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Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies

Andrew T. McGuire^{#1,†}, Anita M. Dreyer^{#1,‡}, Sara Carbonetti¹, Adriana Lippy¹, Jolene Glenn^{1,†}, Johannes F. Scheid², Hugo Mouquet³, and Leonidas Stamatatos^{1,4,†,§}

¹Seattle Biomedical Research Institute, Seattle, WA 98109, USA.

²Laboratory of Molecular Immunology, The Rockefeller University, New York, NY 10065, USA.

³Laboratory of Humoral Response to Pathogens, Department of Immunology, Institut Pasteur and CNRS-URA 1961, 75015 Paris, France.

⁴Department of Global Health, University of Washington, Seattle, WA 98109, USA.

These authors contributed equally to this work.

Abstract

Some HIV-infected individuals develop broadly neutralizing antibodies (bNAbs), whereas most develop antibodies that neutralize only a narrow range of viruses (nNAbs). bNAbs, but not nNAbs, protect animals from experimental infection and are likely a key component of an effective vaccine. nNAbs and bNAbs target the same regions of the viral envelope glycoprotein (Env), but for reasons that remain unclear only nNAbs are elicited by Env immunization. We show that in contrast to germline-reverted (gl) bNAbs, glNAbs recognized diverse recombinant Envs. Moreover, owing to binding affinity differences, nNAb B cell progenitors had an advantage in becoming activated and internalizing Env compared with bNAb B cell progenitors. We then identified an Env modification strategy that minimized the activation of nNAb B cells targeting epitopes that overlap those of bNAbs.

During HIV-1 infection, the viral envelope glycoprotein (Env) elicits a polyclonal antibody response that targets diverse epitopes (1, 2). Antibodies that display narrow breadth of neutralization (narrow neutralizing antibodies; nNAbs) develop during the first months of infection whereas those capable of neutralizing heterologous viruses (broadly neutralizing antibodies; bNAbs) develop several years later in ~10 to 30% of HIV-1–positive individuals (3). bNAbs isolated from HIV-1–infected patients are more protective than nNAbs in experimental HIV-1/SHIV infection (4) and will likely be a key component of an effective HIV-1 vaccine. Even though nNAbs and bNAbs target the same regions of Env (2, 5–7), recombinant Env (rEnv) immunogens are poorly recognized by germline-reverted (gl)

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§Corresponding author. lstatata@fhcrc.org.

†Present address: Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.

‡Present address: GlycoVaxyn, Grabenstrasse 3, CH-8952 Schlieren, Switzerland.

SUPPLEMENTARY MATERIALS www.sciencemag.org/content/346/6215/1380/suppl/DC1 Materials and Methods Author Contributions Figs. S1 to S9 Tables S1 to S3 References (32–43)

bNAbs (glbNAbs) and their corresponding B cell receptors (BCRs) (5, 8–21), suggesting that the lack of bNAb generation during immunization may be due to inefficient stimulation of naïve bNAb BCR progenitors (17, 20). In contrast, little is known about the recognition of rEnv by the naïve BCR progenitors of nNAbs. Understanding why B cell responses against nNAb epitopes dominate over those targeted by bNAbs in the context of rEnv immunization will inform on basic immunological mechanisms of epitope competition and provide new information relevant to the development of an effective HIV-1 vaccine.

Here we investigated whether glnNAbs from distinct clonal lineages that targeted the CD4-binding site (BS) and V3 regions of Env (2) also display minimal rEnv recognition. Amino acid differences between the mutated and gl sequences of nNAbs range from 2.4 to 7.3% for the heavy chains and 2.7 to 5.6% for the light chains for the nNAb CD4-BS antibodies (table S1 and fig. S1). In contrast, prototypic CD4-BS bNAbs, VRC01 (33.9% heavy, 23% light), NIH45-46 (a clonal relative of VRC01; 39.8% heavy, 26.1% light), b12 (21% heavy, 19% light), 8ANC131 (33% heavy, 24% light), and CH103 (12.7% heavy, 10% light) are more mutated (5, 8, 16, 22). The anti-V3 nNAbs are more mutated (11.6 to 21.6% heavy, 9.7 to 13.8% light) than the anti-CD4-BS nNAbs.

In contrast to the anti-CD4-BS glbNAbs, which do not bind rEnv (5, 8, 16, 17, 20) (table S2), glnNAbs displayed broad Env recognition (from 51 to 100%) (table S2). The binding affinities of the glnNAbs were generally weaker than those of the corresponding mutated antibodies, owing to increased off rates in most cases (fig. S2). Whereas the glVRC01 class bNAbs were unable to neutralize any of the viruses tested, three of the five glnNAbs exhibited neutralizing activity against tier 1 viruses (table S3). Overall, we conclude that the glnNAbs and glbNAbs recognize the CD4-BS on soluble and virion-associated Env differently (23, 24). Two of the three anti-V3 glnNAbs displayed neutralizing activity against several tier 1 viruses (table S3).

