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Adaptation of HIV-1 envelope gp120 to humoral immunity at a population level

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By comparing HIV-1 variants from people who became infected at the beginning of the epidemic and from people who have recently contracted the virus, we observed an enhanced resistance of the virus to antibody neutralization over time, accompanied by an increase in the length of the variable loops and in the number of potential *N*-linked glycosylation sites on the HIV-1 envelope gp120 subunit. The enhanced neutralization resistance of HIV-1 in contemporary seroconverters coincided with the poorer elicitation of neutralizing antibody responses, which may have implications for vaccine design.

The majority of HIV-1–infected individuals develop neutralizing antibodies (NAbs) against the gp120 and gp41 subunits of the envelope glycoprotein (Env), which drive the rapid selection of antibody escape variants[1,2]. Env uses multiple mechanisms to escape from antibody neutralization, including amino acid substitutions, insertions in the variable domains of gp120[2–4] and increasing the number of glycan moieties on its outer surface[2,5,6].

Recently, evidence has been provided that HIV-1 adapts over time to host cellular immune responses by losing epitopes restricted by the most abundant human leukocyte antigen types in a population[7]. Here we tested the hypothesis that, over the course of the epidemic, HIV-1 has also become more resistant to antibody neutralization.

We compared the neutralization sensitivity of clonal subtype B HIV-1 variants that we isolated within 4.5 months after seroconversion from individuals who seroconverted either between 1985 and 1989 (historical seroconverters) or between 2003 and 2006 (contemporary seroconverters) (**Supplementary Table 1** and **Supplementary Methods**). Sensitivity to neutralization by two HIVIg preparations, which are pools of purified IgG obtained in 1995 from chronically HIV-1–infected individuals[8], tended to be higher for viruses from historical seroconverters than from contemporary seroconverters (HIVIg batch 1, $P = 0.020$, **Fig. 1a**; HIVIg batch 2, $P = 0.089$, **Fig. 1b**). As this difference in neutralization sensitivity may result from the fact that the antibodies in these preparations were more specific for historical viruses because they were elicited in response to virus variants that were circulating more than 15 years ago, we also determined the sensitivity of historical and contemporary viruses to neutralization by antibodies that were elicited by contemporary viruses. Individual sera from 22 unrelated contemporary HIV-infected individuals had a higher mean neutralizing antibody titer against viruses from historical seroconverters than from contemporary seroconverters ($P = 0.050$; **Fig. 1c**), which was particularly evident for serum from subject M31281 ($P = 0.0001$; **Fig. 1d**). These observations excluded the possibility that the lower neutralization sensitivity of contemporary HIV-1 variants was related to the calendar time from which the HIVIg preparations or the sera originated.

HIV-1 variants from historical seroconverters were also more sensitive to neutralization by the CD4 binding site–directed monoclonal antibody (mAb) b12 ($P = 0.005$; **Fig. 1e**) but not the glycan-binding mAb 2G12 or the gp41-directed mAb 2F5 (**Fig. 1f**,**g**). In contrast, we observed a trend toward increased sensitivity of contemporary viruses to neutralization by gp41-directed mAb 4E10 ($P = 0.070$; **Fig. 1h**). Altogether, these data suggest that HIV-1 may have evolved toward a more neutralization-resistant phenotype and, in particular, toward resistance to CD4 binding site–directed neutralizing activity.

The mechanism underlying the increased resistance to antibody neutralization could potentially influence immunogenicity and the development of NAb responses in newly infected individuals. To study this, we analyzed sera that we obtained approximately 3 years after seroconversion from individuals who seroconverted in 1985–1986 ($n = 31$), 1987–1989 ($n = 25$) or 1990–1996 ($n = 25$) for their neutralization breadth against a multiple-subtype panel of 23 virus variants[9] (**Supplementary Methods**). This revealed a decreasing ability over the course of the epidemic to neutralize heterologous virus variants ($P = 0.004$; **Fig. 1i**). Moreover, sera from individuals who seroconverted in 1990–1996 showed less neutralization potency against subtype B and C viruses ($P = 0.040$ and $P = 0.029$, respectively; **Supplementary Fig. 1a**) and a trend toward a decrease in neutralizing titers compared to sera from the earliest seroconverters ($P = 0.054$; **Supplementary Fig. 1b**). These findings suggest that individuals who became HIV-1–infected more recently developed poorer NAb responses than did individuals who seroconverted early in the epidemic.

