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REVIEW

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Search for antiviral functions of potentially protective antibodies against V2 region of HIV-1

Miroslaw K. Gorny

Department of Pathology, New York University Grossman School of Medicine, New York, NY, USA

ABSTRACT

In the only successful RV144 vaccine trial to date, high levels of antibodies (Abs) against the V2 region of the virus envelope protein gp120 correlated with reduced HIV-1 infection. The protective role of V2 Abs has not yet been determined, and the antiviral function of V2 Abs that mediate protection against HIV-1 in humans or SHIV infection in rhesus macaques remains unclear. V2 Abs do not neutralize resistant tier 2 viruses; their Fc-mediated activities are modest and similar to those of another anti-envelope Abs, and inhibition of the gp120–α4β7 integrin interaction is ineffective in both animals and clinical trials. Moreover, in protection experiments in monkeys, levels of V1V2 vaccine-induced V2 Abs do not correlate with plasma viral load. Together, these observations suggest that V2 Abs may not control SHIV infection in rhesus macaques and that V2 Abs may instead be a surrogate marker of other protective immune responses.

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Introduction

The resistance of HIV-1 to neutralization is the primary reason that several HIV vaccines have yielded disappointing results. To date, only one clinical trial of an HIV vaccine, RV144 in Thailand, has been modestly successful, with protective efficacy of 31.2%.[1] In that study, there were two independent immune correlates: a high level of antibodies (Abs) against the V1V2 region of the HIV gp120 envelope (Env) glycoprotein, which correlated with reduced infection rates; and the binding of IgA Abs to HIV gp120s, which was associated with an elevated rate of HIV infection.[2]

Among V1V2 Abs, V2 Abs are most relevant for vaccinees because anti-V1 Abs are sequence-specific and do not recognize heterologous viruses.[3,4] Two types of V2 Abs were detected in plasma samples of RV144 vaccinees: V2 conformational Abs, which are dependent on glycosylation (V2i) and bind to V1V2 proteins but not V2 peptides, and Abs against linear epitopes represented by V2 peptides (V2p). The levels of both types of Abs correlated with reduced risk of HIV-1 infection.[5,6] The V2 region also contains trimer-dependent and trimer-specific epitopes (formed by two and three gp120 monomers, respectively), known as V2/apex, which are recognized by broadly neutralizing antibodies (bnAbs). However, these and other bnAbs were not detected in plasma samples from RV144 vaccinees.[2] Many bnAbs have been isolated from chronically HIV-infected individuals, but due to their unusual characteristics, they have not yet been elicited by any HIV vaccine in humans or animal models.

The V2 Abs correlate was both intriguing and surprising because these Abs have functions that may not effectively protect against transmitted/founder tier 2 viruses. These V2 Abs do not neutralize HIV-1, and as monoclonal Abs (mAbs) have only weak neutralization activity, and mediate Fc-activities similarly to other anti-Env Abs. The unique function of V2 Abs *in vitro* is inhibition of binding of gp120 or V2 peptides to α4β7 integrin, which was initially thought to prevent infection of Th17 T cells expressing CD4, CCR5, and α4β7 integrin. However, anti-α4β7 mAb was inactive in three protection experiments in rhesus macaques and one clinical trial.[7–10]

This discrepancy between the V2 Abs immune correlate in RV144 and the modest or lack of anti-viral functions of V2 Abs prompted us to test the role of V1V2 vaccine-induced anti-V2 Abs in a protection experiment in rhesus macaques challenged with SHIV virus. The results of the two experiments suggest that V2 Abs alone may not be involved in the prevention or control of virus infection (manuscript in preparation).

Immunogenicity of the V2 region

The V2 region is weakly immunogenic due to its exceptional flexibility and the number of glycosylation sites in the hypervariable part of the V2 loop, which may inhibit the recognition of this domain by immune cells.[4,11] Stabilization of the V2 region by adding the BG505 SOSIP.664 trimer disulfide bonds within the V2 loop and cross-linking V2 and V1 yielded new variants that were more reactive with, and more readily neutralized by, V2 bnAbs.[12] The simian immunodeficiency virus (SIV) V2 region contains two cysteines that stabilize this domain. Immunization of rhesus macaques with SIV Env induces a higher titer of anti-V2 Abs than in monkeys vaccinated with HIV Env immunogens, which do not have the additional disulfide bonds.[13] Structurally constrained V1V2 regions fused to scaffold proteins are more immunogenic: in rabbits, these constructs induced higher titers of anti-V2 Abs

CONTACT Miroslaw K. Gorny mirek.gorny@nyulangone.org New York University Grossman School of Medicine, New York, NY 10016

than unconstrained V1V2 fused to an Fc fragment, which was intended to mimic the unconstrained V1V2 conformation.[14]

The poor immunogenicity of the V2 region is reflected by the broad range of plasma V2 Abs levels in vaccinees and HIV-infected individuals, including some patients who completely lack of V2 Abs against both conformational and linear V2 epitopes. Abs reactive with gp70-V1V2$_{CaseA2}$ protein were detected in 67 of 80 (84%) plasma samples from RV144 vaccine recipients, at relatively low optical density, as measured by ELISA. In another study, reactivity with cyclic V2$_{92TH023}$ peptide was detected in 97% of vaccinees plasma samples.[15,16]

In plasma samples from chronically HIV-infected and antiretroviral treatment (ART)-naïve individuals from Cameroon, cross-reactive Abs against four V1V2 scaffold proteins were detected in 72% to 85% of patients, and Abs against various biotinylated cyclic V2 peptides were detected in 40% to 61%. Among the 79 plasma samples, six (7.6%) lacked anti-V2 Abs against eight different V1V2 proteins and V2 peptides, tested longitudinally for 11 to 54 months after HIV-1 diagnosis.[4] The V2 region of gp120 HIV-1 was comparable between V2-deficient and V2–cross-reactive groups except that it was longer in the V2-deficient panel of viruses. The most striking difference was the significantly lower 50% titers ($p < .0001$) of plasma Abs against gp120, gp41, and V3 peptide in the V2-deficient group relative to the cross-reactive V2 Ab group.[4] Considering additional data showing a strong correlation ($p < .0001$) between the levels of V1V2 Abs and those of gp120 and gp41 Abs, these results suggest that the level of V2 Abs represents a surrogate marker for the general Ab response against HIV-1. Thus, a high level of plasma V2 Abs can indicate that a given HIV$^+$ individual is mounting a strong anti-Env immune response.[4]

This conclusion is supported by the association between V2 Abs and the percentage of CD40 L-expressing CD4$^+$ T cells reactive to HIV-1 Env vaccine strain 92TH023. This observation was made during an evaluation of the RV144 regimen tested in South Africa, sponsored by the HIV Vaccine Trials Network (HVTN) 097.[17] CD4$^+$ Env–specific T cells were selected for analysis because they provide help to B cells,[18] and their relative abundance (i.e., as a percentage of all CD4$^+$ cells) is associated with binding of IgG and IgG3 Abs to gp120 antigens and clade C V1V2 proteins (all four associations, $p < .01$).[17]

Another study revealed a parallel HIV vaccine-induced Ab response to gp120 and V1V2 antigens. In phase 1 clinical trial aimed at testing the immunogenicity of four variants of the RV144 vaccine in which the viral vector was substituted for DNA, the trends in IgG response rates and geometric mean response magnitudes for binding of IgG, IgG3, and IgG4 Abs to gp120 and V1V2 antigens were very similar over time. The global distribution of humoral immunogenicity as a function of response rate was illustrated using radar plots; the correlation was not analyzed. The IgG response rate was 81–100%. The IgG3 binding response peaked at the second vaccination but waned at the final (fourth) vaccination in month 6, and the IgG4 response rate was at ~75%.[19] Vaccines rarely induce IgG4 Abs, but in that study, such response was induced by a variant of the HIV vaccine, emphasizing the parallel Ab responses to gp120 and V1V2 proteins.

