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NOTES

Development of AIDS in a Chimpanzee Infected with Human Immunodeficiency Virus Type 1

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The condition of a chimpanzee (C499) infected with three different isolates of human immunodeficiency virus type 1 (HIV-1) for over 10 years progressed to AIDS. Disease development in this animal was characterized by (i) a decline in $CD4^+$ cells over the last 3 years; (ii) an increase in viral loads in plasma; (iii) the presence of a virus, termed HIV-1_{JC}, which is cytopathic for chimpanzee peripheral blood mononuclear cells; and (iv) the presence of an opportunistic infection and blood dyscrasias. Genetic analysis of the V1-V2 region of the envelope gene of HIV-1_{JC} showed that the virus present in C499 was significantly divergent from all inoculating viruses (≥16% divergent at the amino acid level) and was suggestive of a large quasispecies. Blood from C499 transfused into an uninfected chimpanzee (C455) induced a rapid and sustained $CD4^+$-cell decline in the latter animal, concomitant with high plasma viral loads. These results show that HIV-1 can induce AIDS in chimpanzees and suggest that long-term passage of HIV-1 in chimpanzees can result in the development of a more pathogenic virus.

Experimental infection of nonhuman primates with human immunodeficiency virus type 1 (HIV-1) has been described for gibbons (19), pig-tailed macaques (1), and chimpanzees (3, 8). However, the development of disease (AIDS) has not been previously documented in these animals or in any animal species infected with HIV-1. For many years, chimpanzees have been a major focus in the development of animal models for HIV-1 infection and therapy. The ability to consistently infect chimpanzees with several HIV-1 subtypes and to reisolate the virus over extended periods (8, 18) has made chimpanzees useful for testing vaccine candidates (4, 12, 14). However, the lack of disease in HIV-1-infected chimpanzees has raised concern over the relevance of these vaccine studies. More recently, investigations of HIV-1-infected chimpanzees have focused on analyzing a number of potential mechanisms involved in the lack of disease progression (7, 22, 25). Over 100 chimpanzees have been infected with HIV-1 worldwide. In this study we report the development of AIDS in a chimpanzee infected with HIV-1 for over 10 years and the rapid development of immunosuppression in a chimpanzee transfused with blood from this animal.

All animals were maintained in accordance with the guidelines established by the Animal Welfare Act and the NIH guide for care and use of laboratory animals. The Yerkes Center is fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

To investigate the ability of HIV-1 to induce AIDS in nonhuman primates, a cohort of 12 chimpanzees were inoculated with several strains of HIV-1 at the Yerkes Center in the mid-1980s. As a member of this cohort, C499 was described as part of a previously reported superinfection study (9) and was inoculated on three separate occasions with three different HIV-1 isolates: HIV-1_{SF2} in 1985, HIV-1_{LAV} in 1986, and HIV-1_{NDK} in 1987. The first inoculation resulted in infection, as determined by positive virus isolation and persistent HIV-1-specific antibody response. Clinically, the animal remained healthy until recently, except for the development of thrombocytopenia and lymphopenia in 1988, which resolved without treatment (10). (All HIV-infected chimpanzees are maintained in Biosafety level 3 isolation facilities.) In 1993, after the resumption of yearly monitoring (monitoring of all HIV-1-infected chimpanzees at the Yerkes Center was suspended from March 1990 to May 1993), a decrease in the levels of platelets and $CD4^+$ cells in C499 was observed (24,000 and 390/μl, respectively) (Table 1). Thrombocytopenia and $CD4^+$-cell lymphopenia were persistent in this animal from this point onward. In addition, C499 displayed other significant clinical signs of disease. Beginning in March 1995, C499 developed chronic, intermittent diarrhea for which no enteric pathogens were identified and which was not resolved with antibiotic treatments. In September 1995, this animal developed acute fulminant diarrhea which was associated with large numbers of *Blastocystis hominis* and *Balantidium coli*. At this time, $CD4^+$ cells decreased to extremely low levels (minimum of 10/μl; 2% of total T cells) (Table 1), indicative of severe immunosuppression. Similarly, declines were observed in the levels of total lymphocytes and $CD8^+$ cells (Table 1). Treatment with fluid replacement and antimicrobial and antiprotozoal therapy (doxycycline, ceftriaxone, enrofloxacin, gentamicin, and albendazole) resulted in the resolution of acute diarrhea within 5 days. Because this was the first chimpanzee to develop AIDS,

