Published in final edited form as:

Immunol Rev. 2017 January ; 275(1): 271–284. doi:10.1111/imr.12510.

Survivors Remorse: antibody-mediated protection against HIV-1

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Summary

It is clear that antibodies can play a pivotal role in preventing the transmission of HIV-1 and large efforts to identify an effective antibody-based vaccine to quell the epidemic. Shortly after HIV-1 was discovered as the cause of AIDS, the search for epitopes recognized by neutralizing antibodies became the driving strategy for an antibody-based vaccine. Neutralization escape variants were discovered shortly thereafter, and, after almost three decades of investigation, it is now known that autologous neutralizing antibody responses and their selection of neutralization resistant HIV-1 variants can lead to broadly neutralizing antibodies in some infected individuals. This observation drives an intensive effort to identify a vaccine to elicit broadly neutralizing antibodies. In contrast, there has been less systematic study of antibody specificities that must rely mainly or exclusively on other protective mechanisms, although non-human primate (NHP) studies as well as the RV144 vaccine trial indicate that non-neutralizing antibodies can contribute to protection. Here we propose a novel strategy to identify new epitope targets recognized by these antibodies for which viral escape is unlikely or impossible.

Keywords

AIDS vaccine; HIV-1; neutralizing antibodies; non-neutralizing antibodies

1 INTRODUCTION

From the beginning, neutralization has provided the dominant context for studies of antibody-mediated protection infection against HIV-1 [(1–7) and reviewed recently in (8–10)]. Here, antibody-mediated neutralization is defined as the direct blocking viral entry by foiling productive interactions between the HIV-1 envelope glycoprotein (Env) and CD4, co-receptors, or both. In essence, neutralization promotes the view of antibodies as straightforward natural antiviral “drugs” that simply inhibit infection. Such a straightforward concept naturally captures the imagination. Yet antibodies have evolved to be multitaskers, capable of employing an array of effector mechanisms that guide the balance between protection and infection by HIV-1 [reviewed in (10–16)]. With these mechanisms in mind,

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CONFLICTS OF INTEREST

Drs. Lewis and DeVico own shares in Profectus Biosciences. This conflict is managed by the University of Maryland Baltimore pursuant to state and federal laws.

this review will address the broader picture of how anti-Env antibodies impact HIV-1 infection, which is based on an increasingly large body of new information. In addition, we identify a statistical problem, survivor bias, in the selection of which epitopes to target with an HIV-1 vaccine and suggest an alternative strategy to overcome this problem.

2 ANTIBODY-MEDIATED PROTECTION AGAINST HIV-1: THE NEUTRALIZING ANTIBODY PARADIGM

Because HIV-1 is a retrovirus, it integrates irreversibly into the genome of CD4⁺ cells establishing a latent reservoir that is thought to persist for life.^{17,18} Although anti-Env antibodies do not eliminate this persistent infection, it is widely accepted that the “right kind” of antibody activities and specificities at the time of transmission will protect against infection. This view is supported by a large number of successful passive immunization experiments in NHP models of HIV infection [(19–41) and discussed below]. The barrier to antibody-mediated protection against HIV-1 is not high. It is now clear that only one or few variants (so called transmitted/founder viruses or T/F viruses) are capable of establishing nascent infection across a “transmission bottleneck”, which is imposed by the recipient via mechanisms only partially understood.^{42–50} Viewed conversely, it is clear that the majority of variants circulating in the transmitting host lack the qualities needed to transmit. Although the existence of a bottleneck in HIV-1 transmission has been known for a number of years,^{42–44} the advent of single genome analysis (SGA) opened the way to quantification of the number of T/F variants^{45–50} as well as their genotypic and phenotypic properties.^{47,51–57} In addition to using the CCR5 co-receptor, the initial SGA study showed that T/F variants are characterized by preferential growth in CD4⁺ T cells [see also (47, 58)], consistent co-receptor masking, resistance to non-broadly neutralizing monoclonal antibodies, and sensitivity to broadly neutralizing monoclonal antibodies.⁴⁵ Earlier transmission studies suggested that T/F variants have shortened V1/V2 loops and reduced N-linked Env glycosylation^{59–61} although it is not clear whether these characteristics are shared among all T/F variants.⁵⁷

Perhaps the most striking feature of T/F variants is that they are consistently marked by signature amino acid residues IAKN at positions 33, 55, 64, and 88 (HXB2 numbering) in the C1 region of gp120 where A55 and N88 are completely conserved across SIV, SHIV, and HIV-1 T/F variants in both naïve and immune hosts.^{57,62–65} Currently, connections between T/F variants and humoral immunity are studied mainly in the context of neutralizing epitopes and their structure on the T/F envelope. As with all HIV strains, neutralization sensitivity is rank ordered (i.e. in “Tiers”) by standardized neutralizing antibody panels in the TZM-bl assay.^{66,67} Tier 1 viruses express many vulnerable sites, have flexible structures and are subject to neutralization by a wide array of anti-Env antibodies.^{68–71} In contrast, Env trimers on Tier-2/3 viruses are more conformationally constrained, expose relatively few vulnerable sites, and are resistant to neutralization by all but a few potent anti-Env antibodies.^{68–71} It is now thought that T/F variants fall into the Tier-2/3 rankings of neutralization sensitivity.^{72,73} Thus, they likely present a limited number of neutralizing epitopes on a relatively constrained envelope structure.

