SHORT COMMUNICATION

Virologic and Serologic Characteristics of a Natural Chimpanzee Lentivirus Infection

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This study set out to characterize the unique features of natural lentivirus infection in chimpanzees over time. The virologic and serologic characteristics of this infection were followed longitudinally in a naturally infected chimpanzee together with a small cohort of experimentally HIV-1-infected chimpanzees. The subsequent isolates from the naturally infected chimpanzee were all non-syncytium forming (NSI) versus syncytium forming in the experimentally infected animals. In contrast to HIV-1-infected chimpanzees virus load was higher and plasma viremia occurred but in a cyclic pattern. Serologic follow-up suggested the development of neutralizing antibodies with subsequent escape of new isolates. Interestingly, the sequence of the principal neutralizing (V3 loop) domain (of HIV-1) remained constant over time. Antibodies to peptides from the V3 loop were type specific. The occurrence of persistent, fluctuating plasma viremia and NSI-type virus variants of this natural lentivirus infection are unique characteristics not previously reported in experimentally infected chimpanzees. © 1995 Academic Press, Inc.

Lentiviruses have been isolated from a variety of different primate species (1–10). Current evidence has revealed that chimpanzees are occasionally naturally infected with a lentivirus and isolates from chimpanzees represent the closest known relatives of human immunodeficiency virus type 1 (HIV-1) based on genetic, biological, and antigenic similarities (9–12). Chimpanzees are man's closest relative and one of the few species susceptible to HIV-1 infection. However, experimentally infected chimpanzees appear to be resistant to disease (13–15). Several naturally infected chimpanzees have been identified. One is now in captivity providing us with a rare opportunity to characterize the course of natural lentivirus infection in this species. To accomplish this we undertook a longitudinal study of the virologic and serologic features of natural chimpanzee immunodeficiency-like virus (SIVcpz-ant) infection and compared these findings with those from a small group of HIV-1 experimentally infected chimpanzees.

The naturally SIVcpz-ant-infected chimpanzee (chimp No) has been previously described (9) and is currently housed at the Biomedical Primate Research Center (BPRC) in Rijswijk, The Netherlands. The animal was first identified as seropositive on illegal import into Belgium.

This animal is now approximately 9 years of age. Three experimentally infected chimpanzees are also housed at the BPRC and were studied for comparison. The three animals were infected intraveneously. Two of these animals (chimp Bu and chimp Ma) were infected with cell-free virus from the prototype laboratory strain of HIV-1 (Lai) in 1984. The third (chimp Co) was not infected with an isolate, but rather directly with peripheral blood mononuclear cells (PBMCs) taken from an AIDS patient. Sequence of the V3 loop from this virus reveals that this patient was infected with an Mn-like virus from genetic subtype B of which HIV-1Lai is a member also.

To characterize this natural lentivirus infection in vivo, we first set out to determine the biological phenotype of virus isolates and to examine possible differences in viral load between naturally and experimentally infected chimpanzees. Virus was isolated by cocultivation of PBMCs from the seropositive chimpanzees with phytohemagglutinin-stimulated lymphocytes from healthy (HIV-negative) human donors. Cultures were monitored as previously described (9). Briefly, the medium [RPMI 1640 supplemented with 10% fetal calf serum, 20 U/ml recombinant interleukin-2 (Boehringer Mannheim, Germany), antibiotics, and polybrene] was refreshed twice a week to maintain an approximate cell concentration of $1–2 \times 10^6$ cells/ml. A total of 16 virus isolations from PBMCs of the naturally infected animal were performed between 1989 and 1994 and all were positive as deter-

