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Specificity of the autologous neutralizing antibody response

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Abstract

Purpose of review—It has long been known that autologous neutralizing antibodies (AnAbs) exert pressure on the envelope of HIV, resulting in neutralization escape. However, recently, progress has been made in uncovering the precise targets of these potent early antibodies.

Recent findings—AnAbs primarily target variable regions of the HIV-1 envelope, explaining the strain-specificity of these antibodies. Despite high neutralizing potential and cross-reactivity, anti-V3 antibodies do not contribute to autologous neutralization. The V1V2 is commonly immunogenic in early HIV-1 and SHIV infections, though the nature of these epitopes remains to be determined. In subtype C viruses, the C3 region is a neutralization target, possibly as a result of its more exposed and amphipathic structure. Autologous neutralization appears to be mediated by very few AnAb specificities which develop sequentially suggesting the possibility of immunological hierarchies for both binding and neutralizing antibodies. The role of AnAbs in preventing superinfection and in restricting virus replication is re-examined in the context of recent data.

Summary—New studies have greatly contributed towards our understanding of the specificities mediating autologous neutralization and highlighted potential vulnerabilities on transmitted viruses. However, the contribution of AnAbs to the development of neutralization breadth remains to be characterized.

Keywords

Autologous neutralization; strain-specific; variable loops; C3; escape

Introduction

The development of autologous antibody responses during early HIV-1 infection has become the subject of increasing interest, based on the premise that such specificities may define exposed vulnerabilities on the transmitted envelope, and inform the development of neutralization breadth. The appearance of autologous neutralizing antibodies (AnAb) occurs within months of infection in most HIV-1 infected individuals [1,2,3,4] and while such responses are often potent and rapidly drive neutralization escape, they are extremely strain-specific. The specificities and number of these early antibodies driving escape has recently become clearer.

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Development of anti-HIV-1 antibodies

The first B-cell response to transmitted HIV-1 comprises binding antibodies that first develop within 8 days of detectable viremia, and initially exist as antigen-antibody complexes [5**]. These are followed by circulating anti-gp41 antibodies 5 days later, with anti-gp120 antibodies, primarily targeting the V3 loop, delayed a further 14 days. AnAb responses to HIV-1 develop later in infection, at about 12-20 weeks post-infection in most individuals, with antibodies invariably showing strain-specificity [1,2,3,4,6] especially in subtype C where relatively higher titers and increased strain-specificity have been described [2]. Such AnAb responses persist during most of the course of disease, but may wane during the symptomatic phase of infection perhaps reflecting the inability of the dysfunctional humoral immune system to respond to *de novo* viral variants [4,7*,8]. The strain-specificity of AnAbs [1,2,3,4] and the genetic pressure evidenced on later *env* sequences [4,7*] suggests that these antibodies target the variable regions rather than more conserved structures of the envelope glycoprotein.

Anti-V3 antibodies do not contribute to autologous neutralization

There is now increasing evidence that especially the V1V2 loop, and to a lesser extent the V4 and V5 loops, play a role as direct AnAb targets (reviewed below). In contrast, it has become clear that anti-V3 antibodies, which are among the first antibodies to be elicited in HIV-1 infection, do not contribute to autologous neutralization [9,10*,11*]. This is despite the finding that such antibodies have broadly cross-reactive envelope binding capacity and extremely high neutralizing activity against viruses with artificially exposed V3 regions (such as the HIV-2 chimeric envelope engrafted with HIV-1 V3 loop) [9,10*,11*]. Similar observations using SHIV chimeras suggest that anti-V3 antibodies also play no substantial role in autologous neutralization during SHIV infection of monkeys [12*]. This supports evidence showing that anti-V3 antibodies play a minimal role in neutralization [13,14] due to occlusion of the V3 loop within the trimeric Env [9,10*,15,16,17].