Emphasis on neutralization profiles stems from the widely held belief that these structures will provide key templates for developing protective HIV-1 vaccines and other prophylactic methods.^{9,10} The reasoning is that neutralizing activity is the only way to “mop up” the few variants that do not fall by the wayside during transmission, resulting in sterilizing protection. This rationale follows from multiple studies showing that neutralizing antibodies (albeit not necessarily by neutralizing activity, see below) impose significant immunological pressure on HIV-1 during the course of infection^{72–80} as well as passive immunization studies in non-human primates (NHP) demonstrating protection against high-dose SHIV challenges via neutralizing antibodies.^{19–21,23–31,33,34,36–41} Despite these strengths, the “neutralization only” approach to an HIV-1 vaccine includes three caveats that have significant practical implications. First, it is based on only one possible mechanism for protection, the direct blocking of viral entry, and assumes the other possible mechanisms are secondary in importance, though mounting evidence suggests that Fc-mediated effector function contributes significantly to protection mediated even by broadly neutralizing antibodies.^{29,30,81}

Second, it focuses selectively on highly mutable Env epitopes that enable a perpetual dance between viral and immunological evolution in the host. It is well-known that early HIV-1 transmission is characterized by an autologous neutralizing antibody response to the T/F virus that rapidly elicits neutralization escape variants.^{59,60} These neutralization escape variants elicit new neutralizing antibodies, setting up a predator-prey cycle (repetitive cycles of neutralization and neutralization escape) after HIV-1 transmission^{72–80} that is a common characteristic of persistent lentiviral infections.^{82,83} Recent structural studies indicate that Env trimers of T/F viruses are largely obscured by a glycan cloud that limit exposure of epitopes recognized by broadly neutralizing antibodies.^{84–89} Although these epitopes are targets of broadly neutralizing antibodies, they can clearly escape even the most potent of these antibodies.^{90–93} Thus, the neutralization only approach focuses on epitopes for which escape mutants remain replication competent, though it is possible that escape comes with a fitness cost. Theoretically, such a vaccine might select for vaccine-resistant T/F variants on a global population level.

Third, the neutralization only approach does not address the significance of emerging T/F signatures in the HIV-1 envelope amino acid sequence.^{57,62–65} These signatures may have functional and structural significance in the gp120-gp41 interaction and associated replicative capacity⁵⁷ but are not direct targets of potent broadly neutralizing antibodies.^{57,62,64,65} As noted above, these signatures emerge in immunologically naïve subjects and are not necessarily driven by immune pressure, although this certainly does not preclude them from being exploited as targets for protective antibody responses. An initial report showed that these T/F sequences are associated with neutralization resistance in a SIV model⁶²; however, subsequent studies showed that these T/F sequences are found on neutralization sensitive SIV that is transmitted in vaccinated NHP.^{64,65} Further studies are required to resolve these differences but it is highly likely that whatever the effect of the T/F sequences it lies outside epitopes recognized by neutralizing antibodies.

Although we strongly support efforts to find an HIV-1 vaccine that elicits potent, broadly neutralizing antibodies, the above concerns led us to develop an alternative hypothesis that

we term “the non-survivor hypothesis”. The non-survivor hypothesis is founded on a statistical problem known as survivor bias,^{94,95} that takes into account immunological “hits” on transmitted HIV-1 that are not survivable by mutation and escape. Survivor bias is best illustrated by classical work on defensive aircraft shielding during World War II.^{94,95}

Deep in the throes of war, the mathematician Abraham Wald made a seminal contribution to the war effort by determining how to best shield Allied bombers against enemy anti-aircraft fire.^{94,95} Wald was born to a Jewish family in Hungary, educated in Austria, and by 1937 had found his way to the United States through an invitation by the Cowles Commission for Research in Economics, in the nick of time to escape the Nazi persecution of Jews that came with the invasion of Austria in 1938.⁹⁶ Wald’s contribution was the ingenious use of statistical modeling taking survivor bias into account to reduce the high levels of bomber attrition in the European theater. Survivor bias confounds statistical modeling when predictions are based on “survivors” of a process and not counting non-survivors because that data does not exist. Wald’s seminal work was carried out in 1943 at Columbia University under the aegis of the Applied Mathematics Panel of the National Defense Research Committee, and remained classified for a number of years. An earlier analysis of shrapnel damage on returning aircraft, carried out by the Center for Naval Analyses, suggested shielding the regions of the aircraft receiving maximal damage. This approach, however, produced no effect on attrition. Wald recognized this as an example of survivor bias. In a series of monographs, he developed a statistical model to deduce the vulnerable regions on downed aircraft, absent direct data, that led to increased survival once the planes were shielded appropriately. Although this work was never published in the peer-reviewed literature, his models were used in both the Korean and Vietnamese conflicts and are relevant today. Survivor bias is a well-known statistical artifact that occurs in many settings, with Wald’s work being just one example [for this and other examples see (95)].