mined by the presence of HIV p24 antigen in the culture supernatants. All of the isolates from this animal were non-syncytium inducing (NSI) as tested on PBMCs and MT-2 cells. In contrast, when the same virus isolation procedure was used with human donor PBMCs from the same batch, for the experimentally infected chimpanzees, virus could not be recovered unless CD8 cells were first depleted, revealing syncytia (data not shown) (16). The observation of syncytium-inducing (SI) versus NSI type of virus infections is not dependant on the CD8 depletion method and has been confirmed by using the MT-2 cell line as an indicator or by using human PBMCs. In addition, the SI versus NSI type of virus infections in these experimentally infected chimpanzees has been evaluated independently by another laboratory (19). The differences in these biological variants (NSI vs SI) are important to consider. In humans, HIV-1 isolates differ in biological properties such as replication rate, cell tropism, and SI capacity and are well correlated with disease progression (17, 18). Until now chimpanzees which are infected with NSI variants have not been identified. Previous reports have attributed the lack of NSI variants in chimpanzees (which predominate early in infection in humans) to their resistance to disease (19, 20).

Subsequently we set out to determine if there was any evidence that this naturally infected animal differed in its potential to progress to disease by examining its viral load characteristics. Virus load was determined by assessing plasma viremia and the number of infected PBMCs in circulation by end-point dilution cultures at 3- to 4-month intervals as previously described by Ho et al. (21). Plasma viremia (extracellular virus) in experimentally infected chimpanzees was altogether undetectable. Interestingly, persistent plasma viremia has not been observed in other chimpanzees studied to date (14, 22–25). Titers of infectious virus in plasma from the naturally infected animal fluctuated more over time than did the number of infected PBMCs. Six of the 10 plasma cultures were negative, while at other time points the plasma titers were high (100 and 500 TCID/ml plasma) as shown in Fig. 1. The observed PBMC titers correspond to the average HIV-1 PBMC titers in asymptomatic individuals with HIV infection (26), but are higher than those in chimpanzees experimentally infected with HIV-1, as observed in the present study and by others (14, 15, 22). As in humans the titer of infectious virus in plasma was not consistent and fluctuated from <1 to 500 TCID/ml of plasma. High plasma virus titers are common among AIDS patients but rare in long-term asymptomatic individuals (26).

To determine if fluctuations in plasma viremia were correlated with the host's humoral response we set out to determine neutralizing antibody responses to various HIV-1 isolates. Consecutive serum samples from the naturally infected chimpanzee were subsequently tested against serial isolates for the occurrence of neutralizing antibodies and development of resistance over time.

Neutralization assays with the sequential SIVcpz-ant isolates required the use of human PBMCs because of a lack of replication at high titers on continuous cell lines. Briefly, in each assay 50 $TCID_{50}$ doses of the different virus isolates were incubated for 2 hr with eight different twofold dilutions (starting at 1/10) of each serum sample before adding cells. One hour after adding cells (5 × 10^4 PBMCs), they were washed three times with RPMI 1640 medium and viral growth was measured by the presence of p24 antigen with an HIV p24 antigen capture assay 7 days postculture (27). All neutralization assays were done in duplicate. Table 1 shows the results of assays for neutralizing antibodies (NA) in consecutive serum samples tested against sequential virus isolates. Sera collected at the start of the study (time 0), and at 7, 15, 18, 22, 25, 29, and 33 months were tested for NA against the corresponding virus isolates from the same time point. Again assays were performed using PBMCs. In five of the eight sera tested, neutralizing antibodies to the virus strains were detected, isolated at the same time point, with titers ranging between 1/20 and 1/40. Sera collected at 15 and 18 months were exceptional in that they could neutralize isolates collected after the sera were taken as well as all prior isolates. After the 18-month period no NA were detected which could neutralize subsequent isolates. The titers of NA to the other SIVcpz-ant strains ranged from 1/20 to 1/320. The observation of neutralization-resistant viruses in this naturally infected chimpanzee is consistent with findings in HIV-1-infected humans as well as previous reports on chimpanzees experimentally infected with HIV-1 (28–31).

We next asked if the fluctuations in plasma viremia were due to neutralization escape and if so was escape due to changes in the principal neutralizing domain. When no infectious virus was detected in the plasma, NA titers to the simultaneous isolates ranged from <1/10 to 1/40. Only in two instances when virus titers in plasma were high, were NA to the respective autologous isolates also present with titers 1/20 and 1/40 (Fig. 1). Perhaps due to 3- to 4-month sampling intervals no clear inverse relationship between development of NAs to new isolates and declines in plasma viremia was evident.

