HIV Superinfection and Immune Control: Implications for Vaccine Development?

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On the last day of the 7th conference on Retroviruses and Opportunistic Infections in San Francisco in February 2000, shockwaves reverberated through the Moscone Convention Center. No, it was not an earthquake attributed to the cantankerous San Andreas Fault but rather an earth-shattering case report stemming from a Canadian HIV clinic situated 2500 miles away (Angel, 2000). The case report, presented by Dr. Jonathan Angel and his colleagues from the University of Ottawa, involved an antiretroviral-naive HIV-positive male (patient A) who experienced rapid disease progression and high levels of viral resistance to multiple drugs after engaging in unprotected sexual activity with another HIV-positive male harboring a drug-resistant, possibly more virulent strain of HIV (patient B). Dr. Angel concluded that "Patient A was very likely infected with a resistant strain of HIV-1 by Patient B," and went on to say: "I think there's enough information here to raise awareness regarding HIV superinfection and to say that this should be a public health issue if we can prove it."

HIV superinfection is defined as a second infection, after a primary infection has been established, with a heterologous strain belonging to the same subtype as the primary strain (intrasubtype superinfection) or to a different subtype (intersubtype superinfection).

While there have been several reports demonstrating HIV coinfection—the simultaneous transmission of two or more HIV variants—data supporting the possibility of superinfection have been limited, with the Ottawa case report being one of the only examples to draw upon. However, there has been much consternation regarding the conclusions drawn from the Ottawa case report. For starters, the full details of this case have not been published in a peer-reviewed medical journal. It is also unclear whether the strain causing the primary infection, the superinfecting strain, or a potential recombinant form had resulted in the patient's rapid disease progression, given that comprehensive genetic analyses were not conducted. There has also been some speculation that the purported superinfection reported by Dr. Angel and his colleagues was a case of laboratory error because of contamination—which speaks more to the challenges of identifying superinfection than about superinfection itself.

There is now fresh evidence from two new, heavily deconstructed case reports to conclude, beyond a reasonable doubt, that intersubtype and intrasubtype HIV superinfection can occur. What remain, however, are serious concerns surrounding these findings—deep-seated anxieties that HIV superinfection may have a significant impact on public health initiatives and vaccine development.

A Mystery at Mass General

As has been reviewed in several past issues of *The PRN Notebook*, the Partners AIDS Research Center at the Massachusetts General Hospital has been conducting some extraordinary research involving the long-term control of HIV viremia with the early initiation of antiretroviral therapy in acutely infected patients and subsequent supervised treatment interruptions. There have been numerous successes—nine of 14 newly infected patients who initiated HAART, followed by one or more treatment interruptions, have maintained robust HIV-specific CD4+ and CD8+ cell responses and low viral loads in the absence of treatment; for more than two years in some cases. Yet there have also been cases where this control has not proven durable. Some patients have not been able to maintain appreciable control of HIV replication, irrespective of the number of treatment interruptions attempted. Most intriguing has been one subject in particular—a patient who initially experienced virologic control in response to early therapy and treatment interruptions, only to experience a subsequent sudden loss of virologic control and disease progression. The case report was first presented by Dr. Bruce Walker of the Partners AIDS Research Center at the XIV International AIDS Conference (Walker, 2002) and will be discussed more fully in a pending issue of *Nature*.

The individual in question—dubbed subject AC-06—presented to the Massachusetts General Hospital clinic with symptomatic acute infection. He was negative for HIV antibodies but had a viral load approaching 9 million copies/mL. Four days after learning of his infection, he initiated a HAART regimen consisting of nelfinavir, stavudine, and lamivudine. His viral load rapidly decreased to undetectable levels (see Figure 1).

Five hundred forty-six days after beginning treatment, he initiated his first treatment interruption. Sixty-three days into his first STI, his HIV-RNA level exceeded 50,000 copies/mL, meeting requirements for reinitiation of therapy. After three and one-half months of successful retreatment with the

same HAART regimen, a second STI was initiated. Although there was a noticeable burst in viremia two months into the second STI, his viral load spontaneously declined, remaining below 5,000 copies/mL for more than seven months.

Two hundred-ninety days after stopping therapy for the second time—1005 days after initiating treatment—patient AC-06 experienced a sudden rapid increase in his viral load and a marked decrease in his CD4+ cell count. Consequently, HAART was reinstated for the third time.

Once again, subject AC-06's viral load decreased to undetectable levels in response to therapy. Four months later, a third STI was attempted. This time, there were no signs of immunologic control—his viral load exceeded 50,000 copies/mL within three weeks of stopping treatment. Treatment was again restarted, and a fourth STI was attempted four months later. Although his viral load did not peak above 50,000 copies/mL, he was unable to keep his viral load below 5,000 copies/mL as seen in three sequential blood draws—a secondary criteria for restarting therapy. The patient, however, refused therapy and his viral load has continued to increase (and his CD4+ cell counts decrease) over time.