The theoretical application of survivor bias analysis toward the development of an effective HIV vaccine is relatively straightforward. As discussed above, vaccine development efforts based on direct neutralizing activity are worthwhile, but they come at a cost. Specifically, such vaccines are designed on epitope structures that escape even the most potent neutralization pressure. Stated in terms of survivor analysis, these epitopes are identified by survival of the virus and not its elimination, i.e. they are “survivors”.^{90–93,97} A corollary is that HIV-1 Env domains in which sequence changes are lethal do not show up in transmission databases (i.e. they are “non-survivors” that are incapable of replication). However, these domains, which are essential to viral replication, may be the ideal targets for antibody responses intended to provide sterilizing protection. Wald deduced the vulnerable structures that were missing from the survivor aircraft database, can we do the same for epitopes on Env? The availability of very large sequence databases for the HIV-1 Env provides an initial avenue to identification of “non-survivor” epitopes.

Since the replication of HIV-1 is extremely error prone, highly conserved sequences indicate not only high functional importance but also high vulnerability to mutation. If such a region could be targeted by functional antibodies, the virus would have two equally fatal choices; remain wildtype and be eliminated or mutate and be eliminated. We propose that such regions can be identified by finding highly conserved sequences of the Env trimer that also

harbor epitopes recognized by antibodies capable of blocking infection, eliminating infected cells, or both. Figure 1 satisfies the first requirement, identification of highly conserved sequences in the Env trimer. It is noted that segmental sequence variation in Env has been known since the early days of the AIDS epidemic⁹⁸ and the analysis in Figure 1 is shown for illustration of the problem using only gp120 for simplicity. Figure 1 shows an entropy plot for gp120 determined using compendium sequences and web-based software from the Los Alamos National Laboratory HIV database [(99) and (<http://www.hiv.lanl.gov>)]. The well-known variable and conserved regions of gp120 are seen readily as peaks and valleys, prompting the question whether the most conserved regions harbor epitopes against which a known immune response implicated in antibody-mediated protection against HIV-1. As pointed out above, the 133, A55, K64, N88 T/F sequences map to a very highly conserved segments of the C1 domain,^{57,62,64,65} suggesting that they are “non-survivor” signatures where mutants are incapable of transmission. Are these sequences associated with epitopes that are recognized by antibodies that can exert immunological pressure on HIV-1?

It is recognized that such epitopes are typically discontinuous and are likely not to be apparent in a linear depiction of sequence conservation absent additional information including epitope associated protection and epitope structure. Fortunately, a wealth of recent structural data defining epitopes recognized by broadly neutralizing antibodies as well as poorly neutralizing antibodies or non-neutralizing antibodies that exert antiviral activities by Fc-effector functions. These functions include complement mediated virolysis, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent phagocytosis (ADCP), and antibody-dependent cellular viral inhibition (ADCVI). For brevity, the reader is referred to recent reviews discussing these mechanisms of antibody-mediated protection against HIV-1.^{11,14,15}

There is an excellent picture of the epitope clusters recognized by broadly neutralizing monoclonal antibodies on the native Env trimer [(85) and reviewed in (8, 9)]. These clusters include the CD4 binding site recognized by the VRC01 class of broadly neutralizing monoclonal antibodies,^{100,101} quaternary V1/V2 epitopes on the Env apex recognized by the PG9/PG16 class of broadly neutralizing monoclonal antibodies,^{102,103} N-332/V3 associated epitopes recognized by the PGT121 class of broadly neutralizing monoclonal antibodies,^{103,104} MPER associated epitopes in gp41 recognized by the 2F5 class of broadly neutralizing antibodies,¹⁰⁵ and hybrid epitopes formed by the gp120-gp41 association recognized by the PGT151 class^{106,107} of broadly neutralizing monoclonal antibodies. Save for the MPER epitopes, these epitopes include residues from the apex of the V1/V2 regions and the C2-V5 regions of gp120 that correspond to the outer domain of gp120. This region includes the most variable residues (V1-V5) of the HIV-1 genome. Thus, these regions include epitope residues that can mutate to escape broadly neutralizing antibody pressure by several mechanisms, point mutations, insertional mutations in the variable regions, and by shifting of N-linked glycosylation sites. Even the most potent broadly neutralizing antibody responses are susceptible to escape via mutation that appears to have a very high upper limit, if any at all. Mechanisms of escape are manifold: including point mutations, insertional mutations in the variable regions, and by shifting of N-linked glycosylation sites.^{90–93} Neutralization escape is also consistent with the high levels of protein sequence variation in the outer domain regions of gp120(V1-V5) harboring most of the epitopes recognized by

broadly neutralizing antibodies.^{108–110} Although it is very clear that neutralization escape drives the emergence of broadly neutralizing antibodies, it should be noted that even the most broadly neutralizing monoclonal antibodies often fail to neutralize contemporaneous viruses circulating at the time of detection or isolation [cf. (77)]. Thus, the epitopes recognized by known broadly neutralizing antibodies appear to be uniformly “survivors”.