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TABLE 1. Platelet counts, lymphocyte subset levels, and plasma HIV-1 loads in C499

Date (mo/day/yr)	Platelet count (cells/μl)	Total lymphocyte count (cells/μl)[a]	Absolute $CD4^+$ cell count (cells/μl)	Absolute $CD8^+$ cell count (cells/μl)	Plasma virus level (RNA equivalents/ml)[b]
10/04/88	63,000	3,136	550	1,020	$<10^4$
06/12/89	122,000	3,360	810	1,750	$<10^4$
01/02/90	112,000	3,600	1,040	1,690	$<10^4$
02/06/90	289,000	5,301	1,480	2,490	$<10^4$
05/11/93	24,000	2,461	390	1,720	0.86×10^5
05/18/94	23,000	1,430	300	770	ND[c]
09/11/95	23,000	2,312	160	1,500	1.27×10^5
09/13/95	12,000	532	10	230	1.84×10^5
09/27/95	20,000	4,508	180	4,060	1.65×10^5
10/19/95	12,000	3,854	120	2,960	1.21×10^5
11/16/95	5,000	5,616	170	4,660	1.05×10^5
12/19/95	20,000	1,920	60	1,520	1.19×10^5
01/23/96	12,000	4,366	90	3,150	1.07×10^5
02/13/96	12,000	11,232	110	6,510	0.90×10^5

[a] Lymphocyte subset counts in peripheral blood were determined by FACScan analysis as described previously (2).
[b] Virus levels were determined using the Chiron B-DNA assay as directed by the manufacturer. Plasma samples were stored at −80°C until use.
[c] ND, not done (no plasma sample was available for this date).

treatment with antiretroviral therapies was not administered in order to more fully characterize virological, immunological, and pathological parameters in this animal. As the acute diarrhea was resolved with treatment, $CD4^+$-cell levels rose to a maximum of 180/μl (4% of total T cells) but declined again. Subsequently, chronic, intermittent diarrhea resumed and continued unresolved. During this period, C499 exhibited no lymphadenopathy or wasting. However, beginning in the latter part of 1995 and extending into 1996, C499 developed progressive nonregenerative anemia (hematocrit levels of 37.5% in December 1995 and 27.2% in January 1996, with respective hemoglobin values of 12.1 and 8.4 g/dl; reticulocyte counts were 0.0% since November 1995). Due to progressive hematologic abnormalities, chronic diarrhea, and continued immunosuppression, the animal was euthanized in February 1996.