The Ab response to Env proteins has been observed as an immune correlate in protection experiments in rhesus macaques immunized with the Env HIV immunogens and SHIV challenge.[20–22] Other protection experiments using SIV immunogens and SIV challenge, which are less relevant to the HIV vaccine but still important for understanding the mechanism of protection, also revealed a correlation between the Env Ab response and control of infection.[23–26]

Binding of V2 mAbs to intact viruses

The function of anti-viral Abs depends on their binding affinity to the intact virus, but anti-V2 mAbs bind virions only weakly. In a study using the virus capture assay, five anti-V2i mAbs were examined for their abilities to bind to intact 26 HIV-1 isolates.[27] Binding of V2 mAbs to the HIV-1 isolates was weak and sporadic relative to binding of mAbs against V3, C5, the cluster I region of gp41, and mAb IgG1b12 against the CD4 binding site (CD4bs). In subsequent experiments, the virus was captured with gp41 and C5 mAbs and incubated with PBMCs, which became infected, revealed that mAbs do indeed bind to intact, native, infectious virions.

Another study tested the ability of plasma IgG from RV144 vaccinees to capture various strains of infectious HIV-1.[28] Fractions of plasma IgG, 64% and 78%, captured the strains MN and CM244, respectively. The virion-capturing Abs were directed against multiple epitopes in gp120, which were identified by testing mAbs generated from RV144/RV135 vaccinees representing specificities for C1 conformational (CH40), V3 (CH22), and V2 epitopes (HG107). The mAbs against C1 and V3 captured high levels of infectious virions, whereas the V2 mAb captured the virus weakly.[28] These results suggested that V2 epitopes are generally not well exposed on the surfaces of virions, probably due to the shielding of epitopes by glycosylation and/or the inherent flexibility of the V2 region.[27]

Neutralization of HIV-1

Due to their weak binding for intact virions, V2 mAbs have a weak neutralizing activity that is limited to certain neutralization-sensitive tier 1 viruses. Both the conformational V2i and linear V2p mAbs have low neutralization activity against HIV-1 and limited cross-neutralization.[29–32] In one study, seven V2i mAbs neutralized four to eight of 41 tier 1 pseudoviruses with a 50% neutralization (IC$_{50}$) falling in a broad range between <0.4 and 88.7 μg/mL.[30] The linear V2p mAb HGP68, derived from an HIV-infected individual, and CH58 and CH59, derived from recipients of an RV144-like vaccine, weakly and sporadically neutralized tier 1 viruses.[31,32]

In a large comparative study of 66 V3, CD4bs, and V2i mAbs, the neutralization potency and breadth of V3 mAbs was higher than that of the V2, and CD4bs mAbs tested ($p = .009$ and 0.023, respectively). Furthermore, the V3 mAbs could neutralize most tier 1 and some tier 2 and 3 pseudotyped viruses, whereas the V2 and CD4bs mAbs could only neutralize tier 1 viruses.[33] These three types of Abs are commonly induced by natural HIV-1 infection and vaccines. However, the V2 and CD4bs Abs do not neutralize tier 2 viruses comprising transmitted/founder viruses and are accordingly

classified as non-neutralizing, in contrast to bnAbs that mediate the neutralization of tier 2 and 3 viruses.

V2 Abs induced by the RV144 vaccine did not exert neutralizing activity against tier 1 viruses as determined in blocking experiments.[34] Plasma samples from the recipients of RV144 vaccine neutralized only tier 1 viruses and neutralization was mediated by Abs against the V3 region. This was demonstrated that the V3 peptides blocked 65% and 100% of plasma neutralizing activity against MN.3 and TH023 viruses, respectively. Peptides from the V2, C1, and C5 regions, however, had little or no blocking effect on neutralization of tier 1 viruses by plasma samples.[34] Given that in the RV144 clinical trial, 89% of infections occurred with neutralization-resistant tier 2 CRF01_AE strains,[35] the neutralization did not play a role in protection against HIV-1 in the RV144 clinical trial.

The V2 Abs induced in rabbits and rhesus macaques upon immunization with V1V2 scaffold proteins had minimal neutralizing activities and were tested only against tier 1 viruses.[14,36] Sera from several rabbits at the lowest dilution (1:10) could neutralize the tier 1A virus MW965 after 30 min or extended 24-h incubation of virus and serum prior to the addition of cells. Greater than 50% neutralization of the slightly more resistant heterologous tier 1B virus ZM109 was achieved by 10 of 25 (40%) tested rabbit sera only in the extended 24-h incubation assay.[14] Similar results were obtained by immunization of rhesus macaques with V1V2 scaffold proteins, which yielded plasma Abs with limited neutralizing activity. At week 22, 2 weeks after the last immunization, plasma Abs induced by three of six tested V1V2 proteins exhibited neutralizing activity against two-tier 1A viruses, whereas 1 of 6 V1V2 immunogens induced V2 Abs neutralizing a little more resistant tier 1B virus at low dilution.[36]

Thus, the neutralizing activity of V2, V3, and CD4bs Abs cannot protect humans against HIV-1, which is transmitted as resistant tier 2 and 3 viruses.[37] Tier 1 viruses are not transmitted because these Abs neutralize them in each patient, and even if they were transmitted, the HIV vaccine–induced Abs in a healthy recipient would neutralize the tier 1 viruses while the tier 2 and 3 viruses initiated a new infection. Therefore, development of HIV vaccines has focused on designing immunogens that can induce bnAbs with the ability to neutralize tier 2 and 3 transmitted/founder viruses. These efforts have yielded some progress: for example, upon immunization of animals, the immunogen RC1 elicited Abs targeting the V3-glycan patch on the envelope protein of HIV-1; the resultant V3-glycan Abs neutralize tier 2 and 3 viruses and are precursors of human bnAbs, which upon further sequential vaccination may develop into potent bnAbs.[38] Two studies took a similar approach: in animal models, they tested the immunogens that bind to rare bnAb B-cell precursors, which upon subsequent immunization steps could produce bnAbs against CD4bs and V3-glycan.[39,40] In each of these studies, the design of the immunogens was critical for induction of Abs targeting B cells with germline immunoglobulin receptors and long complementarity-determining region 3 of the heavy chain (CDR H3).

