Bomsel M, Heyman M, Hocini H, et al. intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. Immunity 1998; 9: 277–87. [PubMed: 9729048]

[79]. Hocini H, Belec L, Iscaki S, et al. High-level ability of secretory IgA to block HIV type 1 transcytosis: contrasting secretory IgA and IgG responses to glycoprotein 160. AIDS Res Human Retroviruses 1997; 13: 1179–85. [PubMed: 9310284]

[80]. Hocini H, Bomsel M. Infectious human immunodeficiency virus can rapidly penetrate a tight human epithelial barrier by transcytosis in a process impaired by mucosal immunoglobulins. J Infect Dis 1999; 179 S3: S448–53. [PubMed: 10099117]

[81]. Bomsel M, Tudor D, Drillet AS, et al. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. Immunity 2011; 34: 269–80. [PubMed: 21315623]

[82]. Leroux-Roels G, Maes C, Clement F, et al. Randomized Phase I: Safety, Immunogenicity and Mucosal Antiviral Activity in Young Healthy Women Vaccinated with HIV-1 Gp41 P1 Peptide on Virosomes. PLoS One 2013; 8: e55438. [PubMed: 23437055]

[83]. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. Science 2011; 331: 44–9. [PubMed: 21212348]

[84]. Di Santo JP. Functionally distinct NK-cell subsets: developmental origins and biological implications. Eur J Immunol 2008; 38: 2948–51. [PubMed: 18979515]

[85]. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 2001; 22: 633–40. [PubMed: 11698225]

[86]. Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. Nat Rev Immunol 2003; 3: 304–16. [PubMed: 12669021]

[87]. Bryceson YT, Long EO. Line of attack: NK cell specificity and integration of signals. Curr Opin Immunol 2008; 20: 344–52. [PubMed: 18439809]

[88]. Webster RL, Johnson RP. Delineation of multiple subpopulations of natural killer cells in rhesus macaques. Immunology 2005; 115: 206–14. [PubMed: 15885126]

[89]. Reeves RK, Gillis J, Wong FE, Yu Y, Connole M, Johnson RP. CD16- natural killer cells: enrichment in mucosal and secondary lymphoid tissues and altered function during chronic SIV infection. Blood 2010; 115: 4439–46. [PubMed: 20339088]

[90]. Vargas-Inchaustegui DA, Xiao P, Tuero I, Patterson LJ, Robert-Guroff M. NK and CD4+ T cell cooperative immune responses correlate with control of disease in a macaque simian immunodeficiency virus infection model. J Immunol 2012; 189: 1878–85. [PubMed: 22798665]

[91]. Vargas-Inchaustegui DA, Demberg T, Robert-Guroff M. A CD8alpha(−) subpopulation of macaque circulatory natural killer cells can mediate both antibody-dependent and antibody-independent cytotoxic activities. Immunology 2011; 134: 326–40. [PubMed: 21978002]

[92]. Reeves RK, Rajakumar PA, Evans TI, et al. Gut inflammation and indoleamine deoxygenase inhibit IL-17 production and promote cytotoxic potential in NKp44+ mucosal NK cells during SIV infection. Blood 2011; 118: 3321–30. [PubMed: 21791421]

[93]. Hughes T, Becknell B, McClory S, et al. Stage 3 immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the TH17 cytokine interleukin-22. Blood 2009; 113: 4008–10. [PubMed: 19244159]

[94]. Crellin NK, Trifari S, Kaplan CD, Cupedo T, Spits H. Human NKp44+IL-22+ cells and LTi-like cells constitute a stable RORC+ lineage distinct from conventional natural killer cells. J Exp Med 2010; 207: 281–90. [PubMed: 20142432]

[95]. Hong HS, Rajakumar PA, Billingsley JM, Reeves RK, Johnson RP. No monkey business: why studying NK cells in non-human primates pays off. Front Immunol 2013; 4: 32. [PubMed: 23423644]

[96]. Boulet S, Kleyman M, Kim JY, et al. A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection. AIDS 2008; 22: 1487–91. [PubMed: 18614872]

[97]. Chung A, Rollman E, Johansson S, Kent SJ, Stratov I. The utility of ADCC responses in HIV infection. Curr HIV Res 2008; 6: 515–9. [PubMed: 18991616]

[98]. Chung AW, Rollman E, Center RJ, Kent SJ, Stratov I. Rapid degranulation of NK cells following activation by HIV-specific antibodies. J Immunol 2009; 182: 1202–10. [PubMed: 19124764]

[99]. Lichtfuss GF, Meehan AC, Cheng WJ, et al. HIV inhibits early signal transduction events triggered by CD16 cross-linking on NK cells, which are important for antibody-dependent cellular cytotoxicity. J Leuk Biol 2011; 89: 149–58.