Looking for Answers

"WHAT WE SAW WITH PATIENT AC-06 WAS very different from what we've seen in our other acutely infected patients who demonstrated signs of virologic control during initial STIs," Dr. Allen said. "The virologic control we saw during the first two STIs in patient AC-06 was subsequently lost, whereas in our other patients we might expect to see prolonged virologic control with each STI. This prompted us to evaluate his virus-specific immune responses at various time points to look for correlates of immune control loss."

Gag-specific CD4+ proliferative responses were detected during the initial round of treatment and the first and second treatment interruptions—at least until the sudden increase in viremia, documented 290 days into the second STI.

The next step was to measure virus-specific CD8+ cell responses—which are a critical component of the immune response to HIV—using an interferon-γ enzyme-linked immunospot assay (ELISPOT). This work was accomplished by Dr. Mar-
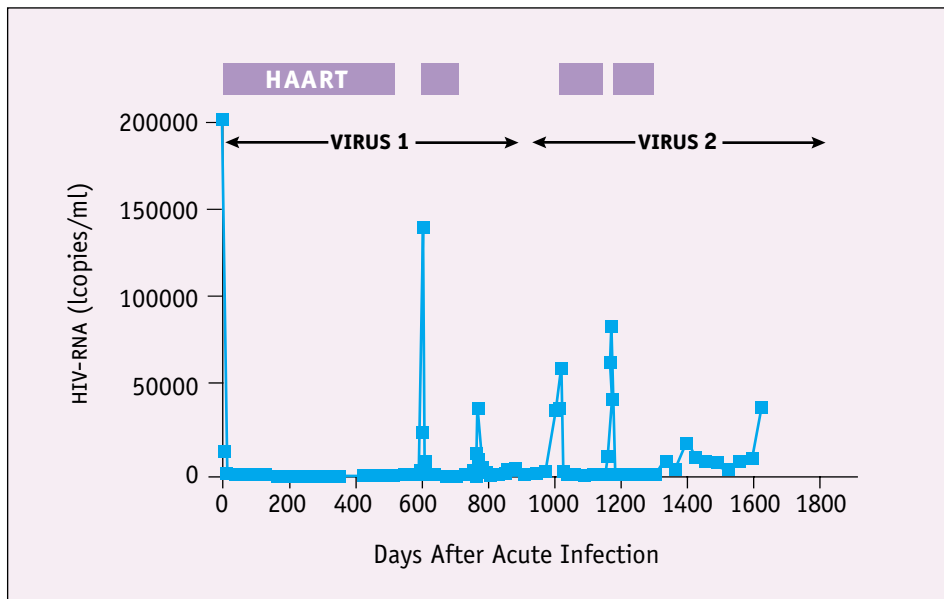
FIGURE 1. Subject AC-06: HIV-RNA Levels During Acute HIV Infection and 60 Months of Follow-Up.
The horizontal bars show the periods during which the patient received highly active antiretroviral therapy (HAART). The horizontal arrows show the change from virus 1 (the first subtype B virus) to virus 2 (the second subtype B virus).

Source: Todd Allen, MD

cus Altfeld and Dr. Xu Yu at the Massachusetts General Hospital. A panel of B clade overlapping peptides spanning all expressed HIV proteins was used to assess the broadening of the CD8+ responses to patient AC-06's HIV. Sure enough, HIV-specific CD8+ cell responses were enhanced during the first and second STI. "The responses we saw were targeting over two dozen distinct CTL epitopes, which really does reflect a broad response to the virus," Dr. Allen explained. However, samples collected during the third STI demonstrated that the epitope-specific CD8+ responses seen during the first two STIs had changed dramatically. "Some of the epitope-specific responses were maintained and others expanded further during the third STI," he said, "but some responses declined, which we couldn't readily explain."

Could it have been that the patient's virus mutated faster than epitope-specific CD8+ cell responses could develop? Dr. Allen's group documented sequence changes with three of the seven targeted CD8 epitopes located with HIV gag, but it was uncertain that changes of this magnitude would result in such a profound loss of immunologic control. Most interesting, however, were a significant number of amino acid changes in the flanking regions that did not contain CD8+ cell epi-

topes. "This tipped us off and we conducted phylogenetic analysis of gag sequences from samples collected during acute infection and during the third STI," elaborated Dr. Allen. "We found that a distinct and unrelated second strain of HIV had emerged. Testing additional samples, we determined that the second virus emerged close to the end of the second STI, when viremia peaked, and persisted through the third STI."