Passive immunization

Passive transfer of anti-Env HIV mAbs to rhesus macaques challenged with SHIV virus provides evidence for the protective role of these Abs during HIV-1 infection. However, passive transfer of a single human anti-V2i mAb, 830A, to rhesus macaques did not confirm that the mAb can mediate protection against tier 1 $SHIV_{BaL.P4}$ challenge.[41] The Ab decreased viral load in plasma (PVL) and viral DNA levels in PBMC and lymphoid tissues but did not significantly reduce the number of infected animals relative to a control mAb DEN3, which targets a surface protein of dengue virus.[41] In two experiments, IgG1 and IgG3 of V2i mAb 830A protected 5 of 18 animals (27%), whereas the control mAb DEN3 protected 1 of 12 animals (8%). V2 mAb 830A lacks protective function due to its low neutralizing activity against the HIV_{BaL} virus as well as its low antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) activities. For example, 830A mediates ADCC modestly *in vitro*, reaching 50% activity at 50 μg/ml against tier 2 $SHIV_{SF162P3}$-infected target cells, whereas two bnAbs, b12 and PGT121, had 50% activity below 1 μg/ml (>50 times lower concentration).[41] The poor accessibility of the V2 region to V2 mAbs results in ineffective neutralization and Fc-mediated activities against the challenging virus.

Similar results were obtained with passively transferred other non-neutralizing human mAbs specific to CD4bs, C1 region of gp120, and against gp41. The mAbs reduced PVL in rhesus macaques, but no protection against SHIV virus challenge was achieved, by contrast, the bnAbs used as positive controls effectively mediated such protection. In one study, a combination of three bnAbs specific for gp120 and gp41 HIV-1 protected against SHIV challenge, whereas two non-neutralizing gp41 mAbs, 246-D and 4B3, only reduced PVL with no impact on SHIV infection.[42]

Two other SHIV challenge studies in rhesus macaques tested non-neutralizing mAbs: one used mAbs against CD4bs and gp41 with positive control bnAb b12, and the other used two non-neutralizing mAbs against gp41 and the C1 region of gp120 compared to control mAb against V3. In both these studies, control mAbs prevented SHIV infection, while the non-neutralizing mAbs neither significantly reduced the number of infected animals nor protected against SHIV infection.[43,44]

The reductions of VL in plasma and viral DNA in PBMCs observed in these studies could be related to the capturing of SHIV virus by transmitted mAbs, particularly gp41 mAbs, although V2 mAb 830A can also weakly bind intact virions.[27] The binding of a mAb to infectious virus enhances its extravascular clearance, which is very rapid in naive animals, with half-lives ranging from 13 to 26 minutes; by contrast, in the presence of neutralizing mAbs, half-lives are reduced to 3.9–7.2 minutes.[45] Furthermore, the immune complex formed upon mAb binding to virions can activate the macrophage system through the Fc receptor, resulting in phagocytosis of the complex, but the underlying mechanisms remain unclear. Non-neutralizing mAbs, including V2 mAb, can bind and capture viruses, resulting in PVL reduction, but this is not enough to protect against SHIV challenge.

Fc-mediated effector functions

Fc-mediated activity, like neutralization, depends on the binding affinity of a mAb for intact virions or virus envelope on infected cells. V2 mAbs and vaccine-induced V2 Abs were tested for two Fc-mediated activities, ADCC and ADCP, and various activity levels were observed depending on the assay used. In a study using primary $CD4^+$ T cells infected with HIV-1 viruses and autologous NK cells, two V2i mAbs mediated ADCC of infected cells at the same level as one gp41 mAb, but were more potent than three bnAbs.[46] Further experiments in the same study tested eight V2i mAbs, all of which mediated ADCC activity comparable to that of bnAb PG9.[46] Two other ADCC studies reported weaker activity mediated by V2i mAb, 830A, relative to two bnAbs, and two V2p mAbs, CH58 and CH59, exhibited a low percentage of specific killing.[32,41] The V2 mAb HG107 mediated ADCC against gp120-coated CD4 target cells, but not against cells infected with HIV-1 AE. CM244.[28,32] These observations suggest that assays using target cells coated with monomeric proteins, e.g., gp120, may not be as biologically relevant as virus-infected cells, which appeared to be more resistant to ADCC activity mediated by V2 mAb.

ADCP is another function mediated by V2 mAbs and vaccine-elicited V2 Abs. This activity can be measured by flow cytometry in THP-1 monocytic cells following uptake of fluorescent beads coated with Env antigens. In a comparative study of 27 human mAbs against V2, V3, CD4bs and gp41 epitopes, mAbs against gp41 exhibited the highest ADCP activity, whereas V2 mAbs mediated dose-dependent activity comparable to those of V3 and CD4bs mAbs.[47] In tests of the ADCP activity against clade C gp120, anti-V2 mAbs were dominant over anti-V3 and anti-CD4bs mAbs, probably due to recognition of conserved V2 epitopes.[47]

V2 Abs induced in rhesus macaques by the prime-boost vaccine, gp120 DNA and V1V2 scaffolds, and tested against $SHIV_{SF162P3}$-infected cells yielded >50% ADCC activity in several animals in almost all tested groups.[36] Each group of animals received either a different construct or were immunized with two or three V1V2 scaffolds. The Ab responses were focused on V1V2 epitopes with little reactivity against gp120 protein, including Abs against V3 and C5 peptides, but it was not determined how these Abs participated in ADCP.[36] These results confirmed an earlier study using rabbits immunized with the same regimen, gp120 DNA and V1V2 scaffolds, which showed modest ADCP activity of plasma Abs depending on V1V2 construct used for immunization.[48]

The role of ADCC and ADCP in protection against HIV-1 or SHIV infection *in vivo* is difficult to determine. In rhesus macaque protection experiments, ADCC and ADCP correlated with the reduced acquisition of either SHIV or SIV infection in only two of 15 experiments analyzed.[20,22,49] Passive transfer of non-neutralizing mAbs specific to V2, CD4bs, C1 region of gp120, and gp41 HIV-1 to rhesus macaques in four different experiments probably mediated the Fc-induced activities, both ADCC and ADCP, due to the Abs' binding properties, but was not able to prevent SHIV infection.[41–44]

These studies show that V2 mAbs and vaccine-induced V2 Abs can mediate ADCC and ADCP activity, but either have activities like those of other non-neutralizing Env mAbs or are induced by vaccine sporadically and with relatively low activity. Moreover, when V2 mAb was passively transmitted to monkeys, it did not protect against SHIV challenge. Thus, the ability of V2 Abs to mediate modest ADCC and ADCP activity does not support the idea that they play a protective role against HIV-1 infection.

Inhibition of α4β7 integrin interaction

The conserved three–amino acid LDI/V motif in the V2 region (aa 179–181), the binding site for α4β7 integrin expressed on Th17 cells, overlaps with the epitope of some anti-V2 mAbs.[50] *In vitro* binding of murine or human V2 mAbs to the α4β7 binding site in the V2 region of gp120 or V2 peptide inhibits their interactions with cells expressing α4β7 integrin.[51,52] These results led to the hypothesis that V2 Abs *in vivo* may block HIV-1 adhesion to Th17 cells, which are located mainly under mucosal tissues, and block subsequent infection of these cells, in which α4β7 integrin is co-localized with CD4 and CCR5.[50,53] These data revealed a unique function of V2 Abs that could explain the correlation between the level of V2 Abs in plasma of RV144 vaccinees and their reduced risk of HIV-1 infection,[2] although expression of α4β7 integrin is not required for infection of target cells.

