Appearance of Autologous Neutralizing Antibody Correlates with Reduction in Virus Load and Phenotype Switch during Primary Infection with Human Immunodeficiency Virus Type 1

To the Editor—Recently, we reported [1] a needlestick injury that resulted in the infection of a health care worker with human immunodeficiency virus type 1 (HIV-1). We now have additional data regarding the autologous neutralizing antibody (ANA) response following transmission.

There are conflicting reports concerning the role of neutralizing antibody during primary infection. Koup et al. [2] reported that only 1 in 5 patients tested during primary HIV-1 infection had measurable ANA, and in that individual, it was undetected until 3–6 months following presentation of symptoms. In contrast, Albert et al. [3] reported that 4 of 4 patients with primary infection developed ANA within 2–4 weeks from presentation of symptoms. In neither of these studies was the exact time of transmission known. Because the time of transmission was known for the health care worker in our report, we had the opportunity to define precisely the development of ANA.

ANA was measured in a peripheral blood mononuclear cell (PBMC) assay using six 5-fold dilutions of autologous serum or plasma. Equal volumes of 100 TCID$_{50}$ of PBMC-derived virus isolate and serum/plasma dilution were mixed and incubated for 2 h at 37°C, 5% CO$_2$, in a humid incubator. After incubation, 0.2 mL of the virus-serum mixture was added in triplicate to wells of a 24-well plate containing $2 \times 10^6$ phytohemagglutinin-stimulated PBMC in 1.8 mL of medium. The next day, cells were washed three times with medium, and 2 mL of fresh medium was added. Supernatant was harvested on days 4 and 7, with medium replaced on day 4. Supernatant was normally assayed for p24 antigen on day 4; however, slower-growing isolates were assayed on day 7. A microwell was considered positive for neutralization if no virus replication was detectable (<30 pg/mL HIV-1 p24 antigen). Neutralization (antibody titer necessary to neutralize all viral infectivity in 50% of culture wells) was calculated by the method of Spearman-Karber; wells were scored negative for neutralization if >30 pg/mL of p24 antigen was detectable. The quantitation of HIV-1 plasma RNA and infected PBMC and the determination of phenotype were performed as described in our previous report [1].

Plasma HIV-1 RNA levels were high 20 days after transmission, with a 2-log reduction by day 33, followed by continued low levels. The concentration of HIV-1–infected PBMC was not tested 20 days after infection; however, virus was culturable in a qualitative culture. The number of infected PBMC was reduced from 40/10$^6$ at day 33 to <0.1/10$^6$ at day 45 and remained low thereafter. ANA was undetectable 20 days after infection. By 33 days, ANA

Figure 1. Association of neutralizing antibody with virus load (A) and viral phenotype (B). A, Appearance of autologous neutralizing antibody correlated with reduction in virus load. Units: IUPM, infectious viral units of HIV-1 per 10$^6$ peripheral blood mononuclear cells; NT-50, antibody titer necessary to neutralize all viral infectivity in 50% of culture wells; RNA/1000, HIV-1 plasma RNA copies $\times 10^3$. B, Cross-neutralizing antibodies were detectable at all sampling points, including switch from syncytium-inducing (SI) to non-SI (NSI); however, none reacted to day 20 isolate. Virus isolates are defined on x axis by day after needlestick injury (A) and phenotype (B). Antibodies (anti-) are defined by day after transmission of blood sample. Blank columns represent untested antibody sample. NT$_{50}$ (NT-50) below level of assay detection (<11) are graphed as 2.
was detectable, and it increased by 45 days. (Antibodies from day 45 were tested against day 33 virus because there was no virus growth from the day 45 sample.) ANA continued to be detectable through the remainder of the year following infection (figure 1A). Therefore, the decrease and maintenance of a low level of virus load was associated with the appearance and continued presence of ANA.