Additional data support the hypothesis that broad neutralizing activity is about survival. There is seemingly paradoxical evidence that HIV-1 envelope domains with highly variable amino acid sequences (i.e. neutralization targets) are actually quite stable at the genetic level. For example, the nucleotide mutation rate in V1 through V5 env gene regions is approximately three-fold less than in the coding regions of the entire HIV-1 genome, including Env codons outside of these regions.¹¹⁰ The decreased mutation rate is due to sequence context and secondary structure of template RNA that increases the fidelity of reverse transcription and decreases the activity of a cytidine deaminase. As suggested in,^{109,110} it is possible that the reduced cytidine deaminase activity preserves GG and GA targets that favor the evolution of neutralization escape variants without loss of replication capacity. Overall, it appears that HIV-1 has evolved a stable genetic platform that ensures survival even as Env trimers bend with the winds of broadly neutralizing antibody responses. Put another way, HIV-1 makes it very hard for broadly neutralizing antibodies to push HIV-1 variants toward a lethal genotype. This is supported further by the recent demonstration that the highly conserved glycan site at N88 restricts access of broadly neutralizing antibodies to epitopes at the gp120-gp41 interface, although it is not part of the epitope itself.⁸⁰

Considering the above, there is a legitimate concern that an exclusive focus on direct neutralizing activity opens the door to survivor bias in interpreting anti-HIV-1 humoral immunity. This issue, by extension, could diminish the chances of developing a successful HIV-1 vaccine. A similar issue afflicts sieve analyses of viruses found in vaccine recipients. The data sets will reflect only what enabled escape and survival from the vaccine effect, not what was lethal. Taking cues from Wald, we can change course and search for evidence of highly conserved, functional, non-survivor epitopes (ones that cannot be mutated without loss of replicative capacity) and openly consider which humoral responses and mechanisms might be directed against them. The concept is that such responses, if sufficiently potent, would be inescapable, as any attempt to do so would be lethal. Below, we argue that such epitopes exist and present a possible example of a highly conserved epitope subregion of Env that is recognized by non-neutralizing antibodies that exert Fc-mediated effector function against HIV-1.

At this juncture, it is useful to consider the semantics of non-neutralizing antibodies. In the discussion to follow, we consider an antibody to be non-neutralizing if consistently fails to neutralize Tier-1 viruses in any standardized assay format in the absence of complement or effector cells. For example, many antibodies will neutralize in the A3R5 assay, which uses a transformed T cell line, whereas they are inactive in the TZM-bl assay that is based on a HeLa cell line.¹¹¹ When it is considered that T/F viruses are Tier-2/3 in neutralization susceptibility, this definition sets a low threshold for categorization of an antibody as neutralizing since it also includes Tier-1 viruses that are not transmitted. Against this backdrop, is there evidence that non-neutralizing antibodies against HIV-1 can protect

against infection? The answer is qualified “yes” and it comes from both vaccination and passive immunization studies.

3 ANTIBODY-MEDIATED PROTECTION AGAINST HIV-1: ARE NON-NEUTRALIZING ANTIBODIES PROTECTIVE?

3.1 Vaccination studies in NHP

There is literature going back almost 30 years stemming from vaccination and SHIV/SIV challenge studies correlating various patterns of protection with non-neutralizing antibodies. The first and most fundamental studies suggesting a role for non-neutralizing antibodies emerged in 1989 via two reports that immunization of rhesus macaques (RM) with inactivated SIV completely protected against challenges with infectious virus.^{112,113} It was known prior to the first studies^{112,113} that virus preparations contain human MHC when they are grown on human cells.¹¹⁴ It was recognized subsequently that this protection was likely mediated by xenogeneic anti-MHC antibodies binding to virion surfaces because the SIVs used for vaccination and challenge were grown in human cells.^{115–118} These studies were followed by reports that anti-MHC antibodies can neutralize laboratory adapted viruses in cell lines^{115,119} but these studies were carried out before the development of rigorous neutralization assays based on the TZM-bl neutralization assay¹²⁰ coupled with standardized viruses with tiered neutralization sensitivities.⁶⁷ At the time, it was difficult to say whether these anti-MHC antibodies were truly neutralizing or not as defined by the TZM-bl assay. It was difficult to see how MHC molecules play a role in virus entry, which is clearly mediated by sequential receptor induced conformational changes in the Env trimer [reviewed in (121)], leading to speculation that effector mechanisms are involved other than interference with requisite conformation changes on the Env trimer critical for entry. A recent study using current methods sheds new light on this issue.¹²²

In that study, NHP were immunized with inactivated SIV and challenged intravenously with SIV, both reagents were derived using human cell substrates.¹²² Control groups also included NHP immunized with “sham” preparations of the human cellular substrate used to grow the SIV. Protection against acquisition was observed for the groups receiving the inactivated SIV vaccine and partial protection was observed for the groups receiving only the sham vaccine. Protection correlated with levels of binding antibodies to framework residues of MHC but not with neutralization in the TZM-bl assay unless complement was included. Based on the absence of potential effector cells in the TZM-bl assay, antibody-dependent complement mediated virolysis is the probable mechanism of this effect and not simply receptor interference.^{123–126} Collectively, these studies strongly suggest that antibodies against host proteins on the viral surface contribute to protection against SIV infection by mechanisms other than classical neutralization, which requires quenching one or more of the receptor-dependent conformational changes in the Env trimer required for entry. The above studies deal only with protection mediated by anti-host protein responses. Nevertheless, they provide compelling proof-of-concept in a widely accepted animal model that the ability of an antibody to bind to a virion surface is in itself sufficient to produce a strong protective effect via various immune mechanisms other than classical direct neutralization. The key question is whether non-neutralizing antibody responses can

similarly protect against HIV-1 transmission by targeting Env epitopes, particularly of the non-survivor variety.

