

IMMUNOLOGIC AND VIROLOGIC STATUS AFTER 14 TO 18 YEARS OF INFECTION WITH AN ATTENUATED STRAIN OF HIV-1

A Report from the Sydney Blood Bank Cohort

JENNIFER C. LEARMONT, ANDREW F. GECZY, D.Sc., JOHN MILLS, M.D., LESLEY J. ASHTON, M.P.H.,
 CAMILLE H. RAYNES-GREENOW, G.D.P.H., ROGER J. GARSIA, Ph.D., WAYNE B. DYER, B.Sc.,
 LARISSA MCINTYRE, M.P.H., ROBERT B. OELRICHS, Ph.D., DAVID I. RHODES, Ph.D., NICHOLAS J. DEACON, Ph.D.,
 AND JOHN S. SULLIVAN, Ph.D., FOR THE SYDNEY BLOOD BANK COHORT RESEARCH GROUP

ABSTRACT

Background and Methods The Sydney Blood Bank Cohort consists of a blood donor and eight transfusion recipients who were infected before 1985 with a strain of human immunodeficiency virus type 1 (HIV-1) with a deletion in the region in which the *nef* gene and the long terminal repeat overlap. Two recipients have died since 1994, at 77 and 83 years of age, of causes unrelated to HIV infection; one other recipient, who had systemic lupus erythematosus, died in 1987 at 22 years of age of causes possibly related to HIV. We present longitudinal immunologic and virologic data on the six surviving members and one deceased member of this cohort through September 30, 1998.

Results The five surviving recipients remain asymptomatic 14 to 18 years after HIV-1 infection without any antiretroviral therapy; however, the donor commenced therapy in February 1999. In three recipients plasma concentrations of HIV-1 RNA are undetectable (<200 copies per milliliter), and in two of these three the CD4 lymphocyte counts have declined by 9 and 30 cells per cubic millimeter per year ($P=0.3$ and $P=0.5$, respectively). The donor and two other recipients have median plasma concentrations of HIV-1 RNA of 645 to 2850 copies per milliliter; the concentration has increased in the donor ($P<0.001$). The CD4 lymphocyte counts in these three cohort members have declined by 16 to 73 cells per cubic millimeter per year ($P<0.001$). In the recipient who died after 12 years of infection, the median plasma concentration of HIV-1 RNA was 1400 copies per milliliter, with a decline in CD4 lymphocyte counts of 17 cells per cubic millimeter per year ($P=0.2$).

Conclusions After prolonged infection with this attenuated strain of HIV-1, there is evidence of immunologic damage in three of the four subjects with detectable plasma HIV-1 RNA. The CD4 lymphocyte counts appear to be stable in the three subjects in whom plasma HIV-1 RNA remains undetectable. (N Engl J Med 1999;340:1715-22.)

©1999, Massachusetts Medical Society.

IT is well recognized that host and viral factors modify the rate at which infection with the human immunodeficiency virus type 1 (HIV-1) progresses to the acquired immunodeficiency syndrome (AIDS). Identified host factors include age¹⁻⁴ and genetic background.⁵⁻⁸ The *nef* gene is a major determinant of virulence in primate lentiviruses. Strains of simian immunodeficiency virus (SIV) lacking the *nef* gene have been shown to be less pathogenic in macaques and to replicate less well in vivo than isogenic strains with an intact *nef* open reading frame.⁹ Kirchhoff et al. reported a single case of long-term, factor VIII-transmitted, nonprogressive infection due to a strain of HIV-1 with deletions in the proximal *nef* gene and the region in which the *nef* gene and the long terminal repeat (LTR) overlap that were distinct in size and exact position from those of the virus infecting persons in the Sydney Blood Bank Cohort.¹⁰ The Sydney Blood Bank Cohort is a group of HIV-1-infected persons with apparently nonprogressive HIV infection, comprising a blood donor and eight persons who received transfusions of blood products from that donor. The group was first described in 1992.¹¹ Subsequently, these subjects were shown to be infected with a strain of HIV-1 (subtype B) with a conserved deletion of 150 or more base pairs (bp) in the *nef*-LTR overlap region and with duplications and rearrangements of nuclear factor- κ B (NF- κ B) and Sp1 transcription factor binding sites in the LTR.¹²

We present a comprehensive analysis of all the longitudinal immunologic and virologic data available on this unique cohort through September 30, 1998.