Cross-neutralizing antibodies (CNA), defined as antibodies obtained from the health care worker at alternate times from the virus isolate, were also measured (figure 1B). CNA were detectable by 20 days after infection and increased as virus load decreased. The inability to measure ANA at day 20, therefore, was not due to the absence of neutralizing antibody, because there was an effective neutralizing response against viruses isolated on days 33 and 75. In fact, none of the CNA could neutralize the virus isolated on day 20. However, the virus that appeared 13 days later (day 33 after infection) was neutralized by all antibodies tested during the first year of infection. Thus, it is possible that the virus variant isolated at day 20 circulated for too short a period to stimulate neutralizing antibody or was resistant to neutralization.

The switch from syncytial-inducing (SI) to non-SI (NSI) phenotype between days 33 and 75 after infection preceded by neutralizing antibodies to both viruses (figure 1B). Replication of the SI virus appeared to be restricted by the antibodies, as virus replication was undetectable at 45 days. The NSI virus that appeared following the negative viral culture at 45 days remained the culturable virus phenotype throughout the first year. This occurred even in the presence of neutralizing antibody, implying that NSI but not SI was partially protected from neutralization.

In summary, we have provided evidence that neutralizing antibodies to HIV-1 can be detected as early as 20 days after transmission of the virus. The appearance of neutralizing antibody correlates with the reduction in virus load as measured by HIV-1 plasma RNA and infected PBMC. Thus, ANAs could have a role in the reduction and maintenance of virus load during primary infection. In addition, we have demonstrated that ANA and CNA preceded the switch from SI to NSI phenotype, possibly adding biologic pressure to influence the switch.

Janet L. Lathey, R. Douglas Pratt,* and Stephen A. Spector
Department of Pediatrics and Center for Molecular Genetics,
University of California, San Diego

References


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Reprints or correspondence: Dr. Janet L. Lathey, Pediatric Infectious Disease, #0672, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92393-0672.

* Current affiliation: FDA, Division of Antiviral Drug Products, Rockville, Maryland.

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Importance of Using Relapse Studies or Tests for Detection of Antibiotic Resistance before Considering that a Treatment for Multidrug-Resistant Enterococcal Experimental Endocarditis Is Effective

To the Editor—The effectiveness of two combinations of cell wall--active agents (ampicillin plus imipenem and ampicillin plus imipenem plus vancomycin) in the treatment of an experimental endocarditis due to a multidrug-resistant Enterococcus faecium isolate was recently reported by Brandt et al. [1]. That study was designed without a relapse period and without tests for detection of a resistant bacterial subpopulation. Regimens were considered effective because, in animals sacrificed shortly after 3 days of treatment, they provided dramatic (i.e., > 7 log_{10} cfu/g) reductions in colony counts in cardiac vegetations compared with controls, even if (as expected from short-term treatment) only a low proportion of animals (15%) had apparently sterile valves. In our opinion, whether such regimens could definitively cure endocarditis and therefore be considered really effective remains to be demonstrated.

Previous studies with enterococcal endocarditis models suggest that a reduction of at least 4 log_{10} cfu/g in cardiac vegetations immediately after short-term treatment (3-5 days) is frequently predictive of the efficacy of the antibiotic regimen. Indeed, a high rate of cure is often observed when treatment is continued for a prolonged period (at least 10 days), even when animals are sacrificed several days after discontinuation of therapy in a so-called relapse study [2, 3]. The predictive value of a strong reduction of vegetation titers after short-term treatment, however, has not been validated for antibiotic regimens and bacterial isolates for which there is a high risk of emergence of resistance.

In an experimental rabbit endocarditis model, our laboratory studied the potential in vivo relevance of the synergistic effect that exists in vitro between β-lactam and glycopeptide against an E. faecium isolate highly resistant to both penicillin and vancomycin. Results showed that different β-lactam–glycopeptide–gentamicin combinations can provide, in animals sacrificed shortly after a 5-day treatment, reductions of 4–5 log_{10} cfu/g in vegetations [4, 5]. However, in the titles and conclusions of the papers, a too-optimistic view was avoided because a bacterial subpopulation resistant to the synergistic effect between β-lactam and glycopeptide was detected in the starting inoculum and in some vegetations. This was responsible for treatment failure in 10%-20% of animals retained for relapse studies.

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