[100]. Min-Oo G, Kamimura Y, Hendricks DW, Nabekura T, Lanier LL. Natural killer cells: walking three paths down memory lane. Trends Immunol 2013; 34(6): 251–8. [PubMed: 23499559]

[101]. Paust S, von Andrian UH. Natural killer cell memory. Nat Immunol 2011; 12: 500–8. [PubMed: 21739673]

[102]. Sun JC, Lanier LL. Versatility in NK cell memory. Immunol Cell Biol 2011; 89: 327–9. [PubMed: 21173784]

[103]. Cooper MA, Colonna M, Yokoyama WM. Hidden talents of natural killers: NK cells in innate and adaptive immunity. EMBO Rep 2009; 10: 1103–10. [PubMed: 19730434]

[104]. Fehniger TA, Cooper MA, Nuovo GJ, et al. CD56bright natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. Blood 2003; 101: 3052–7. [PubMed: 12480696]

[105]. He XS, Draghi M, Mahmood K, et al. T cell-dependent production of IFN-gamma by NK cells in response to influenza A virus. J Clin Invest 2004; 114: 1812–9. [PubMed: 15599406]

[106]. McCall MB, Roestenberg M, Ploemen I, et al. Memory-like IFN-gamma response by NK cells following malaria infection reveals the crucial role of T cells in NK cell activation by P. falciparum. Eur J Immunol 2010; 40: 3472–7. [PubMed: 21072880]

[107]. Horowitz A, Behrens RH, Okell L, Fooks AR, Riley EM. NK cells as effectors of acquired immune responses: effector CD4+ T cell-dependent activation of NK cells following vaccination. J Immunol 2010; 185: 2808–18. [PubMed: 20679529]

[108]. Horowitz A, Newman KC, Evans JH, Korbel DS, Davis DM, Riley EM. Cross-talk between T cells and NK cells generates rapid effector responses to Plasmodium falciparum-infected erythrocytes. J Immunol 2010; 184: 6043–52. [PubMed: 20427769]

[109]. Vantourout P, Hayday A. Six-of-the-best: unique contributions of γδ T cells to immunology. Nat Rev Immunol 2013; 13: 88–100. [PubMed: 23348415]

[110]. Harris LD, Klatt NR, Vinton C, et al. Mechanisms underlying γδ T-cell subset perturbations in SIV-infected Asian rhesus macaques. Blood 2010; 116: 4148–57. [PubMed: 20660793]

[111]. Urban EM, Chapoval AI, Pauza CD. Repertoire development and the control of cytotoxic/effector function in human γδ T cells. Clin Dev Immunol 2010; 2010: 732893. [PubMed: 20396597]

[112]. Riedel DJ, Safadi MM, Armstrong CL, et al. Natural viral suppressors of HIV-1 have a unique capacity to maintain γδ T cells. AIDS 2009; 23: 1955–64. [PubMed: 19609200]

[113]. Agrati C, D'Offizi G, Gougeon ML. Innate gamma/delta T-cells during HIV infection: terra relatively incognita in novel vaccination strategies? AIDS Rev 2011; 13: 3–12. [PubMed: 21412385]

[114]. Angelini DF, Borsellino G, Poupot M, et al. Fc_RIII discriminates between 2 subsets of Vγ9Vδ2 effector cells with different responses and activation pathways. Blood 2004; 104: 1801–7. [PubMed: 15178578]

[115]. Lafont V, Liautard J, Liautard JP, Favero J. Production of TNF-α by human Vγ9Vδ2 T cells *via* engagement of FcγRIIIA, the low affinity type 3 receptor for the Fc portion of IgG, expressed upon TCR activation by nonpeptidic antigen. J Immunol 2001; 166: 7190–9. [PubMed: 11390467]

