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## Analyses of Long-Term Surviving HIV-Infected Japanese Patients with Coagulation Disorders Hint at Novel Means to Prevent and Treat HIV/AIDS

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In the early 1980s, individuals with coagulation disorders such as hemophilia were exposed worldwide to human immunodeficiency virus (HIV) through virus-contaminated coagulation factor preparations. One of the difficulties in conducting a cohort study of HIV-infected individuals is to determine when the individual was exposed to HIV because the HIV infection is often asymptomatic. This problem is circumvented by studying HIV-infected patients with coagulation disorders because of the limited time frame in which these patients could have been infected. Consequently, these patients are useful for studying the natural course of HIV infection and the factors that influence AIDS progression<sup>1-3</sup>.

In Japan, individuals with coagulation disorders were exposed to HIV through virus-contaminated coagulation factor concentrates made from pooled donated human plasma during the late 1970s through 1985. These individuals were exposed to a low dose of HIV, presumably more than once. Approximately 40% (1,431 cases) of Japanese individuals with coagulation disorders became sero-positive. All had a clade B HIV that was most prevalent in Europe and North America. A nationwide surveillance study of these patients that was conducted in Japan in 2006 revealed that 42.2% (604/1,431 cases) had died during the 21 years of follow-up<sup>4</sup>. Recently, the mortality rates of the HIV-infected patients with coagulation disorders in Western countries were reported to range from 60.5 to 67.7% over 15 years<sup>5-8</sup> (Table 1). Thus, the Japanese patients with coagulation disorders had a significantly lower mortality rate despite the similarities in the infecting virus, the route of HIV exposure, and the prevalence of AIDS-related diseases. Comparable figures for HIV-infected Japanese individuals without coagulation disorders are not available. Multiple studies, most of which focused on Caucasian populations, have identified a number of genetic factors that protect against AIDS progression<sup>9-12</sup>. Our studies and those of others have failed to detect any of these major anti-AIDS genetic factors in the Japanese population including CCR5Δ32 and some MHC class II alleles. These observations suggest that a unique genetic factor(s) that is only present in the Asian ethnic group confers resistance to AIDS progression.

Comparison of the death rates of HIV-seropositive individuals with coagulation disorders

Country	HIV-1 infection	Route of HIV-1 exposure	Follow up (years)	No. of patients	No. of death	Death rate (%)	Reference
UK	early 1980s	clotting factor concentrate	1985 - 2000 (15)	1,246	812	65.2%	1
Austria	1970s	clotting factor concentrate	1985 - 2002 (17)	288	195	67.7%	2
Spain	late 70s - early 80s	clotting factor concentrate	1985 - 2003 (18)	585	354	60.5%	3
Canada	1982-1985	clotting factor concentrate	1982 - 2003 (21)	660	406	61.5%	4
Japan	1980-1985	clotting factor concentrate	1985 - 2006 (21)	1,431	604	42.2%	5

1 UK Hemophilia Center Doctors' Organization, AIDS 2004

2 Lichterfeld M, et al., J Infection 2005

3 Amo J, et al., J Acquir Immune Defic Syndr 2006

4 Arnold DM, et al., Blood 2006

5 Annual report of national surveillance for clotting disorders. 2006

Table 1

To test this notion, we examined the well-monitored cohort of 142 HIV-infected patients with coagulation disorders at the Ogikubo hospital in Tokyo, which encompasses approximately 11% of all HIV-infected Japanese patients with hemophilia and clotting factor deficiencies. Of this cohort, 24 (16.9%) were found to fall into the slow progressor/long-term non-progressor (SP/LTNP) category, which is defined as an AIDS-free status with CD4+ T-cell counts of more than 200 mm<sup>3</sup> in the calendar year