V1V2 is a frequent target of autologous nAbs

The role of V1V2 in shielding neutralization determinants is well-recognized [15,16,18,19,20,21,22]. However, V1V2 may also act as a neutralization target in laboratory adapted isolates [23] and primary viruses [1,11*,24,25,²⁶,27,28,29,30]. Use of reciprocal V1V2 chimeras suggested that the V1V2 region was principally responsible for the strain-specific AnAbs detected in plasma from SHIV-infected monkeys [12*]. Similarly in HIV-1 infection there is mounting evidence that the V1V2 is an early target of AnAbs. Transfer of early V1V2 sequences into a heterologous viral backbone resulted in transfer of neutralization sensitivity to autologous plasma [21]. In contrast, transfer of later/chronic V1V2 regions did not result in autologous neutralization sensitivity, suggesting that V1V2 may be a target of early AnAbs, with changes in later V1V2 sequences mediating escape [21]. In subtype C infection, a similar approach using chimeric Env derived from transmission pairs suggested that V1V2 may contain AnAb epitopes in some cases, in addition to the more general role of V1V2 in shielding neutralization determinants [29]. This suggestion was confirmed using chimeras constructed between envelopes derived from early subtype C infection [11*], and by examining neutralization escape variants which also implicated the V1V2 region as a target of AnAbs [11*,31,32,33]. Confirmation of the role of V1V2 as an AnAb target comes from the isolation of anti-V1V2 antibodies recognizing glycan-dependent epitopes from B-cell hybridomas of a subtype C infected individual [33]. The V1V2 region therefore appears to be commonly immunogenic in early HIV-1 and SHIV infections. However, the nature of these epitopes requires further elucidation, as it is still not known whether these neutralizing anti-V1V2 antibodies, like those increasingly isolated

through screening by neutralization rather than binding [34], recognize epitopes only apparent in the trimeric structure of the envelope.

The role of the V4 and V5 regions in autologous neutralization

The role of the V4 and V5 loops as AnAb targets is less clear. The V4 region has been proposed to contribute to the formation of quaternary epitopes in conjunction with the C3 region in subtype C viruses [11*,35] (see below for details), but independently the V4 does not appear to be a significant AnAb target, although changes in this region may mediate neutralization escape [36]. Similarly, the use of chimeras suggested only a marginal role for V5 as a target of AnAbs in early HIV-1 infection [11*], however again changes within the V5 may effectively mediate escape from AnAbs [32,33]. The mechanism whereby such escape occurs remains to be defined, but it may be via modulation of proximal epitopes [32,33] with which V5 interacts, such as the adjacent CD4bs [37].

C3 – a subtype-specific neutralization target?

In addition to the variable regions, the C3 region located in the outer domain of gp120 slightly upstream of the V3 loop has been implicated as a neutralization target in subtype C viruses [35,38] and is under strong diversifying pressure [39]. Furthermore, there are structural differences between subtypes B and C in the $\alpha 2$ ($\alpha 2$)-helix of C3 [38] suggesting increased exposure in subtype C viruses. Also, neutralization resistance mutations were frequently located within the $\alpha 2$ -helix of Zambian subtype C viruses [35], and in Indian subtype C viruses [40]. It has therefore been proposed that AnAbs directly target the $\alpha 2$ -helix in subtype C viruses [11*,35,38]. However, chimeras transferring the $\alpha 2$ -helix between resistant donor Env and sensitive recipient Env from subtype C transmission pairs did not alter the AnAb phenotype, suggesting that the $\alpha 2$ -helix must be associated with other regions of Env for the formation of AnAb epitopes [35], with occasional exceptions where the $\alpha 2$ -helix may serve as an AnAb target independently of V4 [11*,32]. The $\alpha 2$ -helix may contribute to the quaternary structure of the trimer and so affect neutralization epitopes, possibly via the strong interactions this region has with the V4 loop, or the β -10, β -11, β -14 and β -24 strands [35]. That was confirmed by the use of reciprocal chimeras where transfer of the C3 region in conjunction with the V4 loop (C3V4) resulted in transfer of neutralization sensitivity, suggesting that the C3V4 region was a major target of AnAbs in subtype C HIV-1 infection [11*]. The observation that within the $\alpha 2$ -helix in subtype C, amino acid changes frequently resulted in charge changes, unlike subtype B where variable positions tend to maintain similar charge, led to the suggestion that switching of charges may facilitate immune escape [38]. This suggestion was supported by the observation that in subtype C, neutralization escape was associated with mutations within the $\alpha 2$ -helix that resulted in charge changes [32].