Additional analyses demonstrated that the two viruses—discussed herein as viruses 1 and 2—varied by only 12% at the amino acid level. However, half of the known immunologically targeted portions of the two viruses were different. "We were definitely looking at two viruses," added Dr. Allen. "Both viruses were subtype B and possessed the R5 phenotype."

To determine if viruses 1 and 2 established infection at the same time (coinfection) or if virus 2 established itself after primary infection with virus 1 (superinfection), Dr. Allen's group probed further into patient AC-06's medical records and collected samples. Approximately one month before virus 2 was found, subject AC-06 had reported symptoms consistent with acute retroviral syndrome, including fever and nightsweats. These symptoms occurred approximately four to eight
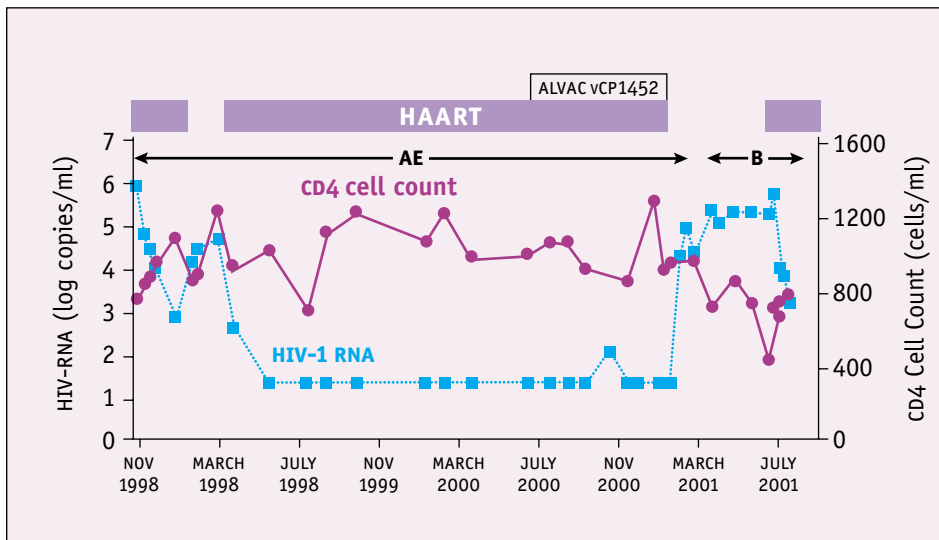
FIGURE 2. The Geneva Subject: HIV-RNA Levels and CD4+ Cell Counts During Acute HIV Infection and 31 Months of Follow-Up.
The horizontal bars show the periods during which the patient received highly active antiretroviral therapy (HAART) and the period of vaccination with ALVAC vCP1452, and the horizontal arrows show the change from subtype AE to subtype B.

Source: Jost, 2002. Reprinted with permission of the *New England Journal of Medicine* and the Massachusetts Medical Society.

weeks after the patient had unprotected sex with an anonymous partner. PCR testing of blood samples revealed virus 1 to be the only detectable virus at the time of acute infection, during the first STI, and for the better part of the second STI. The virus detected during the rebound in viremia toward the end of the second STI was found to be virus 2 (see Figure 1). Analyzing viral DNA from PBMCs also demonstrated that virus 1 was the only virus detected through to the rebound in viremia during the second STI. From that point forward, both viruses 1 and 2 could be detected in PBMCs.

"All of the data point to HIV superinfection," Dr. Allen remarked. "Despite a very strong CTL response, this patient was not able to prevent superinfection with a second virus, to which half of the CD8+ cell responses remained cross-reactive. While we did see an induction of new CD8+ cell responses after superinfection, viremia wasn't well contained and the patient's viral load continued to increase and his CD4+ cells dropped."

A Report From Geneva

WHILE NOT SPECIFICALLY DISCUSSED BY DR. Allen, another noteworthy case of HIV superinfection was reported recently by a team of Swiss investigators. Preliminary data from this intriguing report were first presented at the 9th Conference on Retroviruses and Opportunistic Infections, held last winter in Seattle, updated at the XIV International AIDS Conference this past summer in Barcelona, and then published in September as a brief report in the *New England Journal of Medicine* (Jost, 2002).

On November 18, 1998, a 38-year-old man presented to the University of Geneva with an acute retroviral syndrome. Antibodies to HIV were not yet detectable, although his p24 antigen titer was greater than 100 pg/mL and his viral load exceeded 800,000 copies/mL.

Soon after receiving a diagnosis of primary HIV infection, the patient enrolled in a clinical trial and received a four-drug regimen consisting of amprenavir, abacavir, zidovudine, and lamivudine. Twenty-one months later, he co-enrolled into a therapeutic vaccination trial and was randomized to receive ALVAC's vCP1452.