The V3 loop or the principal neutralizing domain has been reported to be the principal target for type-specific neutralizing antibodies in HIV-1. We therefore tested the sera from the chimpanzees for antibodies to peptides consisting of 20 to 25 amino acid residues, covering the principal neutralizing domain of the gp120 V3 loop of different HIV-1 strains (MN, SF2, IIIB, RF, MAL, ELI, Z6, ANT-70) and chimpanzee lentiviruses (SIVcpz-gab and SIVcpz-ant) (Neosystems, Strasbourg, France). Antibodies to the V3 loop peptides were tested in an ELISA assay as previously described (32). The chimpanzee with SIVcpz-ant infection had only antibodies to the V3 loop peptides derived from the SIVcpz viruses with antibody titers remaining relatively stable over time (1/800–1/3200). Among the experimentally infected chimpanzees,
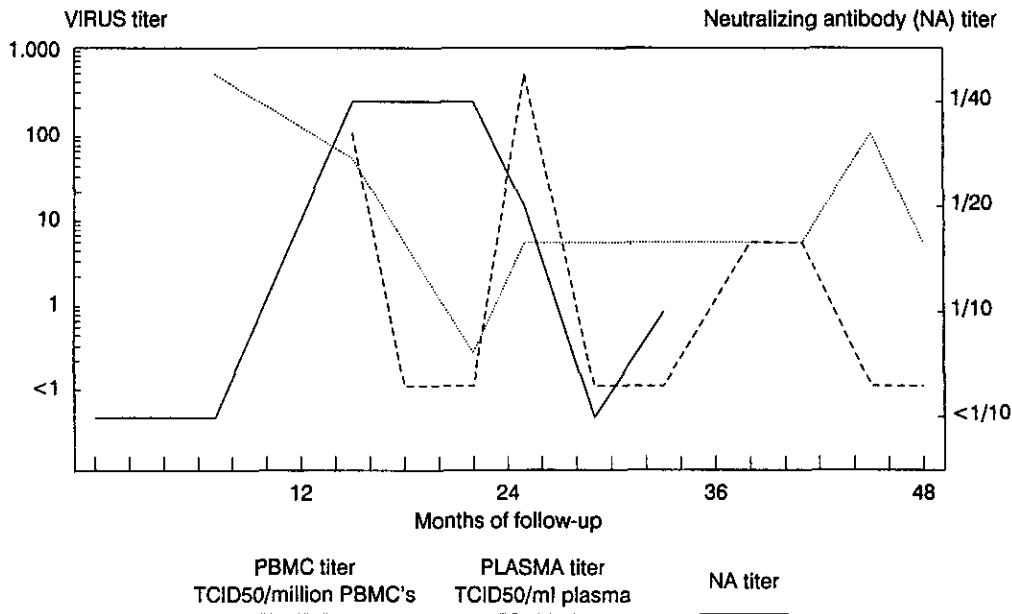
FIG. 1. Titers of infectious virus in plasma and peripheral blood mononuclear cells (PBMCs) and neutralizing antibody titers to the autologous virus isolate in a chimpanzee naturally infected with SIVcpz-ant.

only the HIV-1Lai-infected animals had antibodies to V3 peptides derived from MN and IIIB strains. These results indicate that the naturally and experimentally infected chimpanzees developed a type-specific antibody response to the V3 loop, consistent with the type-specific neutralizing antibody responses found.