This concept was supported by a study in SIV-infected rhesus macaques that received ART and primatized mAb against α4β7 integrin, resulting in sustained control of viremia and protection against infection.[54] However, vedolizumab, a humanized version of the same anti-α4β7 mAb, neither prevented nor controlled HIV_{SF162} infection *in vitro* or in humanized mice.[55] In a repetition of the rhesus macaque experiment, 22 animals were infected intravenously with attenuated SIVmac239-*nef*-stop virus, followed by a 13-week course of ART; 12 animals received primatized anti-α4β7 mAb, and 10 received isotype-matched-control mAb. Plasma viremia decreased due to ART treatment, but during mAb treatment, after discontinuation of ART, it rebounded to a high level, with no differences between active and control groups. Thus, there was no evidence for control of SIV by treatment with an antibody against α4β7.[7]

Two other studies confirmed the results of that experiment.[8,9] In one study, rhesus macaques were infected with wild-type, pathogenic SIVmac251, whereas the other they were infected with attenuated SIVmac239-*nef*-stop. All animals began ART, resulting in decreased viral load; however, anti-α4β7 mAb infusion had no detectable effect on virologic control after ART discontinuation, and thus had no therapeutic efficacy.[8,9]

In the latter study ,[9] two non-neutralizing SIV anti-V2 mAbs, ITS09.01 and ITS12.01, were administered in two experiments by infusion of one mAb or in a combination of two mAbs (five animals per group) during and after ART. Both V2 mAbs blocked the binding of the SIV Env to α4β7 integrin *in vitro* experiment, but as with infusion of primatized anti-α4β7 mAb, no post-treatment control of plasma viremia was observed with SIV anti-V2 mAbs in both protection experiments.

Finally, an open-label phase 1 clinical trial of testing vedolizumab was conducted in 20 HIV-infected individuals. After the interruption of ART, the infusion of vedolizumab did not

prolong the suppression of plasma viremia, indicating that blockade of α4β7 may not be an effective strategy for inducing virologic remission in HIV-infected individuals.[10] Together, these results showed that *in vitro* inhibition of α4β7 integrin is not relevant *in vivo*: V2 mAbs, like to anti-α4β7 mAb in several studies, were unable to suppress plasma viremia in monkeys.

Sieve analysis of HIV-1 breakthrough viruses from the RV144 vaccine trial

The analysis revealed that vaccine-induced immune responses were associated with two signatures in V1/V2 at two amino acid positions: 169, which matched vaccine efficacy, and 181, which was mismatched with efficacy. The vaccine-induced immune responses to V2 may block infection with viruses matching the vaccine at K169 but differing from the vaccine at I181. The genetic signatures in V2 region support the association between V2 Abs and the risk of infection in the RV144 vaccine trial, supporting the hypothesis that V2 Abs can be involved in the partial protection.[56]

The study raised several questions. The first is whether the immune response induced by infection with HIV-1, and not only the vaccine-induced immune response, is also involved in selection of particular versions of the virus. Furthermore, if V2 Abs are involved in blocking infection with viruses matching the vaccine V2 sequence, they should bind and capture the virus efficiently, whereas *in vitro* experiments revealed that these Abs bind to intact virions weakly, especially in the case of tier 2 viruses.[27] Notably in this regard, the RV144 vaccine and natural infection can induce a wide range of V2 Ab levels, including lack of V2 Abs longitudinally, in HIV-1 chronically infected individuals; thus, the levels or titers of V2 Abs should be matched with each breakthrough virus.[4,15] The RV144 study differs from another sieve analysis of breakthrough HIV-1 in the HVTN505 vaccine trial, which observed that immune pressure targeted the CD4bs and CD4-induced mAb footprints.[57] These two-sieve analyzes revealed different genetic signatures in the Env, probably caused by the use of different immunogens for immunization and by different infecting viruses. These observations also indicate that vaccine-induced immune response that targets the V2 region is not commonly achieved.

Protection experiments in rhesus macaques

Protection experiments have been performed in rhesus macaques with two goals: first, to test HIV vaccine efficacy using Env antigens for immunization followed by SHIV challenge; and second, to test the mechanism by which SIV Env immunization protects against SIV challenge. In addition, in the hope of understanding the correlation reported in RV144, these studies also investigated whether vaccine-induced V2 Ab levels correlated with plasma viremia and/or virus acquisition. In this regard, the results of the two experimental protocols differed. Studies using the HIV Env/SHIV protocol did not observe a correlation between the level of anti-V2 Abs and reduced virus acquisition.[20–22] There was one exception in which higher V1V2-specific binding antibody titers correlated with viral control in one arm of an experiment using a vaccine with replicating adenovirus (SAd7) vector, but not in a second arm with nonreplicating Ad4.[58]

By contrast, nine studies using the SIV Env/SIV challenge protocol reported a correlation between V2 Abs and reduced infection risk (reviewed in.[49] Five of these studies were performed in one laboratory, using autologous SIVmac251 immunogens and virus challenge,[59–63] whereas the others used heterologous SIV immunogens and virus.[23–26] Anti-V2 Abs, which correlated with reduced infection, were detected by plasma Ab binding to V2 peptides, V1V2 scaffolds, or both antigens. In addition to the correlation involving V2 Abs, eight of the nine studies reported various other immune correlates involving Abs against Env proteins, T-cell response, mucosal NKp24 cells, and CD14 monocytes. By contrast, correlation between neutralization and protection was reported in only two experiments. In two studies, the correlation depended on the adjuvant used or whether the V2 Abs were measured in mucosal secretions or serum samples. Besides their ability to bind V2 antigens, none of these studies determined which functions of V2 Abs were responsible for their protective effect. Several of these studies suggested that the presence of anti-V2 Abs is a surrogate for another immune response.[21,23,26,62] This response could be mediated either by polyclonal Abs against various Env antigens that mediate Fc activities, including ADCC and phagocytosis, or by trained innate immunity represented by monocytes, macrophages, and NK cells.[63–65]

The difference between the HIV/SHIV and SIV/SIV protocols in the frequency of reported correlation between V2 levels and protection may be due to the higher immunogenicity of the V2 region in SIV relative to HIV-1. The V2 domain in SIV Env contains an additional pair of V2 cysteine residues at positions 183 and 191 that stabilize the Env trimer.[66] Vaccination of nonhuman primates with SIVmac239 and SIVsmE660 Env antigens induces much higher levels of anti-V2 Abs than immunization with HIV-1 Env with sequences from the A244, TH023, MN, 1086, and ZM651 viruses.[13]

Study of the protective capability of vaccine-induced V2 Abs in rhesus macaques

Since RV144 in 2009, no study has selectively investigated the protective capacity of vaccine-induced V2 Abs. We sought to study the vaccine induced V2i and V2p Abs alone, without the interference of other anti-Env Abs to determine whether V2 Abs themselves can protect against SHIV challenge in rhesus macaques. We performed two protection experiments in rhesus macaques and presented the results at four international conferences focused on vaccines, including the International Society for Vaccines Annual Congress, October 27–29, 2019, Ghent, Belgium.[67–70] The results are shown as described in the abstract for the ISV conference, whereas all data, including a third experiment involving mucosal immunization of monkeys, are being further analyzed (manuscript in preparation).