A number of vaccination/challenge studies in NHP support the concept that non-neutralizing antibodies elicited by Env immunogens also play a role in protection against HIV-1 acquisition.^{127–144} It is important to note that all such studies are correlative in nature and cannot establish causality for any mechanism of antibody-mediated protection against HIV-1. To date, most of these studies have been carried out in SIV/SHIV challenge models, which to varying degrees model SHIV/SIV transmission and immunity in humans. Before going further, there are three caveats to consider when interpreting vaccine/challenge studies in RM. First, typical NHP challenge models employ much higher concentrations of virus than those responsible for human infections. For example, it has been estimated that semen contains approximately 10^3 to 10^4 viral copies per milliliter depending on Fiebig stage.¹⁴⁵ High-dose NHP challenge models typically employ inocula of approximately 10^8 copies per milliliter to ensure all animals are infected after a single inoculum.³⁰ Because the transmission frequency of HIV-1 has an upper bound of approximately 138 transmission events per exposure,¹⁴⁶ repeat, low-dose challenge models were developed in NHP to more accurately estimate the ability of interventions to block transmission.¹⁴⁷ These studies typically require doses of approximately 10^6 copies per milliliter to ensure that all animals become infected by a reasonable number of challenges,¹⁴⁸ which is still significantly above upper concentrations of HIV-1 in semen.¹⁴⁶ Thus, protection against acquisition in a high-dose challenge model can identify the most potent protective antibody response but it will underestimate the ability of less potent/lower titer antibodies to protect, although they might do so quite well in an HIV-1 transmission population.

Second, two patterns of protection are possible, sterilizing protection and postinfection control of viremia. Sterilizing protection is the Holy Grail of vaccine development and it is typically difficult to achieve in high-dose challenge studies unless the Env vaccine and the challenge stock are derived from the same virus isolate.¹⁴⁹ Vaccination studies employing heterologous high-dose viruses most frequently exhibit postinfection control of viremia.^{127–135,138,139,141} In contrast, similar vaccine strategies often elicit sterilizing protection when using heterologous repeat, low-dose challenge models,^{132,136,140,141} although some studies still only show postinfection control using these models [cf. (137, 139)].

Third, it is becoming apparent that NHP are highly polymorphic for genes encoding proteins that are involved in Fc-mediated effector function [(150–152) and reviewed in (12)]. Thus, NHP genetic background is very likely to affect protection against SHIV/SIV when it is dependent on ADCC, ADCP, ADCVI, or a combination of mechanisms. Understanding the relationships between humans and NHP at these loci is critical for evaluating HIV-1 vaccines these models as it appears that Fc-mediated effector function contributes to protection by even broadly neutralizing antibodies.^{29,30,81,153} It goes without saying that Fc-mediated effector function will be a major mechanism through which non-neutralizing antibodies exert protection.

With these caveats in mind, Fc-mediated effector functions as well as Env binding titers by non-neutralizing antibodies been correlated with sterilizing protection recent repeat, low-dose vaccination/challenge studies. One report,¹⁴⁰ using Adenovirus-5/Env immunogens elicited sterilizing protection against the Tier-2 SHIV162p3 virus. Protection correlated with binding antibodies and ADCP. A similar study reported in the same manuscript correlated protection in a SIV system with a combination ADCP, ADCC, ADCD antibody-dependent downregulation of CD106a on NK cells, and antibody-dependent production of CCL4 by NK cells. Both studies strongly suggest that Fc-mediated effector function plays a role in protection against HIV-1 transmission.

A second report,¹⁴¹ using the conformationally constrained gp120 immunogen, full-length single chain (FLSC),¹⁵⁴ elicited sterilizing heterologous protection against SHIV162p3 in two independent repeat, low-dose challenge studies as well as against a neutralization resistant swarm of SIVmac251¹⁵⁵ in a third repeat, low-dose challenge study. Protection correlated with antibody specificity for highly conserved CD4-induced epitopes (CD4i) of gp120 (measured in the SHIV162p3 studies) as well ADCC measured in all studies using the rapid fluorometric antibody-dependent cellular cytotoxicity assay (RFADCC).¹⁵⁶ These studies used a gp120 derived from the HIV-1_{BA-L} isolate that is stabilized in the CD4-bound conformation it elicits CD4i antibodies to the co-receptor binding site and Epitope Cluster A¹⁵⁷ in the C1/C2 region of gp120 for which we have the epitope footprints at the atomic level,^{158–162} which permits that survivor analysis that will be discussed in a section below.