We next investigated whether B cells stably expressing glnNAb and glVRC01-class BCRs (fig. S3) could become activated by (Fig. 1A) and internalize (Fig. 1B) rEnv derived from clades A, B, and C. As previously reported, none of the rEnvs tested activated glVRC01-class B cells (17, 20); however, they did activate glnNAb B cells targeting either the CD4-BS or V3 (Fig. 1A). Similarly, glnNAb B cells readily internalized diverse rEnvs, whereas glVRC01 class B cells did not (Fig. 1B). Combined, the above results indicate that rEnv immunogens can activate naïve nNAb B cells but not naïve VRC01-class B cells.

We recently reported that the disruption of three N-linked glycosylation sites (NLGS)—N276D, N460D, and N463D—on the clade C 426c rEnv (herein called 426c.NLGS.TM) confers binding to and activation of glNIH45-46 and glVRC01 B cells (17). In contrast, wild-type (WT) 426c Env is recognized by only the glnNAbs (table S2). gl1-154, gl1-695, gl1-732, and gl4-341 nNAbs inhibited binding of glNIH45-46 to 426c.NLGS.TM (fig. S5A), an indication that the epitopes of the anti-CD4-BS glnNAbs used here overlap those of the VRC01-class glbNAbs. The anti-V3 gl2-59 monoclonal antibody (mAb) caused a modest decrease in the binding of glNIH45-46 (fig. S5A). The gl1-676, gl1-79, and gl2-1261 antibodies, which do not bind 426c.NLGS.TM (Table 1), had no effect on the binding of glNIH45-46 (fig. S5A).

In germinal centers (GCs), B cells expressing higher-affinity BCRs selectively expand, whereas lower-affinity B cells are eliminated (25). The anti-CD4-BS gl1-154, gl1-695, gl1-732, and gl4-341, and the anti-V3 gl2-59 bound 426c.NLGS.TM with a higher apparent affinity than glNIH45-46 (Table 1). glVRC01 bound more strongly to 426c.NLGS.TM than gl1-154 and gl1-695, but had a slightly lower apparent affinity than the gl4-341 and gl1-732 mAbs. The gl2-59 V3-directed mAb bound more strongly to 426c.NLGS.TM than any of the glCD4-BS Abs, consistent with the higher propensity of gl2-59 B cells to be activated and take up rEnv (Fig. 1). We anticipate that upon immunization with the 426c.NLGS.TM, naïve 2-59-like B cells, or B cells that target immunodominant epitopes on the variable regions of Env (V1, V2 and V3), would have an advantage over naïve VRC01-class B cells in becoming activated, taking up antigen, and obtaining T cell help.

To investigate the effect that differences in binding affinity have on the relative activation of B cells expressing different anti-Env BCRs, we developed an assay that allows for the concurrent monitoring of B cell activation in distinct B cell populations (fig. S6). As expected, WT 426c activated glNAb B cells (Fig. 2A top row, and Fig. 2B), but not glVRC01-class B cells (Fig. 2A top row, and Fig. 2B). 426c.NLGS.TM activated gl VRC01-class B cells, but with the exception of gl1-154, it activated glNAb B cells more strongly (Fig. 2A, middle row). 426c.NLGS.TM activated gl1-695 more strongly than glNIH45-46 and comparably to glVRC01 (Fig. 2A, middle row, and Fig. 2B).

Because 426c.NLGS.TM showed the strongest activation of gl2-59 B cells, we aimed to abolish activation of B cells targeting the V1, V2, and V3 regions of 426c.NLGS.TM, by deleting these regions to create 426c.NLGS.TM. V1-3. As expected, 426c.NLGS.TM. V1-3 did not bind to gl2-59 mAb (Table 1) and did not activate gl2-59 B cells (Fig. 2, A and B). With the exception of glNIH45-46, deletion of the variable loops reduced the apparent binding affinities of the anti-CD4-BS antibodies tested (Table 1). Consistent with these changes in binding, 426c.NLGS.TM. V1-3 activated glNIH45-46 B cells more robustly than 426c.NLGS.TM (Fig. 2, A and B). The relative activation of all but one anti-CD4-BS glNAb B cell (gl4-341) by 426c.NLGS.TM. V1-3 was less than that observed for glVRC01-class B cells (Fig. 2, A and B). gl4-341 B cells were activated comparably to glVRC01 class B cells, although the activation was reduced relative to that observed with WT 426c and 426c.NLGS.TM (Fig. 2, A and B). At lower concentrations of 426c.NLGS.TM. V1-3, only glVRC01-class and gl4-341 B cells were activated (fig. S7). To represent a more physiological B cell repertoire, we challenged glVRC01-class B cells mixed with an excess of pooled glNAb, and non-Env reactive B cells (fig. S8). WT 426c only activated nNAb B cells, and although 426c.NLGS.TM could activate the minority glVRC01-class B cells, a preferential activation of the nNAb B cell pool was observed. In contrast, 426c.NLGS.TM. V1-3 activated glVRC01-class B cells preferentially at higher concentrations and exclusively at lower concentrations (fig. S8). When the internalization of rEnv by B cells was quantitated, 426c.NLGS.TM was preferentially internalized by nNAb B cells, whereas 426c.NLGS.TM. V1-3 was preferentially internalized by glVRC01-class and gl4-341 B cells (Fig. 2C). The differential impact of removing the variable Env regions on the binding affinities, B cell activation, and antigen internalization for the bNAbs versus nNAbs to the CD4-BS is likely due to differences in the angle of approach to the CD4-BS

