Lessons learned from humoral responses of HIV patients

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Abstract

Purpose of review: Since 2009 many broadly neutralizing antibodies against HIV have been identified yet there is still no vaccine capable of inducing such antibodies in humans. This review considers the early observations of HIV sera neutralization in light of more recent studies and highlights areas for future research.

Recent findings: Large clinical cohort studies using standardized neutralization assays and pseudoviruses derived from primary isolates have shown that 10-30% of HIV infections result in some level of serum neutralization breadth. However, less than 10% of individuals develop a greater breadth of neutralization and are termed elite neutralizers.

Summary: During HIV infection many individuals develop strain-specific neutralization against their viral quasispecies, and similar immunogen-matched activity can now be induced in animal models. However, only in a minority of infections do broadly neutralizing antibodies develop. Therefore, understanding how the viral diversity, host immune environment and antibody repertoires intersect to support the generation of neutralization breadth in elite neutralizers could provide guidelines as to how to improve immunization responses.

Keywords: HIV, neutralization, antibody, serum, infection

Introduction:

The unusual degree of genetic variability of HIV-1, especially in the *env* gene that encodes the target for neutralizing antibodies, became apparent early on in the epidemic (1-5) and brought with it the concern that it would be difficult to develop an efficacious vaccine that could induce broadly neutralizing antibodies (bnAbs). Early studies that examined the neutralization of HIV-1 by human sera from infected individuals set the agenda for the next 30 years of HIV vaccine research (6-14). These studies, allayed some fear; as although much neutralizing activity was strain-specific at least some individuals developed neutralizing antibodies that were effective across diverse HIV-1 strains from around the globe (15, 16). Thus, despite the diversity in the HIV envelope protein (Env) there were conserved neutralizing epitopes. This review will provide an overview of the subsequent 30 years of research that resulted in a detailed picture of the development of neutralizing humoral responses during HIV infection.

Early studies of HIV neutralization

An obvious target for neutralizing antibodies was the viral interaction with the cell surface receptor CD4, which had already been identified as the major receptor for HIV entry into cells (17). Indeed the 'group specific' response was shown to include antibodies that inhibited CD4-envelope binding (18, 19). The entry of HIV into its target cell is mediated by the highly glycosylated gp120 Env subunit, which is secured on the virion surface by the transmembrane gp41 Env subunit (20). Comparison of the gp120 sequences from various HIV isolates distinguished five variable regions (V1–V5) (4). The first four variable regions are exposed and held in loops by disulphide bonds (20). While the conserved regions of gp120 form a discontinuous structure, identified by mutagenesis to interact with CD4 (21-23). Thus, the CD4 binding site (CD4bs) was a likely candidate for conserved neutralizing epitopes. It was not understood at this time, but later became known, that when viral

gp120 binds to CD4 it induces conformational changes that expose the V3 loop (24), which is the principal determinant of chemokine co-receptor interaction (CCR5 and CXCR4) (25-27). This CD4-induced exposure is indicated by the enhanced binding of several gp120-specific antibodies, in addition to those that recognize the V3 loop and CD4 binding site (28-31). These CD4-induced (CD4i) antibodies (32), similar to V3-loop antibodies, efficiently block the binding of gp120–CD4 complexes to the chemokine receptor (33-35).

Despite the early optimism that broadly neutralizing targets could be identified a stumbling block quickly became apparent. Namely, that bnAb responses would be difficult to recapitulate in a vaccine using recombinant Env (6, 36). This was suggested because the first neutralizing antibodies to arise in human infection or in experimental infection of chimpanzees, targeted the V3 loop and only neutralized in a type-specific manner (37-39). Furthermore, group-specific antibodies (now termed broadly neutralizing) developed only slowly, between one and two years after infection and not in all patients (15). These early studies were usually too small to make accurate estimates of how long it takes to develop bnAbs or in what fraction of infected patients they arose (40-42). Subsequent studies used viral isolates or Env clones derived directly from patients (autologous viruses) (43-45). The same picture emerged; variability among patients with respect to the time needed to develop autologous neutralization and a robust broadening of the response with time in only a minority of individuals. However, these early studies were plagued by cohort variability and a lack of consistency between neutralization assays employed including the use of viruses with varying degrees of neutralization sensitivity (46). Thus, the molecular details underpinning these responses would be required to generate a clear picture of the development of HIV neutralizing antibodies *in vivo*.

How do bnAbs develop during infection?