3.2 Vaccination studies in humans

Subgroup studies of the Vax004 trial, which tested a gp120-based vaccine concept, suggested that Fc-mediated ADCVI correlated inversely with infection risk [as defined (163) in and referred to hereafter as correlate of risk (CoR)]. This relationship indicated that non-neutralizing antibodies can prevent HIV-1 transmission by Fc-mediated effector function,¹⁶⁴ although there was no overall efficacy in the trial.¹⁶⁵ In contrast, the RV144 trial was the first HIV-1 vaccine trial to demonstrate efficacy,¹⁶⁶ which peaked at approximately 60% efficacy in the first 18 months¹⁶⁷ and decaying quickly to background resulting in an overall efficacy of 31.2%.¹⁶⁶ The primary CoR were anti-V1/V2 binding antibodies that correlated inversely with CoR and IgA anti-C1 antibodies that correlated directly with CoR.¹⁶⁸ In secondary analyses, IgA anti-C1 antibodies interacted with ADCC, neutralization of Tier-1 viruses, and CD4⁺ intracellular cytokine responses,¹⁶⁶ revealing inverse CoR for these three variables when the highest tertile of IgA anti-C1 binding was excluded from the dataset.¹⁶⁶ The anti-V1/V2 antibodies neutralize Tier-1 viruses very weakly although their cognate epitopes overlap the apex-binding broadly neutralizing antibodies, PG9/PG16.¹⁶⁹ In contrast, representative anti-V1/V2 from RV144 vaccinees mediate ADCC and can synergize with anti-C1 monoclonal antibodies¹⁷⁰ for potent ADCC.¹⁷¹ The V1V2 domains recognized by the vaccine responses exhibited vaccine-mediated selective pressure in sieve analyses¹⁷² and are thus unlikely to represent non-survivor epitopes.

Anti-C1 antibodies that recognize the A32-subregion of Epitope Cluster A^{157,160,162,173–175} were readily elicited in RV144 vaccinees.¹⁷⁰ It was shown subsequently that IgA antibodies against the A32-subregion of Epitope Cluster A directly correlated with infection risk. This

relationship may be linked to findings that these antibodies block ADCC mediated by IgG specific for the same epitope cluster,¹⁷⁶ assuming that ADCC contributed to reduced risk. Unfortunately, ADCC responses to the A32-subregion of Epitope Cluster A were not specifically measured in the case–control studies. At the same time, vaccination/challenge studies in NHP^{132,139,141} suggest that responses against the A32-subregion may contribute to protection by reducing the numbers of transmitted variants (see below). Although antibody responses to the A32-subregion were not immediately apparent as a CoR in RV144, it should be recognized that the competition binding assays used were somewhat rudimentary and could not distinguish antibody responses to these epitopes by mediated by IgG vs IgA.

3.3 Passive immunization studies in NHP

As pointed out above, vaccine studies can only identify correlates of protection, they cannot establish causality. To do so requires passive immunization with selected antibodies and cognate specificities. Such studies in NHP have established unequivocally that antibodies which neutralize the challenge stock can protect against high-dose SHIV challenges^{19–29,31,33,34,36–39,41} as well as repeat, low-dose SHIV challenges.^{30,35} In contrast, save for one early study using non-neutralizing antibodies against SIV,^{177,178} no other passive immunization study using non-neutralizing antibodies as immune sera^{179,180} or monoclonal antibodies^{33,37,181} has protected against a high-dose SHIV/SIV challenge. These studies show clearly that neutralizing antibodies, and broadly neutralizing antibodies in particular, have a wide dynamic range for protection against HIV-1. They also show that non-neutralizing antibodies are not effective for blocking SHIV infection at the top end of this range, which is typically around 10^8 copies per challenge.³⁰ These three studies also provide evidence that non-neutralizing antibodies do impact high-dose SHIV challenges.^{33,37,181} All three studies included non-neutralizing monoclonal antibodies specific for the immunodominant disulfide region (IDR) in the ectodomain of gp41^{182–184}, whereas one study also included A32¹⁸¹ the canonical monoclonal antibody that defines the A32-subregion of Epitope Cluster A.^{157,160,162,174,175} The first two studies showed that monoclonal antibodies specific for the IDR of gp41 afforded postinfection control of viremia after high-dose SHIV challenges.^{33,37} The third study¹⁸¹ showed that both the monoclonal antibody specific for the IDR of gp41 and A32 significantly reduced the number transmitted viral variants, although there was no postinfection control of viremia. We have suggested elsewhere that postinfection control and sterilizing protection are a continuum in passive immunization studies using high-dose SHIV challenges that is affected dramatically by antibody concentration.^{11,185–187} On the basis of these considerations, we propose that the differences between neutralizing and non-neutralizing antibodies to protect against HIV-1 are quantitative rather than qualitative. This hypothesis is consistent with the patterns of protection observed using the FLSC immunogen in high-dose SHIV challenges,¹³² where only postinfection control was observed, compared with repeat, low-dose SHIV/SIV challenges where sterilizing protection was observed,¹⁴¹ in all cases, protection correlated with CD4i antibody specificity and ADCC.

In summary, these studies suggest that using high-dose SHIV/ SIV challenge studies runs the risk of “throwing the baby out with the bathwater” as they appear to underestimate

quantitative differences in the dynamic range of protection. This hypothesis can be tested in repeat, low-dose challenge studies that use SHIV inocula in the range of 10^5 to 10^6 copies per challenge, which more closely approximates the likely per exposure of HIV-1 inoculum of HIV-1 in at risk populations.¹⁴⁵ These studies are sorely needed as the bulk of the immunization literature cited above for both NHP and humans strongly suggest that vaccine elicited non-neutralizing antibodies should protect against inocula that are closer to those in natural infection.¹⁴⁵ If this hypothesis is correct, the choice of epitopes to be targeted is critical.