by these two types of antibodies (16, 22, 24, 26–29). Additionally, the nNAb may make contacts with portions of the variable loops.

Antibodies can enter GCs and inhibit BCR binding to an immunogen unless the affinity of the BCR for the immunogen is higher than for the soluble antibody (30). Through this feedback mechanism, higher-affinity antibodies hinder the ability of low-affinity, antigen-specific B cells to receive T cell help and thus select for higher-affinity B cell clones (30). Because the apparent affinities of gIVRC01-class bNAbs for 426c.NLGS.TM V1-3 are higher than those for the glnNAbs studied here (Table 1), we expect that such a feedback mechanism will provide a selective advantage to gIVRC01-class B cell responses if this rEnv is used as an immunogen. Inversely, owing to the higher apparent affinities of the glnNAbs for the 426c.NLGS.TM relative to the gIVRC01 class bNAbs (Table 1), we would expect this immunogen to drive the development of nNAb responses through the same antibody-feedback mechanism. Indeed, the binding of anti-CD4-BS nNAbs to 426c.NLGS.TM reduced the activation of gIVRC01-class B cells by that rEnv (Fig. 3A and fig. S9). In contrast, activation of gIVRC01 class B cells by 426c.NLGS.TM V1-3 rEnv was observed even in the presence of the g11-154, and g11-695 Abs (Fig. 3A and fig. S9). Thus, removal of the V1, V2, and V3 regions reduces the ability of some (but not all) anti-CD4 nNAbs to block rEnv activation of gIVRC01 class B cells.

Similar observations were made when the internalization of these two Envs by gIVRC01 class B cells was determined in the absence and presence of glnNAbs (Fig. 3, B and C). g1NIH45-46 B cells more readily internalized 426c.NLGS.TM V1-3 compared to 426c.NLGS.TM, even in the presence of inhibitory antibodies, with the exception of the g14-341 mAb (Fig. 3B, compare white and gray bars). Overall, we conclude that the presence of anti-CD4-BS glnNAbs can inhibit the activation of, and the antigen internalization by, gIVRC01-class B cells in response to the 426c.NLGS.TM, but that these effects are less pronounced when the variable regions V1, V2, and V3 are removed from this rEnv. Thus, selecting which immunogen is used during the priming phase of immunization (e.g., 426c.NLGS.TM versus 426c.NLGS.TM V1-3) will be critically important to the eventual elicitation of anti-CD4-BS bNAbs.

In sum, our study provides a mechanistic explanation as to why rEnv immunogens elicit narrow rather than broadly neutralizing antibody responses to the CD4-BS of Env. This is primarily due to the broad Env-recognition properties of glnNAb BCRs, which contrasts with the inefficiency by which bNAb BCRs recognize rEnv. Notably, we demonstrate that rational immunogen modifications can reduce (and in certain cases eliminate) the activation of naïve B cells that give rise to such nNAbs, while promoting the activation of naïve B cells that give rise to gIVRC01-class bNAbs, even when the epitope of these antibodies substantially overlap. As such, our results are relevant to efforts to elicit protective antibody responses against not only HIV-1 but potentially other pathogens as well.

Supplementary Material

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31. Cells expressing the g1-732 BCR did not internalize the clade A Q168a2 and Q461e2 rEnvs that activated B cells expressing that BCR. This discrepancy can be attributed to pHrodo labeling of these two rEnvs, which reduced the binding of the g1-732 mAb (fig. S4).