Next, we studied the molecular basis for this increased neutralization resistance and decreased immunogenicity. The median total length of the variable regions of gp120 sequences increased from 144.5 amino acid residues in HIV-1 variants from historical seroconverters to 151.5 amino acid residues in HIV-1 variants from

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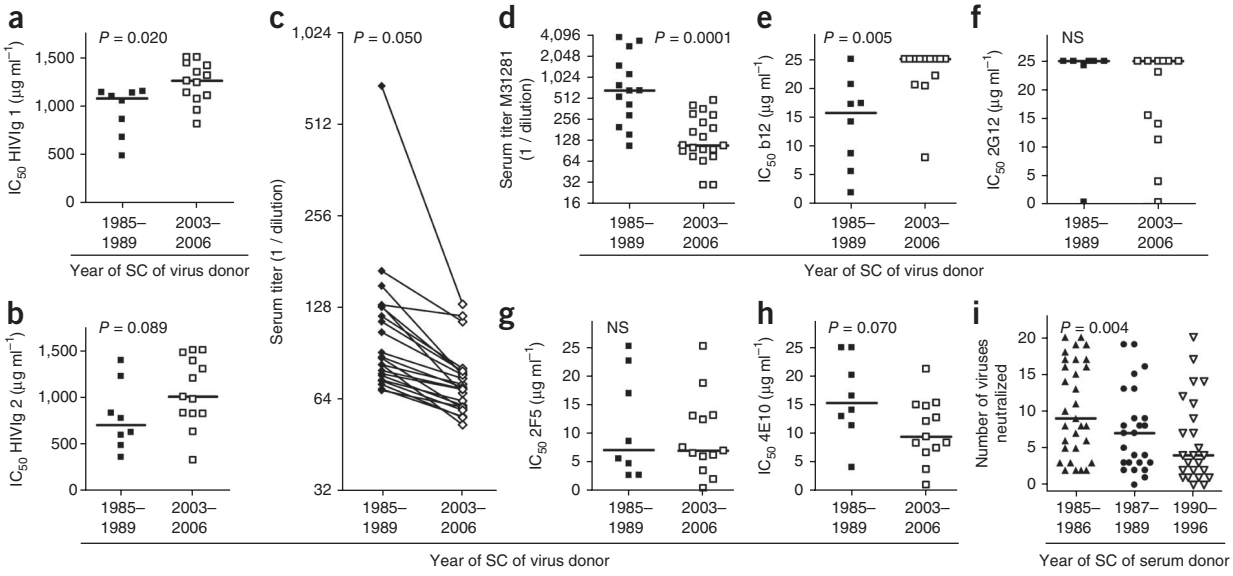

Figure 1 Increased neutralization resistance and reduced immunogenicity of contemporary subtype B HIV-1 as compared to historical HIV-1 isolates. (**a**,**b**) Sensitivity to neutralization by HIVIg batch 1 (**a**) and HIVIg batch 2 (**b**) of clonal HIV-1 variants isolated during primary infection from participants of the Amsterdam Cohort Studies who seroconverted between 1985 and 1989 (historical seroconverters, $n = 8$) or between 2003 and 2006 (contemporary seroconverters, $n = 13$), using one to four virus variants per participant. Each data point shows the average 50% inhibitory concentration (IC_{50}) of all virus variants from one seroconverter. Horizontal bars represent the median IC_{50} of all viruses per group. (**c**) Sensitivity of clonal HIV-1 variants isolated from historical seroconverters ($n = 14$) or contemporary seroconverters ($n = 20$) to neutralization by individual sera from 22 unrelated contemporary seroconverters. Each data point represents the average IC_{50} of a single serum sample tested on all viruses from either historical or contemporary seroconverters. Differences between viruses from both groups of seroconverters were evaluated with a paired Student's *t* test. (**d**) Sensitivity of same virus panel in **c** to neutralization by serum from contemporary seroconverter M31281. Each data point shows the average IC_{50} of all virus variants from one seroconverter, and horizontal bars represent the median IC_{50} of all viruses per group. (**e**–**h**) Sensitivity of the same virus panel used in **a** and **b** to neutralization by monoclonal antibodies b12 (**e**), 2G12 (**f**), 2F5 (**g**) and 4E10 (**h**). Each data point shows the average IC_{50} of all virus variants from one seroconverter. Horizontal bars represent the median IC_{50} of all viruses per group. For all panels, neutralization sensitivities were determined with a peripheral blood mononuclear cell–based assay. Differences between groups in panels **a**, **b** and **d**–**h** were assessed with a Mann-Whitney *U* test. (**i**) Number of heterologous virus variants from a multiclade virus panel ($n = 23$) that could be neutralized by sera obtained approximately 3 years after SC from participants of the Amsterdam Cohort Studies who seroconverted in the period 1985–1986 ($n = 31$), 1987–1989 ($n = 25$) or 1990–1996 ($n = 25$), as determined by a U87-based neutralization assay. Horizontal bars represent the medians. The changing breadth of heterologous neutralizing serum activity over calendar time was evaluated for statistical significance by a Jonckheere-Terpstra test. SC, seroconversion; NS, not significant.