[116]. Poonia B, Pauza CD. Gamma delta T cells from HIV+ donors can be expanded in vitro by zoledronate/interleukin-2 to become cytotoxic effectors for antibody-dependent cellular cytotoxicity. Cytotherapy 2012; 14:173–81. [PubMed: 22029653]

[117]. Couzi L, Pitard V, Sicard X, et al. Antibody-dependent anti-cytomegalovirus activity of human γδ T cells expressing CD16 (FcγRIIIa). Blood 2012; 119: 1418–27. [PubMed: 22180442]

[118]. Wu Y, Wu W, Wong WM, et al. Human gamma delta T cells: a lymphoid lineage cell capable of professional phagocytosis. J Immunol 2009; 183: 5622–9. [PubMed: 19843947]

[119]. Nimmerjahn F, Ravetch JV. Fc-receptors as regulators of immunity. Adv Immunol 2007; 96: 179–204. [PubMed: 17981207]

[120]. Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. Nat Rev Immunol 2008; 8: 34–47. [PubMed: 18064051]

[121]. Siberil S, Dutertre C-A, Fridman W-H, Teillaud J-L. Fc R: the key to optimize therapeutic antibodies? Crit Rev Oncol Hematol 2007; 62: 26–33. [PubMed: 17240158]

[122]. Wines BD, Hogarth PM. IgA receptors in health and disease. Tissue Antigens 2006; 68: 103–14. [PubMed: 16866880]

[123]. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. J Pathol 2006; 208: 270–82. [PubMed: 16362985]

[124]. van Sorge NM, van der Pol W-L, van de Winkel JGJ. Fc R polymorphisms: implications for function, disease susceptibility and immunotherapy. Tissue Antigens 2003; 61: 189–202. [PubMed: 12694568]

[125]. Brouwer KC, Lal AA, Mirel LB, et al. Polymorphism of Fc receptor IIa for immunoglobulin G is associated with placental malaria in HIV-1-positive women in western Kenya. J Infect Dis 2004; 190: 1192–8. [PubMed: 15319871]

[126]. Meletiadis J, Walsh TJ, Choi EH, et al. Study of common functional genetic polymorphisms of FCGR2A, 3A and 3B genes and the risk for cryptococcosis in HIV-uninfected patients. Med Mycol 2007; 45: 513–8. [PubMed: 17710620]

[127]. Lehrnbecher T, Foster CB, Zhu S, et al. Variant genotypes of FcγRIIIA influence the development of Kaposi's sarcoma in HIV-infected men. Blood 2000; 95: 2386–90. [PubMed: 10733511]

[128]. Brouwer KC, Lal RB, Mirel LB, et al. Polymorphism of Fc receptor IIa for IgG in infants is associated with susceptibility to perinatal HIV-1 infection. AIDS 2004; 18: 1187–94. [PubMed: 15166534]

[129]. Forthal DN, Landucci G, Bream J, Jacobson LP, Phan TB, Montoya B. FcγRIIa genotype predicts progression of HIV infection. J Immunol 2007; 179: 7916–23. [PubMed: 18025239]

[130]. Poonia B, Kijak GH, Pauza CD. High affinity allele for the gene of FCGR3A is risk factor for HIV infection and progression. PLoS One 2010; 5: e15562. [PubMed: 21187939]

[131]. Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB. Association of Fcγ receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. Blood 2012; 120: 2836–42. [PubMed: 22915639]

[132]. Takada A, Kawaoka Y. Antibody-dependent enhancement of viral infection: molecular mechanisms and *in vivo* implications. Rev Med Virol 2003; 13: 387–98. [PubMed: 14625886]

[133]. Bakema JE, van Egmond M. The human immunoglobulin A Fc receptor Fc RI: a multifaceted regulator of mucosal immunity. Mucosal Immunol 2011; 4: 612–24. [PubMed: 21937986]

[134]. Robinson JK, Blanchard TG, Levine AD, Emancipator SN, Lamm ME. A mucosal IgA-mediated excretory immune system *in vivo*. J Immunol 2001; 166: 3688–92. [PubMed: 11238608]

[135]. Van Egmond M, van Garderen E, van Spriel AV, et al. Fc RI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity. Nat Med 2000; 6: 680–5. [PubMed: 10835685]