In addition to the $\alpha 2$ -helix, the C3 region also contains the highly variable β -14 strand, as well as the conserved β -15 strand and $\alpha 3$ -helix which contain elements of the CD4 binding site (CD4bs), so it is also possible the anti-C3V4 reactivity is mediated by anti-CD4bs antibodies which are strain-specific. Strain-specific anti-CD4bs antibodies may not be entirely surprising as even the monoclonal CD4bs nAb IgG1b12, which is characterized as broadly cross-neutralizing, neutralizes only approximately two-thirds of primary viruses [13]. Furthermore, there is evidence that the functional CD4 binding region is smaller than the structural epitope of IgG1b12 [41], perhaps allowing some flexibility in secondary binding sites to other non-conserved residues [42]. This may be explained in part also by recent data comparing IgG1b12 with a non-neutralizing monoclonal antibody b13. Although these mAbs share overlapping binding sites, the angle at which these antibodies approach the trimer, and the compatibility of such binding with the trimeric spike, including the

quaternary location of the V1V2 loops [43], may determine the neutralizing capacity and specificity of such CD4bs antibodies [44].

gp41 as a target of autologous neutralizing antibodies

The majority of early AnAb responses to transmitted HIV-1 variants seem to be directed at the gp120 in line with the high levels of positive selection focused here [7*]. Neutralizing antibodies targeting the MPER region of gp41 may develop within the first year of infection in some individuals [1] however their contribution to autologous neutralization has not been well-defined. Recent data in one individual where adsorption of anti-MPER antibodies resulted in abrogation of cross-neutralization, showed that the effect of adsorption on autologous neutralization was marginal, suggesting that these broadly cross-reactive anti-MPER did not substantially contribute to autologous neutralization in this case [45]. In contrast, the identification of 4E10-resistant variants in an HIV-1 infected individual exhibiting an anti-MPER antibody response suggested immune escape from AnAb responses targeting this region [46]. Analysis of neutralization escape in subtype C infected individuals suggested a role for the ectodomain of gp41 in neutralization escape though it was not clear whether the AnAbs in this case targeted gp41 directly or whether escape occurred via modulation of distal epitopes [33]. It is noteworthy that in the above cases, anti-gp41 antibody pressure was observed after one year of infection and considerably delayed compared to the primary autologous neutralization response.

AnAb pressure and evolution of the envelope

Much of the variation that occurs in the Env during early infection is thought to be the result of pressure exerted by AnAbs [3,4,47]. Later in infection, selection pressure on the envelope is decreased [7*], perhaps reflecting the diminishing AnAb response [4,7*]. Neutralization escape has been documented in early HIV-1 subtype B viruses [3,4,7*,48,⁴⁹,50,51,52,53] and more recently subtype C viruses [32,33] and in SIV [54,55,56] with contemporaneous viruses showing less sensitivity to autologous neutralization than earlier viruses. Neutralization escape may occur through single amino acid substitutions [6,32,33], insertions and deletions [21,29], and through an “evolving glycan shield”, where shifting glycans prevent access of AnAbs to their cognate epitopes [4,21,22]. However, in studies of chronically infected mothers transmitting HIV-1 to their children [57] and in long-term non-progressors [8,58*], both sensitive and relatively resistant variants were detected among the quasispecies. A similar pattern of persistence of neutralization sensitive variants was observed in a typical progressor who had notably broad and potent antibody responses (Bosch and Overbaugh, unpublished). The persistence of sensitive variants may result from continuous reversion of less fit escape variants, or persistent release of pre-escape variants from cell reservoirs. This suggests that the envelope glycoprotein may have limited plasticity with which to mediate continuous escape from the autologous antibody response.