To determine if neutralization-resistant isolates from the naturally infected chimpanzee arose due to amino acid changes in the V3 region we sequenced 11 consecutive SIVcpz-ant isolates. This was performed by amplification of the V3 region with specific primers on sequential primary PBMC samples and sequencing reactions on the obtained PCR fragments were done by the Direct Solid Phase sequencing strategy (33). Electrophoresis

and data collection were done on a Pharmacia ALF automatic sequencer. A variant (CIRPGNRTVRNLQIGPGM-TFYNVEIATGDTRRKFC), already present in the first isolate showing one nucleotide substitution at the 3' end of the V3 loop coding sequence (CIRPGNRTVRNLQIGPGM-TFYNVEIATGDTRRAFC) which became dominant after 15 months. This nucleotide substitution resulted in an amino acid substitution where arginine was replaced by lysine. This substitution persisted for the duration of the observation period. The sequence of the V3 loop was also studied on primary PBMCs, collected in March 1993, of the two chimpanzees experimentally infected with Lai in 1984. Compared to the original Lai sequence, no nucleic acid substitution occurred in the V3 region during the 9

TABLE 1

Titers of Neutralizing Antibodies to Sequential SIV cpz-ant Isolates of Sequential Serum Samples
from a Chimpanzee with SIV cpz-ant Infection

Time of serum collection (months)[a]	Neutralization titer against isolates obtained at respective months							
	0	7	15	18	22	25	29	33
0	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
1	1/20	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
7	1/40	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
15	1/160	1/80	1/40	1/40	1/40	<1/10	1/10	<1/10
18	1/80	1/160	1/80	1/40	1/80	1/40	<1/10	<1/10
22	1/40	1/80	1/160	1/80	1/40	<1/10	<1/10	<1/10
25	1/40	1/80	1/160	1/40	1/20	1/20	<1/10	<1/10
29	1/40	1/160	1/320	1/80	1/40	1/20	<1/10	<1/10
33	1/80	1/160	1/160	1/40	1/320	1/20	1/40	1/20
38	1/40	1/320	1/160	1/80	1/80	1/40	1/20	1/20
41	1/80	1/320	1/160	1/40	1/160	1/40	1/40	1/40

[a] After initiation of study.

years that chimp 2 had been infected. For chimp 1 only two amino acids changed in the 9-year period. It has been reported in HIV-1-infected chimpanzees and humans that neutralization-escape mutants may arise from changes of the amino acid sequence in the V3 loop (34–36). However, more recent studies have demonstrated that the mechanism for virus escape from neutralizing antibodies is more complex, as it is in humans which may or may not have an altered V3 amino acid sequence (28, 33, 37). This has also been observed in experimentally infected chimpanzees (29). The remarkably stable V3 sequence of the 11 consecutive SIVcpz-ant isolates suggests that in this infection other regions than the V3 loop play a role in the emergence of neutralization-resistant viruses. Given that the viral population in plasma may be more dynamic, we would have expected more extensive changes in the V3 region sequences in the course of the 40-month observation period.

In summary, there are a number of unique features in this naturally infected animal which distinguish it from other HIV-1 experimentally infected chimpanzees. Firstly, all sequential isolates were NSI. In contrast with the experimentally infected chimpanzees a higher viral load with plasma viremia was observed in the naturally infected animal. The amount of virus present in the plasma greatly fluctuated over time. Both the naturally and the experimentally infected chimpanzees developed type-specific neutralizing antibodies. Virus variants (all NSI) escaped neutralization by autologous sera in the naturally infected animal. The sequence of the V3 loop remained constant over time, suggesting that other envelope regions were involved in neutralization escape.

The neutralizing epitopes of this virus remain to be identified and the extent to which neutralizing epitopes correspond to those of HIV-1 is not known. Persistent infection without clinical manifestations in this chimpanzee offer a rare opportunity to assess the evolution of this virus in its natural host, as well as to define host immune responses which may play a role in preventing disease progression. Questions concerning monocyte/macrophage tropism, cytopathogenic mechanisms, and localization in lymphoid, hematopoetic, and other tissues need to be investigated. Despite the apparent absence of disease in this naturally infected chimpanzee, this animal appears to be unable to restrict viral replication and extracellular viremia, necessitating constant adaptation of the host's humoral immune response to new virus variants. The close relationship of this virus to HIV-1 may help to improve our understanding of the interaction between the host and the virus and may provide valuable insight into HIV-1 pathogenesis.

ACKNOWLEDGMENTS

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