[136]. Mota G, Manciulea M, Cosma E, et al. Human NK cells express Fc receptors for IgA which mediate signal transduction and target cell killing. Eur J Immunol 2003; 33: 2197–205. [PubMed: 12884294]

[137]. Kitamura T, Garofalo RP, Kamijo A, et al. Human intestinal epithelial cells express a novel receptor for IgA. J Immunol 2000; 164: 5029–34. [PubMed: 10799857]

[138]. Lamkhioued B, Gounni AS, Gruart V, Pierce A, Capron A, Capron M. Human eosinophils express a receptor for secretory component: role in secretory IgA-dependent activation. Eur J Immunol 1995; 25: 117. [PubMed: 7843220]

[139]. Heystek HC, Moulon C, Woltman AM, Garonne P, and van Kooten C. Human immature dendritic cells efficiently bind and take up secretory IgA without the induction of maturation. J Immunol 2002; 168: 102–7. [PubMed: 11751952]

[140]. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 2007; 25: 21–50. [PubMed: 17029568]

[141]. Okazaki A, Shoji-Hosaka E, Nakamura K, et al. Fucose depletion from human IgG1 oligosaccharide enhances binding enthalpy and association rate between IgG1 and FcγRIIIa. J Mol Biol 2004; 336: 1239–49. [PubMed: 15037082]

[142]. Ferrara C, Stuart F, Sondermann P, Brunker P, Umana P. The carbohydrate at Fc RIIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. J Biol Chem 2006 281: 5032–6. [PubMed: 16330541]

[143]. Niwa R, Natsume A, Uehara A, et al. IgG subclass-independent improvement of antibody-dependent cellular cytotoxicity by fucose removal from Asn^{297}-linked oligosaccharides. J Immunol Methods 2005; 306: 151–60. [PubMed: 16219319]

[144]. Peipp M, van Bueren JJL, Schneider-Merck T, et al. Antibody fucosylation differentially impacts cytotoxicity mediated by NK and PMN effector cells. Blood 2008; 112: 2390–9. [PubMed: 18566325]

[145]. Forthal DN, Gach JS, Landucci G, et al. Fc-glycosylation influences Fcγ receptor binding and cell-mediated anti-HIV activity of monoclonal antibody 2G12. J Immunol 2010; 185: 6876–82. [PubMed: 21041724]

[146]. Moldt B, Shibata-Koyama M, Rakasz EG, et al. A nonfucosylated variant of the anti-HIV-1 monoclonal antibody b12 has enhanced FcγRIIIa-mediated antiviral activity *in vitro* but does not improve protection against mucosal SHIV challenge in macaques. J Virol 2012; 86: 6189–96. [PubMed: 22457527]

[147]. Ackerman ME, Crispin M, Yu X, et al. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. J Clin Invest 2013; 123(5): 2183–92. [PubMed: 23563315]

[148]. Nguyen DC, Scinicariello F, Attanasio R. Characterization and allelic polymorphisms of rhesus macaque (Macaca mulatta) IgG Fc receptor genes. Immunogenetics 2011; 63(6): 351–62. [PubMed: 21327607]

[149]. Rogers KA, Scinicariello F, Attanasio R. IgG Fc receptor III homologues in nonhuman primate species: genetic characterization and ligand interactions. J Immunol 2006; 177(6): 3848–56. [PubMed: 16951347]

[150]. Miller CJ, Genesca M, Abel K, et al. Antiviral antibodies are necessary for control of simian immunodeficiency virus replication. J Virol 2007; 81: 5024–35. [PubMed: 17329327]

[151]. Corti D, Lanzavecchia A. Broadly neutralizing antiviral antibodies. Annu Rev Immunol 2013; 31: 705–42. [PubMed: 23330954]

[152]. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009; 361: 2209–20. [PubMed: 19843557]

[153]. Moir S, Fauci AS. B cells in HIV infection and disease. Nat Rev Immunol 2009; 9: 235–45. [PubMed: 19319142]

[154]. DeMilito A. B lymphocyte dysfinctions in HIV infection. Curr HIV Res 2004; 2: 11–21. [PubMed: 15053337]

