HIV-1 vaccine design: Learning from natural infection
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Chapter 3

Early development of broadly reactive HIV-1 neutralizing activity in elite neutralizers

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Abstract

Broadly reactive neutralizing activity (brNA) against HIV-1 is observed in 10-30% of infected individuals and generally takes 2-4 years to develop. Here, we show that two elite neutralizers, infected through injecting drug use, developed brNA around the first year post-seroconversion (post-SC), whereas criteria for elite brNA were fulfilled around 30 months post-SC. These results indicate that brNA does not necessarily require multiple years to develop and they should encourage the search for vaccines that can elicit protective humoral immunity.
Despite more than 30 years of intensive research, no HIV-1 vaccine candidate is capable of establishing strong and durable protective immunity. The induction of broadly reactive neutralizing antibodies (bNAb) is high on the wish list for an HIV-1 vaccine, but no immunogen has been able to achieve this. Several passive immunization studies with bNAb in nonhuman primates have shown significant benefit in protection against HIV/simian HIV acquisition, even in low doses and after repeated challenges [1-5], providing the rationale for aiming at the induction of bNAb by vaccines.

Ten to 30 percent of the HIV-1 infected individuals develop broadly reactive neutralizing activity (brNA), from which bNAb can be isolated [6-12]. This percentage can be higher if one uses a less stringent definition for brNA. For example, a recent study by Hraber et al. [13] showed that 50% of the sera from chronic HIV-1 infected individuals could neutralize more than 50% of the viruses included in a 219-virus panel. These data serve as evidence that the human immune system is capable of developing bNAb against the HIV-1 envelope glycoprotein spike (Env). The consensus thought is that the development of brNA, defined as the ability of sera to neutralize the majority of viruses from different clades, takes relatively long, usually between 2 and 4 years post-seroconversion (post-SC) [6, 14], and seems to be fostered by chronic antigen exposure and antigen evolution [15].

For vaccine development, the need for chronic antigen exposure is a challenging problem, as this would imply that vaccination regimens should be continued for at least a couple of years. However, we recently reported that one men who have sex with men (MSM) participant of the Amsterdam Cohort Studies (ACS) on HIV-1 infection and AIDS developed brNA within the first year post-SC [14], which might refute the thought that brNA necessarily requires years to develop [16]. Of interest, this particular individual developed into an elite neutralizer at 29 months post-SC, representing the top 1% of individuals with brNA [9].

Our goal was to investigate whether the development of early brNA in one MSM infected elite neutralizer was an isolated event, or whether it was a common property of elite neutralizers and independent of the transmission route. We previously determined that approximately 18% of HIV-1 subtype B infected injecting drug users (IDUs) from the ACS had brNA in their serum at approximately 36 months post-SC, including three individuals who developed elite brNA (Euler et al., article in preparation) [17]. We defined brNA by the ability of serum to neutralize viruses from different clades with a geometric mean IC$_{50}$ titer between 100 and 500, and elite brNA by the ability to neutralize with a geometric mean IC$_{50}$ titer higher than 500. For this study we selected two elite neutralizers (IDU1 and IDU2) and four individuals with brNA (IDU3 to IDU6) from the ACS, and studied their brNA development longitudinally. All six male individuals were enrolled in the ACS while negative for HIV-1, and seroconverted between 1989 and 1995 during active follow-up. None of the individuals received antiretroviral therapy during the sampling period. There was no correlation between the development of brNA and CD4$^+$ T-cell count, viral load at set-point, progression towards AIDS and therapy use (data not shown). Depending on the
Figure 1: Development of broadly reactive neutralizing activity in HIV-1 infected drug users over the course of infection.