Vaccine-induced anti-V2 Abs were tested in two vaccine experiments in rhesus macaques to determine whether these Abs protect against SHIV challenge. The Anti-Env regimen was designed to mimic the RV144 immunogens: nine rhesus macaques were co-immunized with $gp160_{92TH023}$ DNA plus

$gp120_{A244}$ and $gp120_{MN}$ proteins. The Anti-V2 group focused on the induction of anti-V2 Abs alone by co-immunizations with $V1V2_{92TH023}$ DNA, $V1V2_{A244}$ and $V1V2_{CaseA2}$ trimeric proteins, and a cyclic $V2_{CaseA2}$ peptide. Immunizations were given at 0, 4, 12 and 20 weeks, followed by repeated intrarectal $SHIV_{BaL.P4}$ challenges at a dilution representing low-dose exposures. Plasma Ab responses were evaluated by ELISA against HIV-1 Envs, V1V2 fusion proteins, and V2 peptides, as well as by virus capture assays, neutralization of $SHIV_{BaL.P4}$, ADCC, and phagocytosis. Peak PVL was 10^6–10^7 *gag* RNA copies/mL in the six controls (three animals challenged with each vaccine group). Five macaques (55%) in the Anti-Env group and four (44%) in the Anti-V2 group exhibited tight control or no viremia, with undetectable PVL and viral DNA in PBMCs and lymph nodes. In the Anti-Env study, capture and neutralization of Abs against $SHIV_{BaL.P4}$ inversely correlated with PVL supporting their role in the control of SHIV. Conversely, in the Anti-V2 study, the plasma Abs did not neutralize $SHIV_{BaL.P4}$, and their weak capture of the virus did not correlate with PVL. Antibody levels against V1V2 fusion proteins and V2 peptides were comparable between controllers and non-controllers, as were ADCC activities against SHIV-infected target cells and phagocytosis of gp120-coated beads. These studies revealed partial control of heterologous $SHIV_{BaL.P4}$ infection that was not correlated with ADCC, phagocytosis, or V2 Abs in the plasma, suggesting that these responses may play limited roles in the prevention or control of tier 1 $SHIV_{BaL.P4}$ challenge.

Conclusions

RV144 was the first clinical trial to demonstrate a modest reduction in HIV-1 infection among vaccinees. The correlation between the high level of V2 Abs and the reduced risk of HIV-1 infection emphasized the potential protective role of these Abs. We analyzed the functions of V2 mAbs and vaccine-induced V2 Abs reported in published data, but the results of our search were not consistent with their protective effect. V2 mAbs bind to intact, infectious HIV-1 virions very weakly, suggesting that their functions are also weak. Indeed, V2 Abs are considered as non-neutralizing because V2 mAbs can weakly neutralize only tier 1 viruses, but not tier 2 viruses, which are generally the viruses that are transmitted. Passive transfer of one V2 mAb to rhesus macaques decreased plasma viral load but did not protect against SHIV challenge. Similar effects were achieved by other non-neutralizing mAbs against CD4bs, C1 of gp120, and gp41, which in contrast to bnAbs, did not protect the animals. V2 Abs can mediate ADCC, but on average, their activity is weak, and immune sera from immunized animals have sporadic activity. Phagocytosis is mediated by V2 mAbs, but again weakly, and they do not dominate other non-neutralizing CD4bs and V3 mAbs. The unusual property of V2 mAbs, inhibition of gp120 or V2 peptide binding to α4β7 integrin-expressing cells, was not effective *in vivo* and could not sustain suppressed plasma viremia after ART discontinuation in rhesus macaques or clinical trials when anti-α4β7 mAb or two SIV V2 mAbs were tested. Protection experiments in rhesus macaques showed a correlation between V2 Abs and the outcome of infection in SIV models, possibly due to the different structures of the V2 region, but in the HIV model, this was very rarely observed. Finally, as shown in the ISV conference abstract, our studies revealed that titer of vaccine-induced plasma V2 Abs was comparable in controllers and non-controllers of SHIV challenge and did not correlate with plasma viral load. The results suggested that V2 Abs in this model do not protect against SHIV challenge. Given that the titer of plasma V2 Abs in chronically HIV-infected individuals is strongly correlated with the titer of Abs against gp120, V3, and gp41, the level and correlation of V2 Abs could be a surrogate marker of other Ab responses, as suggested in several previous papers.

Disclosure of potential conflicts of interest

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ORCID

Miroslaw K. Gorny http://orcid.org/0000-0002-2714-8780

References

1. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009;361:2209–20. doi:10.1056/NEJMoa0908492.
2. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. 2012;366:1275–86. doi:10.1056/NEJMoa1113425.
3. He Y, Honnen WJ, Krachmarov CP, Burkhart M, Kayman SC, Corvalan J, Pinter A. Efficient isolation of novel human monoclonal antibodies with neutralizing activity against HIV-1 from transgenic mice expressing human Ig loci. J Immunol. 2002;169:595–605. doi:10.4049/jimmunol.169.1.595.
4. Liu L, Li L, Nanfack A, Mayr LM, Soni S, Kohutnicki A, Agyingi L, Wang XH, Tuen M, Shao Y, et al. Anti-V2 antibody deficiency in individuals infected with HIV-1 in Cameroon. Virology. 2019;529:57–64. doi:10.1016/j.virol.2019.01.011.
5. Zolla-Pazner S, deCamp A, Gilbert PB, Williams C, Yates NL, Williams WT, Howington R, Fong Y, Morris DE, Soderberg KA, et al. Vaccine-induced IgG antibodies to V1V2 regions of multiple HIV-1 subtypes correlate with decreased risk of HIV-1 infection. PLoS One. 2014;9:e87572. doi:10.1371/journal.pone.0087572.
6. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, Shen X, Tomaras GD, Turk E, Imholte G, Eckler L, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. PLoS One. 2013;8:e75665. doi:10.1371/journal.pone.0075665.
7. Di Mascio M, Lifson JD, Srinivasula S, Kim I, DeGrange P, Keele BF, Belli AJ, Reimann KA, Wang Y, Proschan M, et al. Evaluation of an antibody to alpha4beta7 in the control of SIVmac239-nef-stop infection. Science. 2019;365:1025–29. doi:10.1126/science.aav6695.
8. Abbink P, Mercado NB, Nkolola JP, Peterson RL, Tuyishime H, McMahan K, Moseley ET, Borducchi EN, Chandrashekar A, Bondzie EA, et al. Lack of therapeutic efficacy of an antibody to