Sequential specificities arise during early HIV-1 infection

Examination of neutralization escape in HIV-1 infection suggests that there may be limited AnAb specificities driving escape during early infection [31,32]. In 4 subtype C infected individuals, between 1 and 2 antibody specificities appeared to mediate autologous neutralization during the first year of infection [32]. Interestingly, where more than one specificity was present, these arose sequentially during the first year [31,32], sometimes requiring months of infection [32]. This observation suggests the possibility of an immunological hierarchy, with delayed development of selected responses, in line with the hierarchical binding antibody responses which develop in the very early stages of infection [5**], prior to the development of AnAbs. It is also possible that changes which develop across the envelope during the first months of infection in response to neutralization escape,

and other selection pressures, affect the conformation and exposure of the secondary AnAb target, facilitating presentation of this region to the immune system only at a later time-point [32]. This phenomenon may ultimately result in the exposure of more conserved regions that will induce broadly cross-neutralizing antibodies.

AnAbs and prevention of superinfection / control of viremia

The effect of AnAbs on controlling viral load and preventing superinfection is unclear. It has been suggested that AnAbs are ineffective in individuals with high viral loads due to the continuous generation of escape variants but that they may play a role in maintenance of low viral loads in controllers [58*] or during early infection, where viral diversity is relatively low [32]. In one individual, a decrease in the viral load was temporally associated with the development of an AnAb response, followed by a rebound as neutralization escape occurred. This suggested the possibility that AnAbs may in the short-term have impacted on viral load, with this effect abrogated by the emergence of neutralization resistant variants. [32]. Interestingly, relatively high titers of AnAbs were needed before sufficient pressure was exerted on the overall population, forcing escape to occur [32], perhaps reflecting the “soft” selection exerted by AnAbs [6].

The requirement for relatively high antibody titers is supported by the observation that superinfection may occur in the presence of low levels of pre-existing cross-neutralizing antibodies raised against the initial infecting strain [59**]. Furthermore, theoretical calculations of protective *in vivo* titers from passive transfer studies, suggested that nAb titers in excess of 1:200 were required for protection during acute infection, and increasing to titers exceeding 1:1,000 during chronic infection [60**]. These studies are in line with data from animals [61,62,63]. The inhibitory potential of even the best known monoclonal nAbs, namely IgG1b12, 2F5, 4E10 and 2G12, is relatively low compared to most antiretroviral drugs [64**,65], suggesting that high antibody titers may be needed to achieve protective levels.

Conclusion

The now routine use of the recombinant pseudovirus assay [14,66,67], coupled with the use of standardized methodologies to amplify envelopes from single genome templates [68,69], has facilitated detailed characterization of early AnAb responses and resultant escape variants [32,33,69]. In addition, use of chimeric viruses has greatly advanced our understanding of the specificities of AnAbs [1,9,10*,11*,12*,21,29,70]. Future research aimed at understanding the targets of AnAbs should make use of methods which have been recently employed to dissect the specificities of broadly cross-reactive antibodies [71,72,73,74,75]. Specifically the use of methodologies such as adsorptions using soluble gp120 will clarify whether AnAbs recognize epitopes recapitulated on monomeric proteins or whether trimeric structures are required. Perhaps the most direct and powerful method for characterizing specificities mediating autologous neutralization is the isolation of monoclonal AnAbs [33,76]. Use of novel methods for high-throughput molecular generation of AnAbs will facilitate the rapid generation of monoclonal AnAbs [77,78,79,80] which will make fine mapping of epitopes feasible.

Further characterization of the nature of AnAb responses continues to be important for two reasons. Firstly, much attention in the last year has been directed at defining the specificities of broadly cross-reactive nAbs (reviewed by J. Binley in this issue), however the mechanisms that lead to the development of cross-neutralizing capacity are not clear. The development of breadth is likely to be dependent in part on the autologous envelopes to which individuals are exposed [58*,81], and the relationship between autologous and

heterologous neutralization warrants further study. Secondly, identification of AnAb epitopes allows delineation of vulnerabilities on transmitted envelopes which are exposed and immunogenic [76], providing information which may contribute to vaccine design. The identification of common regions [12*,32] involved in autologous neutralization may facilitate the development of multivalent vaccine candidates focused on limited regions of the envelope but better encompassing the diversity therein [12*,32].

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