[155]. Yoon V, fridkis-Hareli M, Munisamy S, Lee J, Anastasiades D, Stevceva L. The gp120 molecule of HIV-1 and its interaction with T cells. Curr Med Chem 2010; 17: 741–9. [PubMed: 20088758]

[156]. Lanzavecchia A, Sallusto F. Human B cell memory. Curr Opin Immunol 2009; 21: 298–304. [PubMed: 19497721]

[157]. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med 2008; 205: 1797–805. [PubMed: 18625747]

[158]. Titanji K, Velu v, Chennareddi L, et al. Acute depletion of activated memory B cells involves the PD-1 pathway in rapidly progressing SIV-infectd macaques. J Clin Invest 2010; 120: 3878–90. [PubMed: 20972331]

[159]. Demberg T, Brocca-Cofano E, Xiao P, et al. Dynamics of memory B-cell populations in blood, lymph nodes, and bone marrow during antiretroviral therapy and envelope boosting in simian immunodeficiency virus SIVmac251-infected rhesus macaques. J Virol 2012; 86: 12591–604. [PubMed: 22973034]

[160]. Brocca-Cofano E, McKinnon K, Demberg T, et al. Vaccine-elicited SIV and HIV envelope-specific IgA and IgG memory B cells in rhesus macaque peripheral blood correlate with functional antibody responses and reduced viremia. Vaccine 2011; 29: 3310–19. [PubMed: 21382487]

[161]. Sundling C, Martinez P, Soldemo M, et al. Immunization of macaques with soluble HIV type 1 and influenza virus envelope glycoproteins results in a similarly rapid contraction of peripheral B-cell responses after boosting. J Infect Dis 2013; 207: 426–31. [PubMed: 23162135]

[162]. Peruchon S, Chaoul N, Burelout C, et al. tissue-specific B-cell dysfunction and generalized memory B-cell loss during acute SIV infection. PLos One 2009; 4: e5966. [PubMed: 19543531]

[163]. Das A, Veazey RS, Wang X, Lackner AA, Xu H, Pahar B. Simian immunodeficiency virus infection in rhesus macaques induces selective tissue specific B cell defects in double positive CD21+CD27+ memory B cells. Clin Immunol 2011; 140: 223–8. [PubMed: 21622026]

[164]. Siegrist CA, Pihlgren M, Tougne C, et al. Co-administration of CpG oligonucleotides enhances the late affinity maturation process of human anti-hepatitis B vaccine response. Vaccine 2004; 23: 615–22. [PubMed: 15542181]

[165]. Cole KS, Rowles JL, Jagerski BA, et al. Evolution of envelope-specific antibody responses in monkeys experimentally infected or immunized with simian immunodeficiency virus and its association with the development of protective immunity. J Virol 1997; 71: 5069–79. [PubMed: 9188572]

[166]. Hammond SA, Raabe ML, Issel CJ, Montelaro RC. Evaluation of antibody parameters as potential correlates of protection or enhancement by experimental vaccines to equine infectious anemia virus. Virology 1999; 262: 416–430. [PubMed: 10502520]

[167]. Barnett SW, Burke B, Sun Y, et al. Antibody-mediated protection against mucosal simian-human immunodeficiency virus challenge of macaques immunized with alphavirus replicon particles and boosted with trimeric envelope glycoprotein in MF59 adjuvant. J Virol 2010; 84: 5975–85. [PubMed: 20392857]

[168]. Lai L, Kwa S, Kozlowski PA, et al. Prevention of infection by a granulocyte-macrophage colony-stimulating factor co-expressing DNA/modified vaccinia Ankara simian immunodeficiency virus vaccine. J Infect Dis 2011; 204: 164–73. [PubMed: 21628671]

[169]. Zhao J, Lai L, Amara RR, et al. Preclinical studies of human immunodeficiency virus/AIDS vaccines: inverse correlation between avidity of anti-Env antibodies and peak postchallenge viremia. J Virol 2009; 83: 4102–11. [PubMed: 19224993]

[170]. Pegu P, Vaccari M, Gordon S, et al. Antibodies with high avidity to the gp120 envelope protein in protection from SIVmac251 acquisition in an immunization regimen that mimics the RV-144 Thai Trial. J Virol 2012; 87: 1708–19. [PubMed: 23175374]