4 ANTIBODY-MEDIATED PROTECTION AGAINST HIV-1: IDENTIFICATION OF POTENTIAL NON-SURVIVOR EPITOPES

Above we argued that neutralizing antibodies recognize “survivor” epitopes that can accommodate continual cycles of neutralization and escape and raised the possibility for the existence of “non-survivor” epitopes for which any mutation to escape an antibody results in a non-functional Env trimer. Thus far, it appears that all neutralizing epitopes can escape, though it is likely that some escapes will have decreased replication/transmission fitness. Escape is also possible for weakly or non-neutralizing anti-V2 antibodies that are a COR in RV144.¹⁷² These antibodies do not neutralize Tier-2 viruses and only narrowly neutralize Tier-1 viruses, so it is unlikely that neutralization played a role in escape. In contrast, antibodies specific for this epitope region mediate potent ADCC and there is no reason to think that “survivor” epitopes are only impacted by neutralizing antibodies. The key point here is that these epitopes are in a highly variable region where was determined by residues at 169 and 181 in RV144.¹⁷² In contrast, NHP vaccination studies using the FLSC immunogen^{132,139,141} as well as RV144^{168,170} suggest that Epitope Cluster-A is also a target of potentially protective non-neutralizing antibodies raising the question of whether these epitopes are survivors or non-survivors.

4.1 Are epitopes in the A32-subregion of Epitope Cluster A non-survivors?

It has been known for many years that the A32 epitope is expressed across HIV-1 clades¹⁸⁸ and that most HIV-1 infected individuals mount antibody responses to this epitope^{173,189} as did RV144 vaccinees.¹⁷⁰ We now have a detailed structural picture of the A32-subregion of Epitope Cluster A that permits the search for potential escape mutations by probing sequence databases from infection cohorts. Epitope Cluster A is comprised of three sub-regions, one that is recognized by A32, a second that is recognized by monoclonal antibody C11, and a third that overlaps both regions.^{157,160,162,175} Monoclonal antibodies A32 and C11 recognize distinct but proximal epitopes that map to the gp41-interactive surface of gp120.^{157,160,162,174,175,187} For this reason, Epitope Cluster A is buried in native Env trimers on virions^{190–192} or the surfaces of HIV-1 infected cells but become antibody accessible after binding to cell surface CD4.^{157,160–162,173,193} Once exposed, Epitope Cluster A becomes a very potent ADCC target.^{157,173,193} Because Epitope Cluster-A becomes an ADCC target only upon CD4-induced conformational rearrangement, the window of time where a target cell can be eliminated is relatively short. Epitope Cluster A is exposed during viral entry for at least several hours^{194,195} where it is a potent ADCC target.¹⁵⁷ Epitope Cluster A is also exposed on productively infected cells where it remains a potent ADCC

target until CD4 is down-regulated by Nef and Vpu.^{16,189,193,196,197} These studies strongly suggest that Nef and Vpu have evolved, in part, to reduce the exposure of Epitope Cluster A during viral budding. It is interesting to note that the ability of Nef to down-regulate CD4 is allele dependent and that Elite Controllers often have defective alleles and increased exposure of Epitope Cluster A for ADCC attack.¹⁹⁷ The mapping of Epitope Cluster A to an Env structure that is indispensable for viral entry and the evolution of a mechanism to reduce its exposure on HIV-1 infected cells raises the possibility that these epitopes are “non-survivors” for which escape is impossible or at least highly improbable.

This question is addressed using the epitope footprint of the A32-subregion of Epitope Cluster A determined recently at the atomic level.^{16,160–162} The A32-subregion maps to the highly conserved C1 region involving residues between 50 and 115 as well as a highly conserved segment in the C2 region between residues 217 and 246. Figure 2 shows the epitope footprint of the A32-subregion derived from ten independent co-crystal structures for A32 and the A32-like monoclonal antibodies, N12-i2, 2.2c, N60-i3, and JR4.^{160,162,175} The A32-subregion epitopes are discontinuous and include the α -0 and α -2 helices and adjacent residues in C1 as well as a short segment of the C2 region, comprising part of the gp41 docking site in the Env trimer. The epitope footprint is displayed on the surface of a gp120-CD4 complex that mimics FLSC [PDB:3JZO (198)] with gp120 regions colored according to protein sequence conservation determined using the Los Alamos HIV Sequence Database (www.hiv.lanl.gov). The most coloring scheme ranges from most conserved (blue) to least conserved (red). The A32-subregion of Epitope Cluster-A is very highly conserved, which is also indicated by a “logo” plot in Figure 3 where the contact residues are in rectangles (depicted for only C1 residues). The high conservation of the A32-subregion and the data above showing that it is a target of potentially protective antibodies strongly suggest that it is comprised of non-survivor epitopes. This is supported by the lack of escape in the A32-subregion in the RV144 trial¹⁷² despite the clear presence of antibody responses to this region in vaccines that have been implicated in protection.^{168,170,176} Further, there was no escape in the A32-subregion in the NHP passive immunization study where A32 significantly reduced the number of transmitted variants of a high-dose SHIV162p3 challenge.¹⁷⁶ As pointed out above, ADCC associated escape has been reported for several regions of gp120, including the C1 region using a synthetic peptide approach.¹⁹⁹ In that study, the ADCC associated escape was at non-contact residues for antibodies that recognize the A32-subregion. Notably, the A32-subregion epitopes are discontinuous and involve two critical cysteine residues in the C1 region and one critical cysteine in the C2 region (not shown in Figure 3) all of which are completely conserved among HIV-1 isolates,^{160,162,175} which might be a key factor in preventing epitope escape. The marked conservation of the A32-subregion across HIV-1 isolates raises the question of its relationship with the I33, A55, K64, N88 T/F signature motif. As pointed out in,⁵⁷ the I33 and N88 residues are distal to this sub-region. Although A55 and K64 are not contact residues for A32-like antibodies, these residues interact with the residues comprising the A32-subregion and likely stabilize its structure. The high conservation of the A55 and K64 residues as well as the residues comprising the A32-subregion suggests that this subregion is comprised of non-survivor epitopes. Future studies analyzing the relationships between the appearance of antibodies to