Concomitant with the decrease in $CD4^+$ cells was an in-

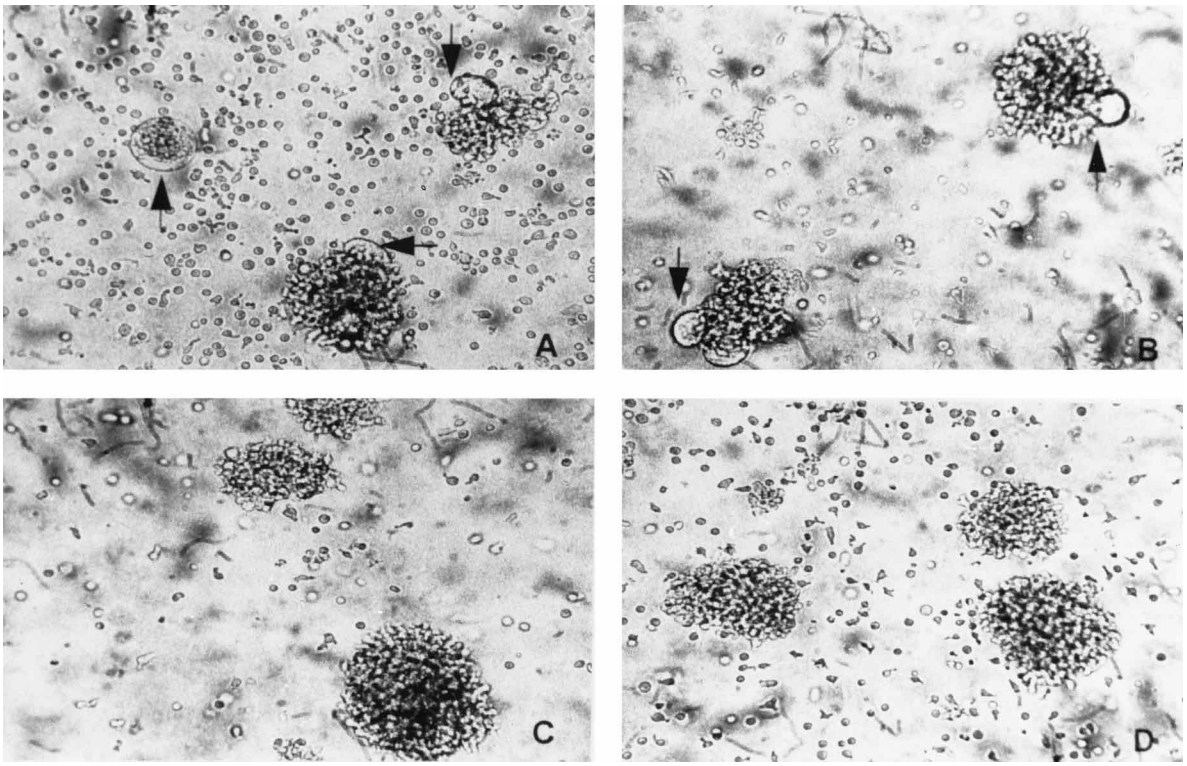

FIG. 1. Syncytium formation in HIV-1-infected cPBMC. The virus strain derived from C499 ($HIV\text{-}1_{JC}$) and other HIV-1 isolates ($HIV\text{-}1_{LAV}$ and $HIV\text{-}1_{SF2}$) were tested for the ability to induce syncytium formation in cPBMC. Virus stocks were prepared in cPBMC ($HIV\text{-}1_{JC}$) or human PBMC ($HIV\text{-}1_{LAV}$ and $HIV\text{-}1_{SF2}$). Cells were incubated with virus overnight and were then washed. Cultures were examined daily for evidence of cytopathic effects. (A and B) cPBMC 4 days after infection with an HIV-1 isolate ($HIV\text{-}1_{JC}$) from C499 showing beginnings of syncytium formation and separated syncytia (arrows). (C) cPBMC 4 days after infection with $HIV\text{-}1_{LAV}$. Note the lack of syncytium formation and the presence of normal cell clusters. (D) cPBMC 4 days after infection with $HIV\text{-}1_{SF2}$, showing no syncytium formation, similar to panel C. The only virus to induce significant cytopathic effects in cPBMC was $HIV\text{-}1_{JC}$. All cultures were examined for 14 days following infection. All cultures, regardless of the virus used, became positive for virus replication by 7 days postinfection.