[171]. Dey AK, Srivastava IK. Novel adjuvants and delivery systems for enhancing immune responses induced by immunogens. Expert Rev Vaccines 2011; 10: 227–51. [PubMed: 21105782]

[172]. Loré K, Karlsson Hedestam GB. Novel adjuvants for B cell immune responses. Curr Opin HIV AIDS 2009; 4: 441–6. [PubMed: 20048709]

[173]. Re MC, Bon I, Grandi N, et al. Recent and long-lasting infections: the need for avidity testing in HIV-1 infected subjects. New Microbiol 2012; 35: 407–14. [PubMed: 23109008]

[174]. Liao HX, Bonsignori M, Alam SM, et al. Vaccine induction of antibodies against a structurally heterogeneous site of immune pressure within HIV-1 envelope protein variable regions 1 and 2. Immunity 2013; 38: 176–86. [PubMed: 23313589]

[175]. Broliden PA, Ljunggren K, Hinkula J, Norrby E, Akerblom L, Wahren B. A monoclonal antibody to human immunodeficiency virus type 1 which mediates cellular cytotoxicity and neutralization. J Virol 1990; 64: 936–40. [PubMed: 2296090]

[176]. Forthal DN, Landucci G, Phan TB, Becerra J. Interactions between natural killer cells and antibody Fc result in enhanced antibody neutralization of human immunodeficiency virus type 1. J Virol 2005; 79: 2042–9. [PubMed: 15681406]

[177]. Burton DR, Hessell AJ, Keele BF, et al. Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. Proc Natl Acad Sci USA 2011; 108: 11181–6. [PubMed: 21690411]

[178]. Hessell AJ, Rakasz EG, Poignard P, et al. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Path 2009; 5: e1000433.

[179]. Alpert MD, Heyer LN, Williams DEJ, et al. A novel assay for antibody-dependent cell-mediated cytotoxicity against HIV-1- or SIV-infected cells reveals incomplete overlap with antibodies measured by neutralization and binding assays. J Virol 2012; 86: 12039–52. [PubMed: 22933282]

[180]. Lambotte O, Ferrari G, Moog C, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. AIDS 2009; 23: 897–906. [PubMed: 19414990]

[181]. Bender BS, Davidson BL, Kline R, Brown C, Quinn TC. Role of the mononuclear phagocyte system in the immunopathogenesis of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Rev Infect Dis 1988; 10: 1142–54. [PubMed: 3206059]

[182]. Tyler DS, Stanley SD, Nastala CA, et al. Alterations in antibody-dependent cellular cytotoxicity during the course of HIV-1 infection. Humoral and cellular defects. J Immunol 1990; 144: 3375–84. [PubMed: 2329275]

[183]. Thomas CA, Weinberger OK, Ziegler BL, et al. Human immunodeficiency virus-1 env impairs Fcγ receptor-mediated phagocytosis *via* a cyclic adenosine monophosphate-dependent mechanism. Blood 1997; 90: 3760–5. [PubMed: 9345063]

[184]. Dugast A-S, Tonelli A, Berger CT, et al. Decreased Fc receptor expression on innate immune cells is associated with impaired antibody-mediated cellular phagocytic activity in chronically HIV-1 infected individuals. Virology 2011; 415: 160–7. [PubMed: 21565376]

[185]. Liu Q, Sun Y, Rihn S, et al. Matrix metalloprotease inhibitors restore impaired NK cell-mediated antibody-dependent cellular cytotoxicity in human immunodeficiency virus type 1 infection. J Virol 2009; 83: 8705–12. [PubMed: 19553339]

[186]. Brunetta E, Hudspeth KL, Mavilio D. Pathologic natural killer cell subset redistribution in HIV-1 infection: new insights in pathophysiology and clinical outcomes. J Leuk Biol 2010; 88: 1119–30.

[187]. Lichtfuss GF, Meehan AC, Cheng W-J, et al. HIV inhibits early signal transduction events triggered by CD16 cross-linking on NK cells, which are important for antibody-dependent cellular cytotoxicity. J Leuk Biol 2011; 89: 149–58.

[188]. He X, Li D, Luo Z, et al. Compromised NK cell-mediated antibody-dependent cellular cytotoxicity in chronic SIV/SHIV infection. PLoS One 2013; 8: e56309. [PubMed: 23424655]