(A) Broadly reactive neutralizing activity (brNA) development in six injecting drug use infected and six men who have sex with men (MSM) infected individuals. Geometric mean IC50 titer values for each individual across the six-viral panel are plotted over the course of infection. Individuals infected via injecting drug use (present study) or via MSM (adapted from Euler et al.[14]) are indicated in blue and gray, respectively. The elite neutralizers are indicated with solid lines; individuals with normal levels of brNA are indicated with dashed lines. The months at which brNA was reached are indicated behind the individuals’ ID. Asterisks mark the two individuals who did not reach brNA level at the last time point tested. Double daggers mark the five MSM infected individuals with normal levels of brNA. (b) Neutralizing activity over the course of HIV-1 infection against the individual viruses of the six-virus panel for the six individuals infected via injecting drug use (IDU1 to IDU6). The IC50 titers per virus
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and the geometric mean IC_{50} titers (dashed red line) are plotted for each individual. The dotted horizontal lines represent a geometric mean IC_{50} titer of 100 (brNA level) and the dashed horizontal lines represent a geometric mean IC_{50} titer of 500 (elite brNA level). Neutralization titers are expressed as the reciprocal of the plasma dilution that inhibited virus infection by 50% (IC_{50}). The lowest serum dilution used in the assay was 1:40. For the calculation of IC_{50}s for viruses that were not inhibited by the 1:40 serum dilution, we assumed that 50% inhibition would have occurred at a 1:20 serum dilution.

serum availability, sera from the six IDU cohort participants, taken every 3 months until 3 years post-SC, were tested for brNA in a pseudovirus assay using 6 tier 2 viruses (92BR020, MGRM-C-026, 92TH021, 93IN905, 94UG103 and JRCSF) by Monogram Biosciences [18]. This six-virus panel covers 93% of the variation in neutralization of a larger pseudovirus panel, consisting of 15 pseudoviruses from different clades [9].

In the sera of IDU1 to IDU5, early low titer heterologous neutralizing activity could be detected between 1.3 and 11.0 months post-SC, although for IDU4 and IDU5, this was mainly against other subtype B viruses (Fig. 1A and 1B). The sera from one individual (IDU6) showed detectable low titer heterologous neutralizing activity only after 28 months post-SC. Two out of six individuals developed brNA between 2 and 3 years post-SC (IDU3 and IDU4), whereas two individuals did not reach the brNA level at the last time point tested (IDU5 and IDU6). The two elite neutralizers (IDU1 and IDU2) already developed brNA around the first year post-SC (after 11 months and 17 months, respectively) and developed into elite neutralizers at 30 and 32 months post-SC, respectively. The response in the elite neutralizers was directed against all viruses tested in the 6-virus panel, and not subtype specific (Fig. 1B), although the activity of IDU1 against the recombinant virus CRF01_AE was moderate and only detectable at the last time point. Previous studies on MSM infected individuals from the ACS, showed early brNA development in one elite neutralizer (Fig. 1A) [14]. Statistical analysis on the six IDU and six MSM infected individuals from the ACS who were followed longitudinally, revealed that brNA before 2 years post-SC was more likely to be observed in individuals who eventually developed elite brNA (P = 0.046).

Thus, we showed that two elite neutralizers, infected via injecting drug use, who developed an unusually potent neutralizing activity against different HIV-1 clades, had already developed brNA around the first year post-SC, which is consistent with our earlier observation in one MSM infected individual with elite brNA [14]. Collectively, our data show that, at least under some circumstances, the human immune system is capable of generating brNA against different HIV-1 clades in a relatively short timeframe and this can occur irrespective of the transmission mode, contradicting the idea that brNA necessarily requires 2-4 years of persistent viral replication and chronic antigen exposure [15]. Explanations for this phenomenon might be found in viral or host factors or the interplay between both. One possibility could be that these elite neutralizers are infected with viruses on which bNAb epitopes are unusually immunogenic. Furthermore, it is possible that the particular Ab specificities in these elite neutralizers require less extensive somatic
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hypermutation than most known bNAb. The characterization of envelope glycoproteins and bNAb from these elite neutralizers therefore requires further study. Another possibility is that host factors play a role in the development of elite brNA, but such associations have not yet been identified [8, 17]. Although many uncertainties remain on the accelerated brNA development in elite neutralizers, these observations should encourage the search for immunogens that elicit brNA that can be used as vaccines in the prevention of HIV-1 infection.

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