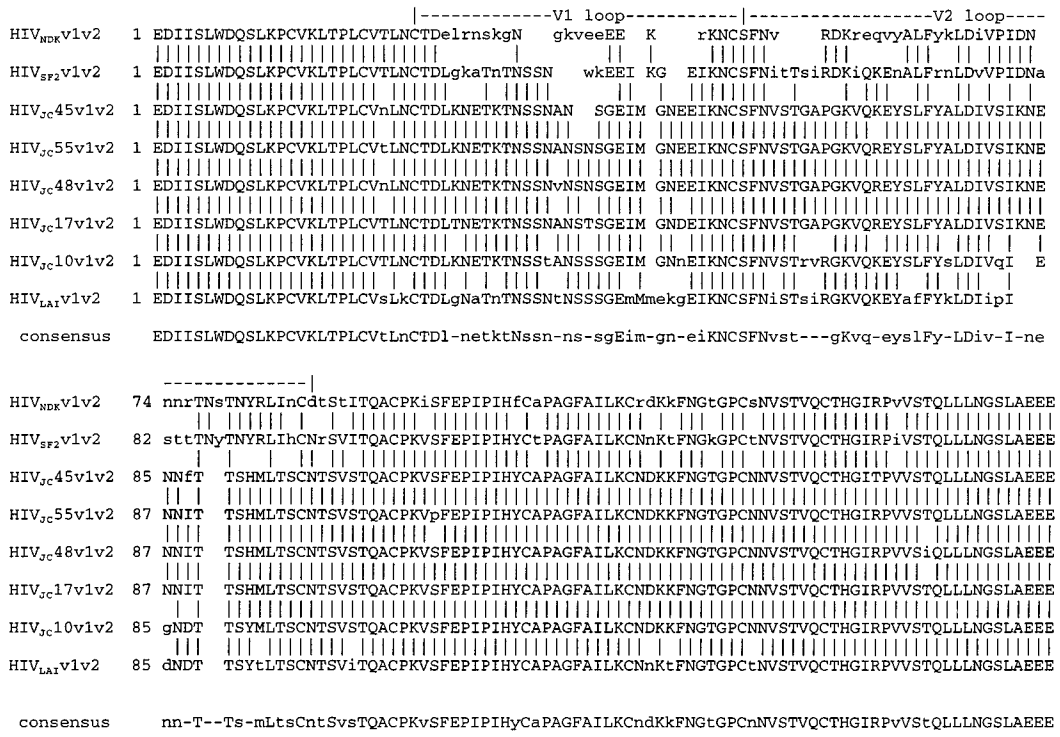

FIG. 2. Amino acid alignment of V1-V2 clones obtained from HIV-1_{JC}-infected cPBMC and prototypes HIV-1_{LAI}, HIV-1_{SF2}, and HIV-1_{NDK}. *env* clones of HIV-1_{JC} encompassing the V1-V2 region were sequenced by the dideoxy chain termination method (Sequenase; Amersham Life Science, Arlington Heights, Ill.). With the Intelligenetics Suite of programs (Intelligenetics, Beaverton, Oreg.), sequences of HIV-1_{JC} *env* fragments were used to derive corresponding amino acid sequences. Deduced amino acid sequences from five of these clones (HIV$_{JC}$10, HIV$_{JC}$17, HIV$_{JC}$45, HIV$_{JC}$48, and HIV$_{JC}$55) were then aligned with the identical region in HIV-1_{LAI}, HIV-1_{SF2}, and HIV-1_{NDK} isolates. Amino acid sequences of HIV-1_{LAI}, HIV-1_{SF2}, and HIV-1_{NDK} were obtained from the Human Retroviruses and AIDS Database (20).

crease in HIV-1 loads in plasma (Table 1). The increase in the level of the virus was detected in plasma samples dating from May 1993, when the $CD4^+$-cell decline was first noted, but not before the suspension of monitoring in 1990. These levels are significantly higher than those for five other chimpanzees at the Yerkes Center which received cell-free or cell-associated HIV-1_{LAV} or HIV-1_{SF2} inoculations (all have undetectable plasma viral RNA levels and are not immunosuppressed [data not shown]). These results suggest that pathogenic effects began to occur sometime between 1990—when $CD4^+$-cell counts were at normal levels and viral loads were undetectable—and 1993—when alterations in the number of $CD4^+$ cells and significant virus loads were present.

The ability to isolate virus from C499 varied since C499's first exposure to HIV-1. In general, early after inoculation, HIV was easily isolated from the peripheral blood mononuclear cells (PBMC) of this animal; however, after several months, virus could no longer be isolated. From August 1988 until the suspension of monitoring in 1990, the virus was consistently isolated from C499 on a monthly basis. Subsequently, after the resumption of monitoring in mid-1993, HIV-1 continued to be easily isolated from this animal. Quantitative titration of PBMC viral load following the development of acute diarrhea in C499 in September 1995 until the time of euthanasia revealed that 10^4 to 10^5 PBMC was consistently required for virus isolation. The immune response of C499 to HIV-1 infection was very strong up to the time of euthanasia. HIV-1 antibody endpoint titers (HIV-1 whole-virus enzyme-linked immunosorbent assay; Genetic Systems, Redmond, Wash.) ranged from 51,200 to 204,800 since 1993. Because of the deteriorating condition of this animal and because of the severe decline in $CD4^+$ cells, it was hypothesized that the HIV-1 present in this animal might have evolved to become more cytopathic for chimpanzee $CD4^+$ cells. Cocultivation of PBMC derived from C499 (obtained at the time of acute diarrhea) with uninfected chimpanzee PBMC (cPBMC) resulted in the isolation of a virus (HIV-1_{JC}) which induced syncytium formation in chimpanzee cells (Fig. 1). This characteristic has been previously described for only three HIV-1 isolates (13, 22, 23), none of which were used for inoculation of C499. Thus, it is reasonable to assume that genetic changes which confer the ability to induce syncytium formation occurred in the virus present in C499.