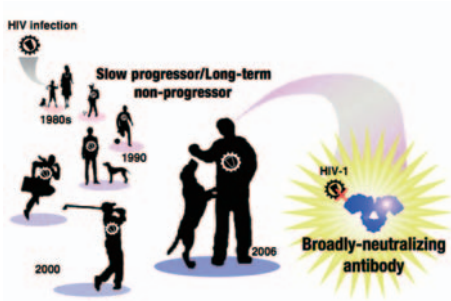


Fig. 1

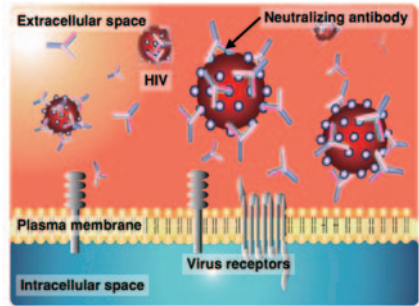


Fig. 2

2000 (15 years after infection). None of these 24 patients take anti-retroviral drugs. They varied in their plasma viral RNA copy level, with some having levels as high as 104/ml. Only one of the 24 subjects fell into the “elite controller” category characterized by undetectable viral RNA copies (0.7%, 1/142 cases). This seemingly high frequency of SP/LTNP patients may be related to the reasons why the HIV-infected Japanese patients with coagulation disorders have a low mortality rate compared to the Western cohorts.

Of the SP/LTNP patients, we identified three individuals with potent neutralizing plasma that was effective against multiple clades of HIV, including the clade/circulating recombinant forms A, B, C, D, 01\_AE and 02\_AG (Fig. 1). This response is remarkable given that all the patients are infected with the clade B virus. This broad HIV neutralization activity was originally determined by using a virus replication assay on peripheral blood mononuclear cells and was subsequently verified by Dr. David Montefiori’s group at Duke University, who used the neutralization assay employing the “pseudotyped” replication-incompetent virus<sup>13</sup>. The HIV-neutralizing activity was recovered in the protein A-bound fraction, which suggests that the HIV was neutralized by neutralizing antibodies (Nabs). Cases of patients with broadly neutralizing antibody are very rare. The Nab-containing plasma was at least as potent as the cocktail of the previously isolated monoclonal Nabs (mNabs) 2F5, b12 and 2G12. The neutralization epitope appeared to involve a novel site of the CD4-binding region of the viral envelope.

Nabs interfere with the ability of HIV to infect cells (Fig. 2). It is now known that while most HIV-infected individuals usually develop low levels of Nabs, these Nabs do not neutralize the “contemporary” circulating “autologous” viruses in the majority of cases. It takes approximately a year to develop neutralization activities against the autologous virus, at which point the virus mutates and escapes from these Nabs. These observations together suggest that Nabs can only play a limited role in controlling autologous virus propagation. This in turn yields the question, why is it significant that some Japanese SP/LTNP patients have elevated levels of highly cross-reactive Nabs that neutralize heterologous viruses?

From the point of view of AIDS vaccine development, we know that it is extremely difficult to develop an AIDS vaccine. Moreover, it has not been feasible to efficiently induce an Nab with inhibitory activity towards multiple HIV strains. However, we are encouraged by the fact that some individuals CAN actually develop the immunological status that we are striving to induce with a vaccine. It gives hope that an AIDS vaccine may be still possible. We speculate that when we understand how the high titer cross-reactive Nabs are induced and maintained in SP/LTNP patients, we may be able to build a successful Nab-based AIDS vaccine.

The Nabs that we detected are also important because they can be used as a therapeutic drug. Several broadly cross-reactive mNabs have been established from HIV-infected individuals. A cocktail of these mNabs actively delays the progression of AIDS<sup>14,15</sup>. By cloning the mNabs from Japanese SP/LTNPs, we might be able to provide another option to treat HIV-infected individuals since they target a novel neutralizing epitope. The novel mNabs may circumvent the emergence of viruses that are resistant to currently available anti-retroviral drugs.

In conclusion, while it was deeply unfortunate that the Japanese patients with coagulation disorders became infected with HIV, we may be able to use our observations to improve not only their fortunes but also those of other HIV-infected individuals. We also speculate that these patients may bear other major genetic determinants (apart from the ability to produce potent Nabs) that may hinder the progression of AIDS. These determinants can be identified by comprehensive analyses of the patients with coagulation disorders that pinpoint the genetic and phenotypic parameters associated with the control of plasma viral RNA copy levels, peripheral CD4+ T-cell counts, and overall disease progression. When performed in the

context of an international framework, these studies may well identify the correlates that could lead to a novel therapy for HIV-infected individuals.

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