contemporary seroconverters ($P = 0.003$; **Fig. 2a**). This change was largely the result of an increased length of the V1 region ($P = 0.009$; **Fig. 2b**) and less so of the V4 region ($P = 0.099$; **Fig. 2c**), whereas the length of V2, V3 and V5 regions remained the same over time (data not shown). Viruses from contemporary seroconverters contained a median of 1.5 additional potential N-linked glycosylation sites (PNGSs) in gp120 as compared to viruses from historical seroconverters ($P = 0.003$; **Fig. 2d**), and these PNGSs were located mainly in the variable regions ($P = 0.046$; **Fig. 2e**) and, again, particularly in the V1 loop ($P = 0.007$; **Fig. 2f**).

We obtained HIV-1 variants from historical and contemporary seroconverters at, on average, 2.3 and 1.1 months after seroconversion, respectively ($P = 0.002$, Student's *t* test). However, virus populations from both subject groups were equally homogeneous with regard to their genetic diversity (average diversity of 0.28% and 0.36% for historical and contemporary gp120 sequences, respectively, $P > 0.1$, Student's *t* test) and had not diversified with respect to length or number of PNGSs, in agreement with the reported limited viral evolution in the first months after infection[10,11]. Moreover, envelope characteristics within each group were not associated with the time point of virus isolation after seroconversion (data not shown). Therefore, differences in sampling time point are unlikely to account for the differences in the envelope characteristics between historical and contemporary HIV-1 variants.

Phylogenetic analysis showed that the gp120 sequences of viruses from our study subjects did not cluster separately from subtype B gp120 sequences from the Los Alamos database (**Supplementary Fig. 2**), indicating that HIV-1 variants from Amsterdam have not evolved differently compared to other subtype B viruses in the world. A subsequent comparison of gp120 sequences in the Los Alamos database from subtype B HIV-1 variants with a documented year of isolation between 1985 and 1988 ($n = 27$) or between 2003 and 2005 ($n = 72$) (**Supplementary Methods**) did not reveal a difference in the total length of the variable loops (**Fig. 2g**). However, the median length of the V1V2 loop tended to increase from 65.0 to 67.5 amino acids in historical compared to contemporary sequences ($P = 0.083$; **Fig. 2h**). Moreover, contemporary gp120 sequences contained a median of one additional PNGS compared to historical sequences ($P = 0.031$; **Fig. 2i**), which again could be attributed mainly to the V1 region ($P = 0.049$; **Fig. 2j**). These results suggest that the adaptation of HIV-1 is not restricted to our cohort. Notably, we also observed an increase in V1 length and number of PNGSs in V1 between historical and contemporary subtype C gp120 sequences obtained from the Los Alamos database (**Supplementary Fig. 3**). It will be worthwhile to confirm whether the adaptation of HIV-1 to humoral immunity has also occurred for other HIV-1 subtypes.