To confirm that the virus present in C499 was different from the viruses used for inoculation, DNA prepared from HIV-1_{JC}-infected cPBMC was used as a template in typical PCR assays with primers (forward, no. 384: 5′CCCTTCGAAGAG GATATAATCAGTTTATGGGATCAAAGC3′; reverse, no. 383; 5′CCCTTCGAACTCTTCTTCTGCTAGACTGCCATT 3′) designed to amplify a 507-bp fragment of the *env* gene containing the V1 and V2 regions. Genetic analysis of 16 HIV-1_{JC} V1-V2 clones (obtained by ligation of the amplification products with the vector pGEM7ZF (Promega, Madison, Wis.) showed amino acid homologies of 80 to 84% with HIV-1_{LAI}, 73 to 80% with HIV-1_{SF2}, and 63 to 68% with HIV-1_{NDK}. Thus, there appears to be considerable divergence between the virus present in C499 at the time of acute disease and the viruses used to inoculate this animal. This divergence is further illustrated in Fig. 2, which shows amino acid alignments of five HIV-1_{JC} clones, HIV-1_{LAI}, HIV-1_{SF2}, and HIV-1_{NDK}. Comparative analyses between the 16 HIV-1_{JC} clones showed that amino acid homologies ranged from 81 to 96%, with no clones being identical. These results suggest that the virus population in C499 consisted of a large quasispecies. Furthermore, the data, when combined with the in vitro analyses described above, suggest that the virus adapted after years of replication and mutation, becoming more pathogenic for the chimpanzee. While no evidence of recombination is evident from analyses