alpha4beta7 in SIVmac251-infected rhesus macaques. Science. 2019;365:1029–33. doi:10.1126/science.aaw8562.
9. Iwamoto N, Mason RD, Song K, Gorman J, Welles HC, Arthos J, Cicala C, Min S, King HAD, Belli AJ, et al. Blocking alpha4beta7 integrin binding to SIV does not improve virologic control. Science. 2019;365:1033–36. doi:10.1126/science.aaw7765.
10. Sneller MC, Clarridge KE, Seamon C, Shi V, Zorawski MD, Justement JS, Blazkova J, Huiting ED, Proschan MA, Mora JR, et al. An open-label phase 1 clinical trial of the anti-alpha4beta7 monoclonal antibody vedolizumab in HIV-infected individuals. Sci Transl Med. 2019;11:eaax3447. doi:10.1126/scitranslmed.aax3447.
11. McLellan JS, Pancera M, Carrico C, Gorman J, Julien JP, Khayat R, Louder R, Pejchal R, Sastry M, Dai K, et al. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature. 2011;480:336–43. doi:10.1038/nature10696.
12. de Taeye SW, Go EP, Sliepen K, de la Pena AT, Badal K, Medina-Ramirez M, Lee WH, Desaire H, Wilson IA, Moore JP, et al. Stabilization of the V2 loop improves the presentation of V2 loop-associated broadly neutralizing antibody epitopes on HIV-1 envelope trimers. J Biol Chem. 2019;294:5616–31. doi:10.1074/jbc.RA118.005396.
13. Shen X, Duffy R, Howington R, Cope A, Sadagopal S, Park H, Pal R, Kwa S, Ding S, Yang OO, et al. Vaccine-induced linear epitope-specific antibodies to Simian immunodeficiency virus SIVmac239 envelope are distinct from those induced to the human immunodeficiency virus type 1 envelope in nonhuman primates. J Virol. 2015;89:8643–50. doi:10.1128/JVI.03635-14.
14. Jiang X, Totrov M, Li W, Sampson JM, Williams C, Lu H, Wu X, Lu S, Wang S, Zolla-Pazner S, et al. Rationally designed immunogens targeting HIV-1 gp120 v1v2 induce distinct conformation-specific antibody responses in rabbits. J Virol. 2016;90:11007–19. doi:10.1128/JVI.01409-16.
15. Zolla-Pazner S, deCamp AC, Cardozo T, Karasavvas N, Gottardo R, Williams C, Morris DE, Tomaras G, Rao M, Billings E, et al. Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. PLoS One. 2013;8:e53629. doi:10.1371/journal.pone.0053629.
16. Karasavvas N, Billings E, Rao M, Williams C, Zolla-Pazner S, Bailer RT, Koup RA, Madnote S, Arworn D, Shen X, et al. The Thai Phase III HIV Type 1 vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. AIDS Res Hum Retroviruses. 2012;28:1444–57. doi:10.1089/aid.2012.0103.
17. Gray GE, Huang Y, Grunenberg N, Laher F, Roux S, Andersen-Nissen E, De Rosa SC, Flach B, Randhawa AK, Jensen R, et al. Immune correlates of the Thai RV144 HIV vaccine regimen in South Africa. Sci Transl Med. 2019;11:eaax1880. doi:10.1126/scitranslmed.aax1880.
18. Laman JD, Claassen E, Noelle RJ. Functions of CD40 and Its Ligand, gp39 (CD40L). Crit Rev Immunol. 2017;37:371–420. doi:10.1615/CritRevImmunol.v37.i2-6.100.
19. Rouphael NG, Morgan C, SS L, Jensen R, Sanchez B, Karuna S, Swann E, Sobieszczyk ME, Frank I, Wilson GJ, et al., Team HP, the NHIVVTN. DNA priming and gp120 boosting induces HIV-specific antibodies in a randomized clinical trial. J Clin Invest. 2019;129:4769–85. doi:10.1172/JCI128699.
20. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, Liu J, Abbink P, Maxfield LF, Seaman MS, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell. 2013;155:531–39. doi:10.1016/j.cell.2013.09.061.
21. Barouch DH, Tomaka FL, Wegmann F, Stieh DJ, Alter G, Robb ML, Michael NL, Peter L, Nkolola JP, Borducchi EN, et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). Lancet. 2018;392:232–43. doi:10.1016/S0140-6736(18)31364-3.
22. Bradley T, Pollara J, Santra S, Vandergrift N, Pittala S, Bailey-Kellogg C, Shen X, Parks R, Goodman D, Eaton A, et al. Pentavalent HIV-1 vaccine protects against simian-human immunodeficiency virus challenge. Nat Commun. 2017;8:15711. doi:10.1038/ncomms15711.
23. Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, Iampietro MJ, SanMiguel A, Seaman MS, Ferrari G, et al. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature. 2012;482:89–93. doi:10.1038/nature10766.
24. Roederer M, Keele BF, Schmidt SD, Mason RD, Welles HC, Fischer W, Labranche C, Foulds KE, Louder MK, Yang ZY, et al. Immunological and virological mechanisms of vaccine-mediated protection against SIV and HIV. Nature. 2014;505:502–08. doi:10.1038/nature12893.
25. Singh S, Ramirez-Salazar EG, Doueiri R, Valentin A, Rosati M, Hu X, Keele BF, Shen X, Tomaras GD, Ferrari G, et al. Control of heterologous Simian immunodeficiency virus SIVsmE660 infection by DNA and protein coimmunization regimens combined with different toll-like-receptor-4-based adjuvants in Macaques. J Virol. 2018;92:e00281–18. doi:10.1128/JVI.00281-18.
26. Kwa S, Sadagopal S, Shen X, Hong JJ, Gangadhara S, Basu R, Victor B, Iyer SS, LaBranche CC, Montefiori DC, et al. CD40L-adjuvanted DNA/modified vaccinia virus Ankara simian immunodeficiency virus (SIV) vaccine enhances protection against neutralization-resistant mucosal SIV infection. J Virol. 2015;89:4690–95. doi:10.1128/JVI.03527-14.
27. Nyambi PN, Mbah HA, Burda S, Williams C, Gorny MK, Nadas A, Zolla-Pazner S. Conserved and exposed epitopes on intact, native, primary human immunodeficiency virus type 1 virions of group M. J Virol. 2000;74:7096–107. doi:10.1128/JVI.74.15.7096-7107.2000.
28. Liu P, Yates NL, Shen X, Bonsignori M, Moody MA, Liao HX, Fong Y, Alam SM, Overman RG, Denny T, et al. Infectious virion capture by HIV-1 gp120-specific IgG from RV144 vaccinees. J Virol. 2013;87:7828–36. doi:10.1128/JVI.02737-12.
29. Gorny MK, Moore JP, Conley AJ, Karwowska S, Sodroski J, Williams C, Burda S, Boots LJ, Zolla-Pazner S. Human anti-V2 monoclonal antibody that neutralizes primary but not laboratory isolates of human immunodeficiency virus type 1. J Virol. 1994;68:8312–20. doi:10.1128/JVI.68.12.8312-8320.1994.
30. Gorny MK, Pan R, Williams C, Wang XH, Volsky B, O'Neal T, Spurrier B, Sampson JM, Li L, Seaman MS, et al. Functional and immunochemical cross-reactivity of V2-specific monoclonal antibodies from HIV-1-infected individuals. Virology. 2012;427:198–207. doi:10.1016/j.virol.2012.02.003.
31. Corti D, Langedijk JP, Hinz A, Seaman MS, Vanzetta F, Fernandez-Rodriguez BM, Silacci C, Pinna D, Jarrossay D, Balla-Jhagjhoorsingh S, et al. Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. PLoS One. 2010;5:e8805. doi:10.1371/journal.pone.0008805.
32. Liao HX, Bonsignori M, Alam SM, McLellan JS, Tomaras GD, Moody MA, Kozink DM, Hwang KK, Chen X, Tsao CY, 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. doi:10.1016/j.immuni.2012.11.011.
33. Li L, Wang XH, Williams C, Volsky B, Steczko O, Seaman MS, Luthra K, Nyambi P, Nadas A, Giudicelli V, et al. A broad range of mutations in HIV-1 neutralizing human monoclonal antibodies specific for V2, V3, and the CD4 binding site. Mol Immunol. 2015;66:364–74. doi:10.1016/j.molimm.2015.04.011.
34. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, McLinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, 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. doi:10.1093/infdis/jis367.
35. Alam SM, Liao HX, Tomaras GD, Bonsignori M, Tsao CY, Hwang KK, Chen H, Lloyd KE, Bowman C, Sutherland L, et al. Antigenicity and immunogenicity of RV144 vaccine AIDSVAX clade E envelope immunogen is enhanced by a gp120 N-terminal deletion. J Virol. 2013;87:1554–68. doi:10.1128/JVI.00718-12.