A32-subregion epitopes and the evolution of sequence changes are required to test the non-survivor hypothesis.

5 CONCLUSIONS

The discussion above frames the protective epitope diversity problem of HIV-1 vaccine development in terms of survivor analysis leading to the key question of whether there are protective, non-neutralizing antibody responses to epitopes that lie in functionally critical regions of Env with a narrow tolerance for mutation. A case is made that such epitopes are non-survivors of multiple humoral effector mechanisms, whereas other epitopes have evolved to escape cycles of neutralizing antibody pressure and are survivors. To date, HIV-1 vaccine development has been driven by the identification of neutralizing antibody responses, which target survivor epitopes that have evolved to accomplish seemingly endless rounds of mutation and escape. While we are strongly supportive of the bnAb approach to develop an HIV-1 vaccine, we also suggest that non-survivor epitopes that cannot escape pressure from humoral immunity are equally important and largely unexplored options. Since the “non-survivor/survivor bias” concept was not recognized earlier in HIV-1 vaccine development, no past study was deliberately configured to use it as a hypothesis or guiding principle to explore protective anti-HIV-1 humoral immunity. However, there are data to suggest ways down this path. As discussed here, the A32-subregion of Epitope Cluster A is the prime candidate formally to test the non-survivor hypothesis. Alternative targets may reside in gp41, as it was shown that non-neutralizing anti-gp41 antibodies correlated with protection against a high-dose SIV challenge in live attenuated vaccine model.^{143,144} The ability of an anti-gp41 monoclonal antibody to the PID to decrease the number of T/F variants in a high-dose SHIV passive immunization study further supports this possibility.¹⁸¹ Further, the identification of the highly conserved fusion sequence in gp41 as an element of an epitope recognized by a new broadly neutralizing antibody is also a possible non-survivor neutralizing epitope candidate.²⁰⁰ Further exploration of these concepts will require refinements of the relatively simple models and systems now being used to study neutralizing activity. Optimized systems would incorporate analyses of multiple humoral effector mechanisms at mucosal and systemic sites, a complete understanding of relevant Fc receptor polymorphisms and related effector mechanisms, low virus inocula, and genetic methods geared toward detecting lethal effects on virus replication. In closing, we suggest the following steps to identify non-survivor epitopes that can be exploited for vaccine development. (i) Determine regions of very low protein sequence variation using bioinformatics and sequence databases; (ii) Identify epitopes targeted in these regions by antibodies that can impose pressure on the virus by neutralization, Fc-mediated effector function, or both, with the requirement that escape mutations are not selected; (iii) Evaluate these antibodies using repeat, low-dose passive immunization models to more accurately reflect the levels of transmitting HIV-1 in humans; and (iv) Use this information to design improved vaccine concepts.

Acknowledgments

The authors thank their Division of Vaccine Research faculty colleagues at the IHV, Robert C. Gallo, M.D., Roberta Kamin-Lewis, Ph.D., Krishanu Ray, Ph.D., Mohammad Sajadi, M.D., and Alan Schmaljohn, Ph.D., for many outstanding discussions leading to the ideas in this review. We also thank our postdoctoral fellows and lab

associates for the outstanding laboratory work supporting our program. Finally, one of us (G. K. L.) thanks his colleague, Dr. Garnett Kelso of Duke University, for pointing out the possibility of survivor bias in HIV-1 vaccine design over 20 years ago; any errors of interpretation are solely ours. This work was supported by grants #OPP1017606 and #OPP1033109 from The Bill and Melinda Gates Foundation. I was also supported by NIH grants P01 AI120756-01, P01A124912, and R01AI116274.

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FIGURE 1.

Entropy plot for gp120 illustrating conserved and variable regions. The individual entropy values are shown indicating the per-residue protein sequence variability and the curve was generated as a moving average to depict the relative regional diversity

FIGURE 2.

Depiction of the relative sequence variability on a model of CD4-triggered gp120. Blue represents the highest protein sequence conservation, whereas red represents the highest protein sequence variability

FIGURE 3.

Logo plots for the per-residue protein sequence variation in the C1 region. The rectangles represent contact residues for the A32-subregion of Epitope Cluster A. For brevity, the C2 contact residues for the A32-subregion are not shown but they are equally conserved as those of the C1 region