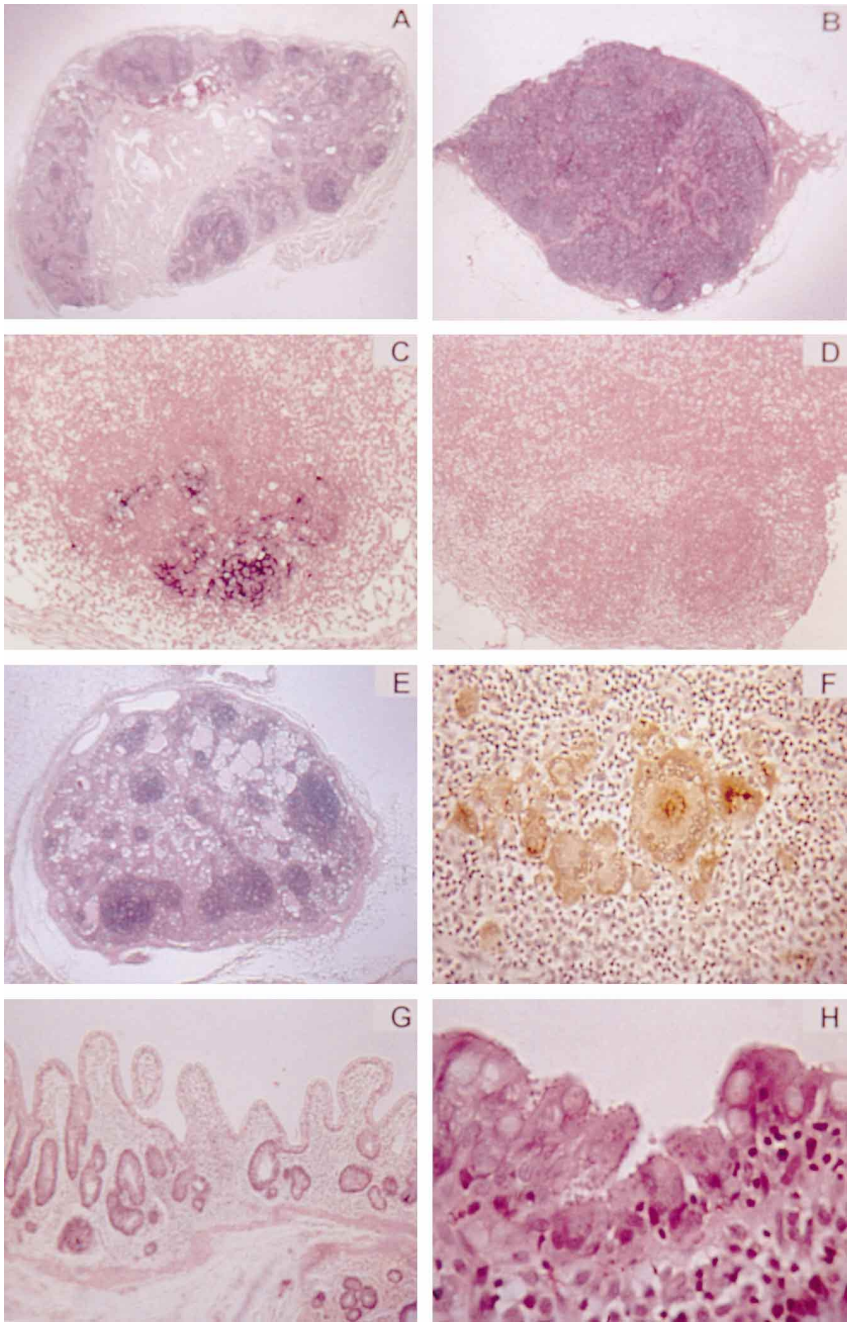

FIG. 3. Histopathologic analyses of tissue obtained from C499. (A) Hematoxylin-eosin-stained section of an inguinal lymph node taken from C499 at the time of acute diarrhea. Generalized lymphoid depletion is present in the cortex and a few germinal centers. (B) Hematoxylin-eosin-stained section of an inguinal lymph node taken from an age-matched, uninfected chimpanzee. This photomicrograph, showing a lack of follicular development and a very cellular cortex, illustrates the typical appearance of a lymph node in uninfected chimpanzees. (C) In situ hybridization analysis of the lymph node section in panel A. Digoxigenin-labeled HIV-1 riboprobes were hybridized to the lymph node section to detect HIV-1-specific RNA as described previously for SIV (16). Results show a diffuse hybridization signal suggestive of dendritic cell trapping of virus within germinal centers. (D) Control in situ hybridization of lymph node section from uninfected chimpanzee with the same anti-sense HIV-1 riboprobes. Note the lack of reactivity. (E) Hematoxylin-eosin-stained section of a mesenteric lymph node taken from C499 after euthanasia. As in panel A, there is lymphoid atrophy and follicular depletion. (F) Immunohistochemical staining of HIV-1 p24 antigen in a deep cervical lymph node obtained from C499 after euthanasia. This section shows a focus of HIV-1 antigen-positive macrophages and multinucleated giant cells. Formalin-fixed, paraffin-embedded tissues were reacted with a monoclonal antibody (KAL-1; Dako) directed to HIV-1 p24. Bound antibody was detected with an immunoperoxidase technique (Vector ABC; Vector Laboratories, Burlingame, Calif.) with DAB as the substrate chromogen, similar to previously described methods (16). (G) Hematoxylin-eosin-stained section of ileum obtained from C499 after euthanasia. Note the blunting of villi and the presence of inflammatory infiltration due to *Cryptosporidium* infection. (H) Higher magnification of a hematoxylin-eosin-stained section of ileum from C499 showing numerous *Cryptosporidium* organisms lining the apical surface of the intestinal epithelial cells.

performed in this small area, the possibility of recombination cannot be ruled out for other portions of the HIV-1_{JC} genome.

To investigate additional pathogenic effects of HIV-1 infection on C499, tissue samples obtained by biopsy during the acute diarrheal stage (September 1995) and at necropsy were subjected to histopathological analyses. First, examination of a peripheral lymph node (obtained in September 1995) revealed marked lymphoid depletion within the cortical area, with a few

follicles remaining (Fig. 3A), compared with a lymph node from an age-matched, uninfected chimpanzee (Fig. 3B). To detect virus expression in tissues, in situ hybridization experiments with digoxigenin-labeled riboprobes encompassing the entire HIV-1 genome (derived from the HIV-1 BH10 molecular clone [15]) were used to probe formalin-fixed lymph node sections. Detection of bound probes was performed with a sheep anti-digoxigenin-alkaline, phosphatase-labeled Fab monoclonal antibody and with Nitro Blue Tetrazolium-5-bromo-4-chloro-3-indolylphosphate toluidinium as the substrate chromogen, according to previously described methods (17). In situ hybridization studies of C499's lymph node (Fig. 3C) demonstrated the presence of HIV-1 RNA in follicular trapping patterns, with few positively staining cells. These findings are similar to changes observed in HIV-1-infected persons developing AIDS (21). Control samples, which included sense riboprobes (Fig. 3D) and lymph nodes from uninfected chimpanzees, did not show any staining. Additional lymph nodes obtained from C499 at necropsy showed a similar depleted pattern (Fig. 3E). However, a few lymph nodes were not as depleted but did contain multinucleated giant cells which stained positive for the HIV-1 p24 antigen (Fig. 3F). These giant cells are often found in the lymph nodes of simian immunodeficiency virus (SIV)-infected macaques and are occasionally found in the lymph nodes of HIV-1-infected persons. Finally, histopathologic analysis of intestinal tissue from C499 revealed pathologic changes in the ileum, with significant blunting of the villi and intense infiltration of mononuclear cells and plasma cells (Fig. 3G). Examination of the intestinal mucosa at higher magnification revealed extensive infection with *Cryptosporidium* (Fig. 3H), which was present throughout the small intestine. This organism is an AIDS-defining opportunistic pathogen (5) and probably accounted for the chronic intermittent diarrhea and intestinal pathology in C499. Most tissues obtained from C499 following euthanasia appeared grossly normal, with no lymphadenopathy or splenomegaly. However, the spleen showed moderate congestion and lacked follicle development. Bone marrow specimens obtained at euthanasia showed some functional impairment in CFU granulocyte macrophage and CFU formation, although levels of $CD34^+$ cells were not altered (24). Virus was isolated from all lymphoid organs including inguinal, axillary, and mesenteric lymph nodes as well as from the spleen and thymus. Virus was also isolated from the kidney and liver but not from the brain or cerebrospinal fluid.