Six weeks after beginning HAART, his viral load dropped to 1,000 copies/mL. Unfortunately, therapy was proving to be a foe to his liver, resulting in therapy discontinuation for six weeks. Upon resuming HAART, his viral load decreased rapidly, to less than 50 copies/mL. After receiving vCP1452, his antiretroviral therapy was again halted as per the vaccine research

protocol. That was in January of 2001, and by February of 2001 his viral load had rebounded to 80,000 copies/mL and then decreased to 21,000 copies/mL.

Blood drawn on April 10, 2001, revealed that the patient had experienced a sharp rebound in viral load, which fluctuated between 200,000 and 400,000 copies/mL for the next four months. He had mild symptoms—including fatigue and fever—and lost approximately 300 CD4+ cells/mm³ during this time. The patient noted that he had been in Brazil three weeks before the April 10 blood draw and that he had had several unprotected sexual experiences. Four months after this second viral load rebound, the patient consented to restart therapy and his viral load decreased rapidly. Soon after restarting treatment for the third time, his alanine aminotransferase levels increased significantly. Using HCV-PCR, it was determined that he was in the initial throes of HCV infection. He is now being successfully treated with pegylated interferon and ribavirin.

A snapshot of this patient's clinical history, beginning with the time of his diagnosis of acute HIV infection to the last date of follow-up data reported in the *New England Journal of Medicine* article, is illustrated in Figure 2.

What Lies Beneath

SUSPECTING THAT A SECOND HIV INFECTION might be behind the second rebound in their patient's viral load, the Geneva team—with the help of investigators at the Hôpital Pitié Salpêtrière in Paris—sequenced the reverse transcriptase genes, gag p17, and the C2 to V3 region of env of samples collected from November 18, 1998, through January 4, 2002. Samples collected in November 1998 and January and February 2001 indicated that the AE HIV subtype was present. In contrast, sequencing of samples collected in April, May, and November 2001 and January 2002—samples collected after the second rebound in viral load—indicated the presence of HIV subtype B.

To rule out the possibility that their patient had been coinfected with two subtypes of HIV at the same time, the research team performed plasma PCR with subtype B- and AE-specific primers. Sure enough, it was determined that the only detectable

virus circulating in the patient's blood from March 1998 to March 2001 was of the AE subtype. In April 2001, when the second rebound in viral load was detected, amplicons from both the AE subtype and B subtype were detected.

Delving further, the research team investigated the responses of CD8+ cells to the patient's AE- and B-specific epitopes using ELISPOT. Only cells directed against subtype AE-specific epitopes were detected between November 1998 and March 2001. They markedly decreased after the switch to the B subtype in April 2001. And to make matters worse, none of the subtype B epitopes derived from the patient's sequences were recognized at any time during either subtype AE or subtype B infection.

Superinfection:
The Great Vaccine Debate

IRREFUTABLE DATA CONCLUDING THAT HIV superinfection can and does occur is only the tip of the iceberg. What is necessary now is to determine its clinical significance, which could very well spell trouble for both people already infected with HIV and those who could potentially benefit from a preventive HIV vaccine in the future.

In an editorial appearing in the September 5, 2002, issue of the *New England Journal of Medicine* accompanying the Geneva superinfection case report, Drs.

Philip Goulder and Bruce Walker of Massachusetts General Hospital attempt to address some of the implications HIV superinfection may have on HIV vaccine initiatives (Goulder, 2002). While Drs. Goulder and Walker wrote, "there are aspects of (the Geneva case report) that need not leave us in despair," it appears that some of their more optimistic comments are refuted by their own, more recent HIV superinfection case report.

For example, the HIV-specific immune responses that were detected in the Swiss patient were narrowly directed and insubstantial, with CTLs targeting only a single region of the virus identified. The typical broadening of immune responses in this patient may have been blunted by early HAART use and may have further waned during the period of effective therapy. In other words, the virus-specific immune response may simply have been too limited to protect against another virus, particularly of a different subtype.

Turning to the Boston patient, limited CTL responses did not appear to be a problem. Prior to the emergence of the second virus, over two dozen CD8+ cell epitopes were detectable, indicating a broad immune response. Still, such responses were not capable of controlling a second subtype B virus that varied by only 12%. A central question thus remains: just how broad will vaccine-induced immune responses need to be to successfully prevent—or rapidly control—primary HIV infection.

On a more positive note, Dr. Allen pointed out that patient AC-06 did generate new CD8+ cell responses against epitopes that had not been previously presented after the emergence of the second virus, which suggests that therapeutic immunization in chronic infection may be possible. "If we are to better understand the obstacles that may face vaccine development or to develop effective therapeutic vaccines, we need to continue studying immune responses in patients who control HIV replication and then lose control," Dr. Allen said. "These types of studies should provide important insights regarding the extent and magnitude of HIV-specific immune responses that are needed to provide cross-protective immunity." *prn*

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