Given our results, we propose that, over a period of 20 years, subtype B HIV-1 has adapted to the humoral immune response by enhancing

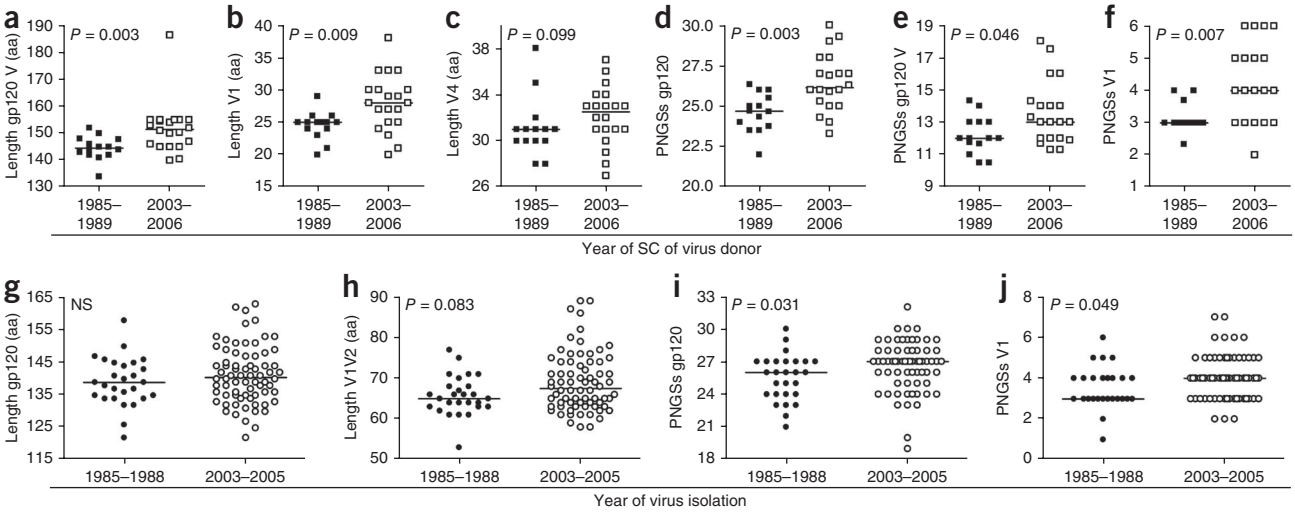

Figure 2 Increased Env length and increased number of PNGSs in the viral envelope in contemporary subtype B HIV-1 as compared to historical isolates. (**a**–**f**) The lengths of gp120 (**a**), the V1 loop of gp120 (**b**) and the V4 loop of gp120 (**c**) and the number of PNGSs in gp120 (**d**), the variable (V) regions of gp120 (**e**) and the V1 loop of gp120 (**f**) are shown for viruses isolated during primary infection from participants of the Amsterdam Cohort Studies who seroconverted between 1985 and 1989 ($n = 14$) or between 2003 and 2006 ($n = 20$). Each data point represents the average value for all viruses from one seroconverter. (**g**–**j**) Similar comparisons are shown for subtype B envelope sequences from the Los Alamos database, with a documented year of virus isolation between 1985 and 1988 ($n = 27$) or between 2003 and 2005 ($n = 72$) for the length of gp120 (**g**), the length of the V1V2 loop of gp120 (**h**) and for the number of PNGSs in gp120 (**i**) and in the V1 loop of gp120 (**j**). In all panels, horizontal bars represent the median. Differences between groups were evaluated with a Mann-Whitney *U* test. aa, amino acid residues. Amsterdam Cohort Studies have been conducted in accordance with the ethical principles set out in the declaration of Helsinki, and written informed consent was obtained from each cohort participant prior to data and material collection. The study was approved by the Academic Medical Center Institutional Medical Ethics Committee of the University of Amsterdam.

the masking of its envelope. We found the largest changes in the V1 loop, which is the prime target of the autologous NAb response[12,13] and is thought to protect underlying vulnerable epitopes from antibody recognition[14]. As the increased neutralization resistance of contemporary HIV-1 seems to coincide with a blunted NAb response in contemporary seroconverters, these findings may be relevant for the choice of envelope in vaccine design.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

E.M.B. designed the study, performed experiments, analyzed data and wrote the manuscript; Z.E. performed experiments and analyzed data; M.R.A.W. performed part of the sequence analyses; B.D.M.B.-N. performed part of the neutralization experiments; M.L.G. and J.M.P. selected and recruited individuals for the contemporary seroconverter cohort and contributed samples and data of these subjects; and H.S. designed the study, supervised the project and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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