36. Hessell AJ, Powell R, Jiang X, Luo C, Weiss S, Dussupt V, Itri V, Fox A, Shapiro MB, Pandey S, et al. Multimeric epitope-scaffold HIV vaccines target V1V2 and differentially tune polyfunctional antibody responses. Cell Rep. 2019;28:877–895 e6. doi:10.1016/j.celrep.2019.06.074.
37. Davis KL, Gray ES, Moore PL, Decker JM, Salomon A, Montefiori DC, Graham BS, Keefer MC, Pinter A, Morris L, et al. High titer HIV-1 V3-specific antibodies with broad reactivity but low neutralizing potency in acute infection and following vaccination. Virology. 2009;387:414–26. doi:10.1016/j.virol.2009.02.022.
38. Escolano A, Gristick HB, Abernathy ME, Merkenschlager J, Gautam R, Oliveira TY, Pai J, West AP Jr., Barnes CO, Cohen AA, et al. Immunization expands B cells specific to HIV-1 V3 glycan in mice and macaques. Nature. 2019;570:468–73. doi:10.1038/s41586-019-1250-z.
39. Saunders KO, Wiehe K, Tian M, Acharya P, Bradley T, Alam SM, Go EP, Scearce R, Sutherland L, Henderson R, et al. Targeted selection of HIV-specific antibody mutations by engineering B cell maturation. Science. 2019;366:eaay7199. doi:10.1126/science.aay7199.
40. Steichen JM, Lin YC, Havenar-Daughton C, Pecetta S, Ozorowski G, Willis JR, Toy L, Sok D, Liguori A, Kratochvil S, et al. A generalized HIV vaccine design strategy for priming of broadly neutralizing antibody responses. Science. 2019;366:eaax4380. doi:10.1126/science.aax4380.
41. Hessell AJ, Shapiro MB, Powell R, Malherbe DC, McBurney SP, Pandey S, Cheever T, Sutton WF, Kahl C, Park B, et al. Reduced cell-associated DNA and improved viral control in Macaques following passive transfer of a single anti-V2 monoclonal antibody and repeated simian/human immunodeficiency virus challenges. J Virol. 2018;92:e02198–17. doi:10.1128/JVI.02198-17.
42. Moog C, Dereuddre-Bosquet N, Teillaud JL, Biedma ME, Holl V, Van Ham G, Heyndrickx L, Van Dorsselaer A, Katinger D, Vcelar B, et al. Protective effect of vaginal application of neutralizing and nonneutralizing inhibitory antibodies against vaginal SHIV challenge in macaques. Mucosal Immunol. 2014;7:46–56. doi:10.1038/mi.2013.23.
43. Burton DR, Hessell AJ, Keele BF, Klasse PJ, Ketas TA, Moldt B, Dunlop DC, Poignard P, Doyle LA, Cavacini L, 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 U S A. 2011;108:11181–86. doi:10.1073/pnas.1103012108.
44. Santra S, Tomaras GD, Warrier R, Nicely NI, Liao HX, Pollara J, Liu P, Alam SM, Zhang R, Cocklin SL, et al. Human non-neutralizing HIV-1 envelope monoclonal antibodies limit the number of founder viruses during SHIV mucosal infection in rhesus Macaques. PLoS Pathog. 2015;11:e1005042. doi:10.1371/journal.ppat.1005042.
45. Igarashi T, Brown C, Azadegan A, Haigwood N, Dimitrov D, Martin MA, Shibata R. Human immunodeficiency virus type 1 neutralizing antibodies accelerate clearance of cell-free virions from blood plasma. Nat Med. 1999;5:211–16. doi:10.1038/5576.
46. Mayr LM, Decoville T, Schmidt S, Laumond G, Klingler J, Ducloy C, Bahram S, Zolla-Pazner S, Moog C. Non-neutralizing antibodies targeting the V1V2 domain of HIV exhibit strong antibody-dependent cell-mediated cytotoxic activity. Sci Rep. 2017;7:12655. doi:10.1038/s41598-017-12883-6.
47. Musich T, Li L, Liu L, Zolla-Pazner S, Robert-Guroff M, Gorny MK. Monoclonal antibodies specific for the V2, V3, CD4-binding site, and gp41 of HIV-1 mediate phagocytosis in a dose-dependent manner. J Virol. 2017;91:e02325–16. doi:10.1128/JVI.02325-16.
48. Zolla-Pazner S, Powell R, Yahyaei S, Williams C, Jiang X, Li W, Lu S, Wang S, Upadhyay C, Hioe CE, et al. Rationally designed vaccines targeting the V2 region of HIV-1 gp120 induce a focused, cross-clade-reactive, biologically functional antibody response. J Virol. 2016;90:10993–1006. doi:10.1128/JVI.01403-16.
49. Duerr R, Gorny MK. V2-specific antibodies in HIV-1 vaccine research and natural infection: controllers or surrogate markers. Vaccines (Basel). 2019;7:E82. doi:10.3390/vaccines7030082.
50. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, Wei D, Xiao Z, Veenstra TD, Conrad TP, Lempicki RA, et al. HIV-1 envelope protein binds to and signals through integrin alpha4-beta7, the gut mucosal homing receptor for peripheral T cells. Nat Immunol. 2008;9:301–09. doi:10.1038/ni1566.
51. Nakamura GR, Fonseca DP, O'Rourke SM, Vollrath AL, Berman PW. Monoclonal antibodies to the V2 domain of MN-rgp120: fine mapping of epitopes and inhibition of alpha4-beta7 binding. PLoS One. 2012;7:e39045. doi:10.1371/journal.pone.0039045.
52. Lertjuthaporn S, Cicala C, Van Ryk D, Liu M, Yolitz J, Wei D, Nawaz F, Doyle A, Horowitch B, Park C, et al. Select gp120 V2 domain specific antibodies derived from HIV and SIV infection and vaccination inhibit gp120 binding to alpha4beta7. PLoS Pathog. 2018;14:e1007278. doi:10.1371/journal.ppat.1007278.
53. Cicala C, Martinelli E, McNally JP, Goode DJ, Gopaul R, Hiatt J, Jelicic K, Kottilil S, Macleod K, O'Shea A, et al. The integrin alpha4beta7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. Proc Natl Acad Sci U S A. 2009;106:20877–82. doi:10.1073/pnas.0911796106.
54. Byrareddy SN, Arthos J, Cicala C, Villinger F, Ortiz KT, Little D, Sidell N, Kane MA, Yu J, Jones JW, et al. Sustained virologic control in SIV+ macaques after antiretroviral and alpha4beta7 antibody therapy. Science. 2016;354:197–202. doi:10.1126/science.aag1276.
55. Ling L, Wu T, To KKW, Cheung KW, Lui KOL, Niu M, Lam KS, Wang CC, Li J, Wang H, et al. Vedolizumab-mediated integrin alpha4-beta7 blockade does not control HIV-1SF162 rebound after combination antiretroviral therapy interruption in humanized mice. AIDS. 2019;33:F1–F12. doi:10.1097/QAD.0000000000002149.
56. Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, deCamp AC, Carrico C, Menis S, Magaret CA, et al. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. Nature. 2012;490:417–20. doi:10.1038/nature11519.
57. deCamp AC, Rolland M, Edlefsen PT, Sanders-Buell E, Hall B, Magaret CA, Fiore-Gartland AJ, Juraska M, Carpp LN, Karuna ST, et al. Sieve analysis of breakthrough HIV-1 sequences in HVTN 505 identifies vaccine pressure targeting the CD4 binding site of Env-gp120. PLoS One. 2017;12:e0185959. doi:10.1371/journal.pone.0185959.
58. Malherbe DC, Mendy J, Vang L, Barnette PT, Reed J, Lakhashe SK, Owuor J, Gach JS, Legasse AW, Axthelm MK, et al. Combination adenovirus and protein vaccines prevent infection or reduce viral burden after heterologous clade c simian-human immunodeficiency virus mucosal challenge. J Virol. 2018;92:e01092–17. doi:10.1128/JVI.01092-17.
59. Pegu P, Vaccari M, Gordon S, Keele BF, Doster M, Guan Y, Ferrari G, Pal R, Ferrari MG, Whitney S, et al. Antibodies with high avidity to the gp120 envelope protein in protection from simian immunodeficiency virus SIV(mac251) acquisition in an immunization regimen that mimics the RV-144 Thai trial. J Virol. 2013;87:1708–19. doi:10.1128/JVI.02544-12.
60. Gordon SN, Doster MN, Kines RC, Keele BF, Brocca-Cofano E, Guan Y, Pegu P, Liyanage NP, Vaccari M, Cuburu N, et al. Antibody to the gp120 V1/V2 loops and CD4+ and CD8+ T cell responses in protection from SIVmac251 vaginal acquisition and persistent viremia. J Immunol. 2014;193:6172–83. doi:10.4049/jimmunol.1401504.
61. Gordon SN, Liyanage NP, Doster MN, Vaccari M, Vargas-Inchaustegui DA, Pegu P, Schifanella L, Shen X, Tomaras GD, Rao M, et al. Boosting of ALVAC-SIV vaccine-primed Macaques with the CD4-SIVgp120 fusion protein elicits antibodies to V2 associated with a decreased risk of SIVmac251 acquisition. J Immunol. 2016;197:2726–37. doi:10.4049/jimmunol.1600674.