At the time of acute diarrhea in C499, a blood transfusion was performed to determine the effects of passage of virus from this animal to an uninfected chimpanzee. Forty milliliters of blood obtained by venipuncture from C499 was immediately transfused intravenously into C455. This chimpanzee, which was bred in captivity, was seropositive for Epstein-Barr virus and cytomegalovirus and was seronegative and PCR negative for HIV prior to the transfusion. Results of titration analysis of PBMC and plasma from C499 show that in the 40 ml of blood, 1×10^4 50% tissue culture infective doses ($TCID_{50}$) of virus was in PBMC and 2×10^4 $TCID_{50}$ of virus was in plasma. Thus, C455 received a total of 3×10^4 $TCID_{50}$ of virus. Analysis of peripheral $CD4^+$ cell levels in C455 revealed a precipitous decline beginning by 2 weeks after transfusion, when absolute numbers of $CD4^+$ cells decreased from 1,240 to 320 cells/μl (Fig. 4). This decline continued, reaching a minimum value of 10 cells/μl (1% of total T cells) by 14 weeks posttransfusion. Since this time, the total percentage of T cells that the $CD4^+$ population encompasses has remained constant at 1%, with only a slight rise in the absolute number of $CD4^+$ cells (20 cells/μl at the latest time point). Levels of peripheral $CD8^+$ cells showed an initial decline from 1,590 to 880 cells/μl in the first 2 weeks after transfusion. However, these levels quickly rebounded to 2,010 $CD8^+$ cells/μl by 8 weeks after transfusion. Since this time, the number of $CD8^+$ cells in circulation has been maintained between 550 and 4,320/μl, with the level in most recent sample being 840 cells/μl. Because $CD4^+$-cell levels declined so rapidly, the level of virus present in C455 was investigated by quantitation of plasma HIV-1 RNA with the Chiron B-DNA assay (Fig. 4). Two weeks after transfusion, plasma viral loads were 2×10^7 RNA equivalents/ml and reached maximum levels by 4 weeks posttransfusion (6.2×10^7 RNA equivalents/ml). Following a decline (which corresponded with the development of anti-HIV-1 specific antibody), plasma HIV-1 levels have been maintained at $\sim 1.1 \times 10^5$ RNA equivalents/ml. Virus has been easily isolated from C455 at all times posttransfusion. Quantitative coculture of PBMC from C455 has shown that early after transfusion, virus could be isolated from as few as 10^2 PBMC, while at more recent time points, up to 10^5 to 10^6 cells was required for virus isolation. In vitro analysis of virus isolated from this animal at several time points has shown that the ability to induce syncytium formation in cPBMC has been retained (data not shown), further implicating this virus in the pathogenesis of $CD4^+$-cell decline. Antibody responses to HIV-1 have been moderate in C455. Enzyme-linked immunosorbent assay titers have been maintained between 1,600 and 6,400 since 4 weeks posttransfusion. Clinically, C455 has appeared normal, except for episodic incidences of a rash on the chest and in the scrotum area. In addition, the animal has experienced no weight loss, lymphadenopathy, or anemia (normal hematocrit levels).