A key and consistent observation in studies of natural infection so far is that the 10-30% of individuals whose serum is broadly neutralizing (Table 1), first make a strain-specific response and then eventually shift to make a broader neutralizing response. Understanding how this shift to broad neutralization is achieved is key to vaccine development. How can it be mimicked to generate bnAb responses, both as an adjunct to therapy and in a classical protective vaccine? Detailed studies of bnAbs from mainly from the 1-10% of individuals with elite serum neutralization (as defined in each cohort – see Table 1) have begun to paint a picture that suggests that there are a number of ways to achieve bnAb activity. To date, five major bnAb sites have been identified: the CD4bs, the high mannose patch, the Env trimer apex, the gp120-gp41 interface and the membrane proximal region (MPER), and these are extensively reviewed elsewhere (57, 58). It will be important know if this broad anti-viral activity is achieved by multiple antibody lineages or by one or a few dominant expanded families. Studies on serum with bnAb-specific mutant viruses suggest that, at least in some cases, a limited number of epitopes are targeted within individuals (55, 59, 60). There are notable examples of expanded bnAb families within particular individuals that reconstiute their serum neutralization response (61-64). For example, one 15-year longitudinal study of the CD4bs-specific VRC01 bnAb family shows how the virus and antibody response mutate in a “cat and mouse” fashion during infection (65). In addition, structurally and genetically similar bnAbs to VRC01 have been identified in other HIV-infected people (66, 67) although it is important to note that neutralizing epitopes can be targeted by genetically unrelated bnAbs (68-70).

How different HIV-specific antibody families impact one another during infection is relatively unknown. However, there is one intriguing example where a separate antibody family appears to help the development and expansion of a bnAb lineage within a particular individual (71). Strikingly, the cooperating antibody lineage was later found to develop neutralization breadth independently (72). This provides

support for the idea that the development of breadth is a stepwise process that will require sequential immunogens to guide the antibody response along the desired maturation pathway. This concept is also supported by a number of observations: Firstly, that early less-mutated bnAb family members have a limited degree of neutralization breadth (73); and secondly, some good news, that specifically designed immunogens (74) can re-elicit this level of breadth in Knockin mice which encode the bnAb precursor (75). Notably, the sequential immunogens used to elicit these bnAb-like antibodies were closely related allowing boosting of the preceding antibody response. However, in genetically outbred animal models, immunization largely only produces autologous neutralization of primary isolates. Recently, this has been found to be due in part to immunogenic autologous neutralizing epitopes accessible due to holes in the glycan shield of Env (76). This is reminiscent of the specificity of autologous neutralization often observed in natural infection (45). Therefore, the field has now produced antibodies by immunization that resemble the early neutralizing specificities induced during infection. Thus, the next step is to understand how neutralization breadth develops and mimic that by vaccination.

Fundamental to this next step is to clearly define the involvement of strain-specific antibodies in the development of breadth. One study has suggested breadth can arise to the same site as the early autologous neutralizing response, but with the broad antibody actually binding the N-linked glycan that enabled the virus to escape from the autologous neutralization (77). It seems unlikely this was due to direct affinity maturation of the autologous antibodies to recognise the N-linked glycan. More feasible is that viral escape resulted in heightened variation in this region (including the additional glycan site) that triggered the generation of *de novo* bnAb lineages. However, apart from this example (77), it has been observed that, some early bnAb family members can only neutralize in the absence of specific glycan sites proximal to their epitopes. Such glycan shields are then tolerated by the related

fully matured bnAbs (78, 79). In summary, the large body of work on bnAb lineages to date has provided clues as to how breadth develops, and the significance of the glycan shield in restricting neutralization breadth. Therefore, understanding what role, if any, strain-specific responses play in the step-wise development of neutralization breadth could suggest potential shortcuts to inducing breadth by immunization.

Features associated with the development of bnAbs

To recapitulate the shift from autologous to broad neutralization in vaccination it will also be valuable to understand why this occurs in some cases of natural infection and not others. To date, there are features associated with the development of neutralization breadth but no absolute predictive markers. The most consistent predictive trait of broad neutralization is a greater time of untreated infection, hence the preponderance of long-term non-progressors (LTNP) in broadly neutralizing cohorts (55, 56, 80, 81). Greater length of untreated infection occurs concurrently with greater viral diversity as quasi-species develop from the estimated 1-3 founder viruses (82). Furthermore, it has been suggested that greater diversity during the earliest stages of infection may have more influence on the development of breadth (83). However, in contrast to its association to the development of breadth, HIV Env sequence diversity poses a great barrier to the elicitation of bnAbs due to the extensive level of variation as reviewed in (84) and (85). In addition, Env adopts diverse conformational states, including non-infectious forms that can act as decoys for the immune response and by combining these multiple levels of variation HIV routinely escapes from host antibodies in ‘a mutational arms race’ (86, 87). Nevertheless, studies have suggested that viral subtype diversity can be overcome (88) and particular Env signatures are associated with the development of breadth (89). Moreover, intra-host Env diversity and viral escape from early neutralizing responses have been suggested to drive the maturation of broader responses (61,

90). Thus, while Env diversity is a major roadblock to developing a protective antibody response following immunization paradoxically it is also a probable trigger of bnAb development.