62. Vaccari M, Gordon SN, Fourati S, Schifanella L, Liyanage NP, Cameron M, Keele BF, Shen X, Tomaras GD, Billings E, et al. Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition. Nat Med. 2016;22:762–70. doi:10.1038/nm.4105.
63. Vaccari M, Fourati S, Gordon SN, Brown DR, Bissa M, Schifanella L, Silva de Castro I, Doster MN, Galli V, Omsland M, et al. HIV vaccine candidate activation of hypoxia and the inflammasome in CD14(+) monocytes is associated with a decreased risk of SIVmac251 acquisition. Nat Med. 2018;24:847–56. doi:10.1038/s41591-018-0025-7.
64. Sui Y, Lewis GK, Wang Y, Berckmueller K, Frey B, Dzutsev A, Vargas-Inchaustegui D, Mohanram V, Musich T, Shen X, et al. Mucosal vaccine efficacy against intrarectal SHIV is independent of anti-Env antibody response. J Clin Invest. 2019;129:1314–28. doi:10.1172/JCI122110.
65. Su B, Dispinseri S, Iannone V, Zhang T, Wu H, Carapito R, Bahram S, Scarlatti G, Moog C. Update on Fc-mediated antibody functions against HIV-1 beyond neutralization. Front Immunol. 2019;10:2968. doi:10.3389/fimmu.2019.02968.
66. Bohl C, Bowder D, Thompson J, Abrahamyan L, Gonzalez-Ramirez S, Mao Y, Sodroski J, Wood C, Xiang SH. A twin-cysteine motif in the V2 region of gp120 is associated with SIV envelope trimer stabilization. PLoS One. 2013;8:e69406. doi:10.1371/journal.pone.0069406.
67. Hessell AJDM, Barnette P, Pandey S, Sutton W, Liu L, Li L, Kong X-P, Haigwood NL, Gorny MK. 2018. Tight control of viremia after SHIV BaL.P4 challenge in rhesus macaques immunized with HIV vaccine mimicking the RV144 regimen. Keystone Symposia, HIV Vaccines; 2018 January 28-February 2; Banff, Canada. Abstract.
68. Hessell AJDM, Barnette P, Pandey S, Sutton W, Liu L, Li L, Kong X-P, Haigwood NL, Gorny MK. 2018. Tight control of SHIV BaL.P4 challenge in rhesus macaques co-immunized with DNA and protein HIV vaccine regimen. HIV Research for Prevention; 2018 Oct 21-25; Madrid, Spain. Abstract.
69. Hessell AJDM, Barnette P, Pandey S, Sutton W, Li L, Tuen M, Gach JS, Forthal DN, Duerr R, Jiang X, et al. 2019. Tight control of SHIV challenge in macaques with vaccine-induced neutralizing and non-neutralizing anti-V2 antibodies. Keystone Symposia, HIV Vaccines; 2019 Mar 21-24; Whistler, Canada. Abstract.
70. Li LDM, Barnette P, Pandey S, Sutton W, Spencer D, Gach JS, Hunegnaw R, Tuen M, Jiang X, Shao Y, et al.. 2019. Impact of vaccine-induced anti-V2 antibodies on virus control in SHIVBaL.P4 challenged rhesus macaques. International Society for Vaccines, Annual Congress; 2019 Oct 27-29; Ghent, Belgium. Abstract.