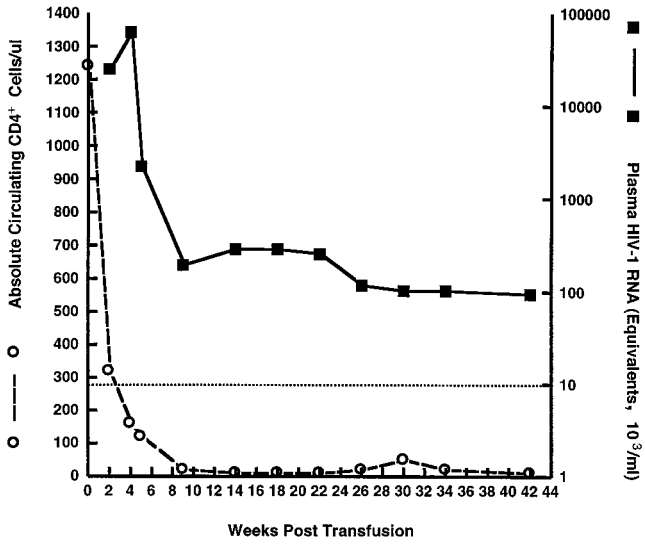

FIG. 4. $CD4^+$-cell decline and plasma virus loads in C455 following transfusion with blood from C499. Immediately before and at various times after transfusion, blood was collected from C455 for in vitro analyses of $CD4^+$-cell levels and plasma virus loads. Absolute peripheral $CD4^+$ cells in C455 showed a dramatic decrease by 2 weeks posttransfusion. This rapid decline continued, and by 14 weeks after transfusion, the number of $CD4^+$ cells decreased to 10/μl. These low cell numbers have been maintained to date (42 weeks posttransfusion). Plasma HIV-1 RNA loads in C455 showed high levels of virus present by 2 weeks after transfusion. Results obtained at the 4-week point suggest very high levels of virus replication in C455. However, HIV-1 levels appeared to be somewhat controlled by 5 to 9 weeks posttransfusion. The cutoff level of 10^4 equivalents/ml (– – –) is the lower limit of the assay.

In summary, the results presented here demonstrate that a chimpanzee infected with HIV-1 developed AIDS as defined by the Centers for Disease Control and Prevention classification system (5). Progression of clinical disease (anemia, throm-

bocytopenia, and chronic diarrhea) in this animal was associated with several key findings, including the following: (i) the presence of a virus which is cytopathic for cPBMC in vitro and in vivo and is genetically distinct from those used for inoculation, (ii) an increase in viral load; (iii) $CD4^+$-cell depletion, (iv) lymph node depletion, and (v) the presence of *Cryptosporidium* organisms in the intestine. It appears that the critical change(s) associated with clinical progression may have developed during the period in which C499 was not monitored (mid-1990 through mid-1993). The precipitous $CD4^+$-cell decline concomitant with high viral loads displayed in C455, transfused with blood from C499, suggest that the HIV present in C499 has evolved to become more pathogenic for chimpanzees. The increased pathogenicity of a lentivirus after passage into a new host has been previously observed with the adaptation of SIV from sooty mangabeys to pig-tailed macaques, resulting in the development of the acutely lethal strain SIVsmmPBj14 (11).

The relevance of the HIV-1-infected chimpanzee as a model for vaccine evaluation has been questioned due to lack of disease development. Indeed, the lack of an animal model which supports pathogenic HIV-1 infection has been a continuing problem for vaccine development. Although the time of progression to disease (>10 years), the currently limited numbers of animals available for use, and the overall high costs associated with working with chimpanzees are deterrents to their widespread use in AIDS research, the potential usefulness of this model should not be disregarded. The development of AIDS in C499, the fact that additional HIV-infected chimpanzees at the Yerkes Center have depressed $CD4^+$-cell counts (<500/μl) and thrombocytopenia, and the rapid progression of the $CD4^+$-cell decline in C455 support and strengthen the role that this animal model may play in future AIDS-related studies. Alternatively, the likely adaptation of HIV-1 from long-term chimpanzee infection to a pathogenic form may provide a critical link for the adaptation of HIV-1 to growth in more readily available nonhuman primate species. Attempts are now in progress to determine whether the adapted virus from C499 is capable of growing in pig-tailed or rhesus macaques. In addition, continued biological and genetic characterization of $HIV\text{-}1_{JC}$ may provide key insights into the pathogenesis of HIV-1 infection in humans and chimpanzees.

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