Another key aspect of the development of broad HIV neutralization is the host immune system. The role of particular bnAb genetic precursor prevalence may play a role but has not been directly assessed in large cohort studies and is expertly reviewed elsewhere (91). There have been limited reports on the role of immune cell subsets, primarily that low CD4 counts correlate with breadth (49, 52, 55), but this could potentially be related to high viral load. In addition, neutralization breadth has been associated with higher levels of T follicular helper (TFH) cells expressing PD1 in human subjects (92). TFH cells have also been found to be important in the development of broad antibody responses in non-human primate infections (93). Recent data from a very large human cohort show a trend to greater likelihood of bnAb-like serum responses in individuals of African ethnicity (56). Furthermore, a separate genome-wide association study in the Amsterdam Cohort found single-nucleotide polymorphism in MHC and HLA-B to be most strongly associated with breadth but not significant at a genome level (94). These observations suggest the overall host immune enivronment plays a role in the generation of bnAbs, as do studies showing that more rapid development of neutralization breadth can occur (<1 year). Particularly as this can occur in immunlogically distinct situations, such as transmission following injecting drug use (95) or from mother-to-child (96). Therefore, there is a need to further elucidate the role of host biology in the development of bnAb responses.

The bnAb revolution

Over the last decade the identification of bnAbs has revolutionized our understanding of the humoral response to HIV (57, 97). The rapid isolation of so many bnAbs was

possible due to advances in screening assays to identify individuals with broadly neutralizing serum as reviewed in (58). Critically, this involved the generation of large panels of Env pseudoviruses derived from primary isolates and categorization of their neutralization sensitivity, to exclude easily neutralized strains (98-100). These tools enabled neutralization breadth to be investigated in large cohort studies as summarized in Table 1 (47, 48, 50, 52-55). A clearer picture emerged; breadth of neutralization develops in 10-30% of individuals generally 2-3 years after infection, despite the use of different definitions of breadth, virus panels and clinical populations between studies. Notably, elite neutralization, where breadth is equivalent to that seen with individual bnAbs, is less frequent, arising in 1-10% of individuals (Table 1). These large cohort studies are largely consistent with early observations of the rapid rise of autologous neutralization activity followed by the later, and less frequent, development of breadth (43, 44). Additionally, longitudinal serum studies have also shown the early development of autologous neutralization and resulting selection of viral escape mutations within individuals (45, 101, 102). These strain-specific responses have been seen as early as two weeks post seroconversion and escape seen in response to low titers of autologous neutralizing antibodies (103). However, despite the fact that viral Env mutation within the host is largely driven by the need to escape the autologous neutralizing response, there is no apparent consequent loss of viral fitness (104) which raises the question of how effective humoral responses are after transmission during chronic infection?

Early studies that attempted to correlate neutralizing antibodies with protection were disappointing. There was no decline in viremia with the development of either autologous or broad neutralization (43, 44, 105, 106). Indeed, one study suggested that patients could harbor viruses sensitive to neutralization despite the presence of serum antibodies that potently neutralize these particular strains (107). Nor is there evidence that development of neutralization breadth improves patient

prognosis (51, 108) . The observation that broad neutralization is predominantly found in LTNP compared to rapid progressors (47, 49, 51, 53) may be attributable to sustained exposure to the viral Env antigen over long periods. In contrast rapid progressors become virally suppressed on treatment, which limits antigenic stimulation Despite these shortcomings the evolution of neutralization escape mutants *in vivo* was often clearly observed (44, 45, 109). Furthermore, contemporary circulating HIV strains are more resistant to bnAbs compared to historical strains (110, 111). This suggests that overall there is evolutionary pressure on HIV to escape the onslaught of neutralization activity within individuals that controls diversification of the virus to some degree. In agreement with this, it is known that bnAbs can protect from infection in animal models, reviewed in (112), and recently human bnAb therapy has increased time to viral re-bound (113). Furthermore, pre-existing anti-HIV antibodies have been reported to prevent the emergence of escape mutants to the administered bnAb (114) further supporting the idea of using bnAbs to treat chronic infection.