Fig. 1. Activation by and internalization of Env by glnNAb and gIVRC01-class B cells
(A) Calcium flux in B cells expressing the gIBCRs of CD4-BS specific nAbs (1-154, 1-676, 1-695, 1-732, 4-341), CD4-BS specific bAbs (NIH45-46 and VRC01), or V3-specific nAbs (1-79, 2-59, 2-1261) challenged with the indicated Env proteins at a 1 μ M final concentration. Black arrow indicates time of Env addition. **(B)** Quantitation of Env internalization in the above B cell lines. Bars represent the mean of two independent experiments (31). Clade A, B, and C rEnvs are colored shades of red, blue and green respectively in (A) and (B).

Fig. 2. Activation by and internalization of Env by glnNAb and gIVRC01-class B cells in response to modified Env immunogens

(A) glnIH45-46 or gIVRC01 B cells were mixed with the indicated glnNAb B cells. The pooled B cells were then challenged with WT 426c (top row), 426c.NLGS.TM (middle row), or 426c.NLGS.TM ΔV1-3 (bottom row) at a 1 μM final concentration. Calcium flux was monitored concurrently in both B cell lines with the strategy depicted in fig. S6. Black arrow indicates time of Env addition. (B) Area under the calcium flux curve determined from duplicate experiments in (A). Error bars represent SD from the mean of 10 (gIVRC01-class) or 4 replicates (other B cell lines). Green asterisks and blue stars over the bars indicate significant differences ($P < 0.05$) from glnIH45-46 and gIVRC01 B cells, respectively, for each protein using a two-tailed Student's t test. (C) Quantification of internalization of the indicated Envs by gIVRC01 class and glnNAb B cells. Error bars represent SD from the mean of two independent experiments each performed in duplicate ($n = 4$). Green astrices and blue stars represent significant differences ($P < 0.05$) from glnIH45-46 and gIVRC01, respectively, for each protein using a two-tailed Student's t test.

Fig. 3. B cell activation and antigen uptake of Env by gIVRC01-class B cells in the presence of soluble anti-HIV antibodies

(A) Calcium flux was monitored in glnIH45-46 (solid bars) or gIVRC01 (patterned bars) B cells incubated with 426c.NLGS.TM (white bars) or with 426c.NLGS.TM. V1-3 (gray bars) in the presence of equimolar concentrations of the indicated antibodies. Bars represent mean and SD of the area under the calcium flux response curve relative to that of Env in the absence of soluble mAb from three independent experiments. Asterisks represent a significant difference ($P < 0.05$) in the calcium flux response between 426c.NLGS.TM and 426c.NLGS.TM. V1-3 using a two-tailed Student's t test. Env internalization by glnIH45-46 (B) or gIVRC01 (C) B cells incubated with 426c.NLGS.TM (white bars) or 426c.NLGS.TM. V1-3 (gray bars) in the absence or presence of equimolar concentrations of the indicated nNAbs. Bars represent the mean of two independent experiments. Asterisk denotes significant difference ($P < 0.05$) in fluorescence compared to cells incubated with Env alone using a two-tailed Student's t test in (B) and (C). Dotted line corresponds to mean 426c.NLGS.TM internalization by B cells in the absence of Ab in (B) and (C).

Table 1

Binding kinetics of germline reverted antibodies to trimeric 426c Env gp140 variants measured by BLI.

Ab		426c NLGS.TM trimeric gp140			426c NLGS.TM trimeric V1 2 3		
		Apparent K_A (1/M)	k_{on} (1/Ms)	K_{off} (1/s)	Apparent K_A (1/M)	K_{on} (1/Ms)	K_{off} (1/s)
CD4-BS	gIPt4-341	2.85×10^8	2.96×10^4	1.04×10^{-4}	1.77×10^8	1.57×10^4	8.85×10^{-5}
	gIPt1-732	6.30×10^8	7.48×10^4	1.19×10^{-4}	2.65×10^8	9.71×10^3	3.66×10^{-5}
	gIPt 1-154	4.12×10^7	1.61×10^4	3.91×10^{-4}	3.31×10^6	7.73×10^3	5.88×10^{-4}
	gIPt 1-695	6.77×10^7	9.89×10^3	1.46×10^{-4}	2.24×10^7	4.66×10^3	2.08×10^{-4}
	gIPt 1-676	NA	NA	NA	NA	NA	NA
	gINI45-46	4.04×10^7	9.87×10^3	2.44×10^{-4}	3.52×10^8	1.40×10^4	3.98×10^{-5}
	gIVRC01	2.67×10^8	5.51×10^3	2.06×10^{-4}	2.64×10^8	9.07×10^3	3.43×10^{-5}
V3	gIPt1-79	NA	NA	NA	NA	NA	NA
	gIPt2-59	1.19×10^{11}	1.85×10^5	1.55×10^{-6}	NA	NA	NA
	gIPt2-1261	NA	NA	NA	NA	NA	NA

NA: binding was undetectable.