Conclusion

Many lessons have been learnt from studying the humoral response to HIV both in terms of serological studies and characterization of bnAbs and their family members. First, antibodies can be effective against HIV from the observation that the presence of early neutralizing antibodies drives the evolution of the viral Env within the host. Second, HIV bnAbs possess a level of neutralization breadth and potency previously thought impossible, renewing hope that a protective vaccine can be developed. Third, although virus can overcome neutralizing antibodies if it is established in the host before antibodies develop, as in natural infection, but not if antibodies are present in advance, as in passive transfer studies. Fourth, development of breadth is a question of balance; not only is diversity important but

so too is time. High enough viral diversity over at least a year of infection is generally needed to stimulate a bnAb response but excessive viral replication, resulting in rapid progression and immune dysregulation, will not favour breadth. Finally, absolute predictors of neutralization breadth have not been identified. This suggests additional factors should be investigated such as the balance of different antibody specificities and lymphocyte subsets, along with genetic host factors. However, the interplay of host and viral factors that result in broad neutralization will likely vary between individuals, as HIV bnAbs are a diverse group which can arise from multiple pathways of antigenic stimulation. In summary, despite great advances in our understanding of humoral responses to HIV, what controls the tipping point that leads to broad neutralization remains to be defined.

Key points:

- Despite early optimism neutralizing responses are difficult to recapitulate in a vaccine
- Clinical cohort studies show that broad neutralization occurs in 10-30% of HIV infections
- Elite HIV serum neutralization is seen in <10% of individuals
- More research is needed to understand the shift from strain-specific to broad HIV neutralization during infection and how it varies between different people.

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Figure legends:

Table 1: Selected serological studies assessing breadth of neutralization response in HIV infection.

Summary data from the indicated cohort studies which have attempted to measure breadth and potency of HIV neutralization in serum samples. Abbreviations: IAVI = International AIDS Vaccine Iniative, NIH = National Institutes of Health.

Cohort	Viruses screened	No. patients	Author defined breadth	Time frame
IAVI Protocol G (47)	Multiple panels:	N =1798		Single time point: 3 years onwards
	101 virus panel	n = 101	Broad = 45% had an IC50 titer of ≥150 for viruses from 1-3 clades	
	15 of above viruses	n = 463	Broad = 36% had IC50 titers of ≥150 for viruses from >4 clades Intermediate = 51% had an IC50 titer of ≥100 for viruse from 1-3 clades	
	6 representative viruses	n = 1234	Broad = 34% had IC50 titer of ≥100 against >4 clades, Elite breadth = 1% had mean IC50 titer of 1:500 to majority viruse from >4 clades.	
NIH (48)	5 virus panel	n = 113	Broad = 33% neutralized 4/5 viruses, Intermediate = 33% 3/5 viruses, no breadth = 29% 0 or 1/5 viruses	Single time point: 1 year onwards
Amsterdam Cohort (49)	28 virus panel	n = 35	Broad = IC50 >100 for >50% of viruses per subtype, from >3 subtypes, 20% broad at 2 years, 31% broad at 4 years	Longitudinal: breadth observed 2 and 4 years after seroconversion
South African National Blood Services (50)	10 virus panel	n = 70	Broad = 23% neutralized all 10 viruses	Single time point: 180 days onwards
Amsterdam Cohort (51)	23 virus panel	n = 82	Broad = 33%, moderate = 48% and no breasth = 20%	Single time point: ~35 months after seroconversion
Caprisa Cohort (52)	44 virus panel	n= 40	Broad = 17.5% neutralized > 40% of viruses	Longitudinal: peak titer at 3 years after seroconversion
Ragon Institute (53)	20 virus panel	n = 17	Broad = 29% neutralized 75% viruses	Longitudinal: breadth observed at 1-2.5 years
Multiple (54)	219 virus panel	n = 205	Broad = 50% neutralized >50% of viruses, Elite breadth = 10% neutralized 90% of viruses	Single time point: chronic HIV infection
IAVI Protocol C (55)	6 virus panel	n = 439	Broad = 2% elite >2, 9% strong (1-2), 25% moderate (0.5-1) and not broad = 46% low (0.1-0.5), 18% no breadth	Longitudinal: 0-4 years
Swiss Cohort (56)	8 virus panel	n = 4484	Cummulative scoring for % neutralization. Elite (score >15) = 1.3%, Broad (score 10-14) = 4%, cross-neutralizing (score 5-9) = 15.6%	Single time point: >1 year off treatment

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