From the Australian Red Cross Blood Service—New South Wales, Sydney (J.C.L., A.E.G., C.H.R.-G., W.B.D., L.M., J.S.S.); the National Centre in HIV Virology Research and the Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria (J.M., R.B.O., D.I.R., N.J.D.); the National Centre in HIV Epidemiology and Clinical Research, Sydney (L.J.A.); and the Royal Prince Alfred Hospital, Sydney (R.J.G.) — all in Australia. Address reprint requests to Ms. Learnmont at the Australian Red Cross Blood Service—NSW, 153 Clarence St., Sydney, NSW 2000, Australia, or at jlearnmont@arcbs.redcross.org.au.

Other authors were Dale A. McPhee, Ph.D., Suzanne Crowe, M.B., B.S., Ajantha E. Solomon, B.Sc., Catherine Chatfield, B.Sc., and Ian R.C. Cooke, Ph.D., National Centre in HIV Virology Research and the Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria; and Sean Blasdall, M.App.Sc., and Harmjan Kuipers, M.Sc., Australian Red Cross Blood Service—New South Wales, Sydney.

METHODS

Subjects

The recipients and the donor in the Sydney Blood Bank Cohort were traced by the Australian Red Cross Blood Service—New South Wales. The recipients had received transfusions of HIV-1–infected blood products donated before May 1985, when universal screening of blood products for HIV was introduced in Australia. For the recipients, the time since infection was calculated from the date of transfusion with the HIV-1–infected blood product. For the donor, it was estimated as the midpoint between the date of collection of the last identified negative donation (i.e., that resulting in no HIV infection in the recipient) and the first identified positive donation (that resulting in HIV infection in the recipient) (Table 1). Three recipients (Recipients 5, 8, and 10) have died, and their medical records have been reviewed. The case of Recipient 8 has already been reported.¹¹ This patient's medical records were reexamined, and further data were obtained. Permission to perform an autopsy on Recipient 5 was refused, but the death certificate was reviewed and the treating physician was interviewed. An autopsy was performed on Recipient 10, and the treating physician was interviewed.

Laboratory Testing

The absolute numbers of circulating lymphocytes were enumerated with a Coulter S Plus IV counter (Coulter, Hialeah, Fla.). The proportions of T-lymphocyte subgroups were determined by the whole-blood lysis method (Q-Prep, Coulter). The percentages of CD4 and CD8 lymphocytes were determined by direct immunofluorescence with monoclonal antibodies (Ortho Diagnostics, Raritan, N.J., and Coulter) and expressed as the numbers of CD4 and CD8 cells per cubic millimeter. Concentrations of HIV-1 RNA in plasma (viral load) were measured with the Amplicor HIV-1 Monitor kit (Roche Diagnostics, Nutley, N.J.). Blood for RNA quantification was collected in acid–citrate–dextrose anticoagulant, plasma-separated within six hours, and stored at -80°C until it was thawed for assay.

The *nef*-LTR region of the HIV-1 provirus was amplified by triple-nested or booster polymerase chain reaction (PCR) from genomic DNA extracted from peripheral-blood mononuclear cells.¹² The region was cloned and sequenced as previously described.¹³ The *CCR5* gene was analyzed for the previously described 32-bp deletion ($\Delta 32$) in genomic DNA from the subjects' peripheral-blood mononuclear cells by the method of Dean and colleagues.⁵ Genotype determination for the stromal-derived factor (*SDF-1*) and *CCR2* alleles was performed by PCR amplification and restriction-fragment–length polymorphism analysis as described in the literature.^{14,15} Virus was isolated by techniques based on those of Neate et al.,¹⁶ with the following modification: peripheral-blood mononuclear cells from selected donors were phytohemagglutinin-activated and then cocultured with fresh peripheral-blood mononuclear cells from the Sydney Blood Bank Cohort that had been separated by Ficoll–Hypaque density-gradient centrifugation, and 20 percent of the cell population was treated on day 0 with ultraviolet irradiation.¹⁷ Viral replication was quantified by extracellular soluble p24 production according to the manufacturer's instructions (Organon Teknica, Durham, N.C.).

Statistical Analysis

Changes in T-cell subgroups, circulating lymphocytes, and viral load with time were assumed to be linear, and regressions were calculated by the least-squares method on the basis of 11 determinations for Recipient 4 and between 17 and 39 determinations for the other members of the cohort. The data were analyzed through September 30, 1998. Statistical analyses were performed with Stata statistical software (release 5.0, Stata, College Station, Tex.). All reported P values are two-sided.

RESULTS

Epidemiology

The donor and six recipients (Recipients 7, 8, 9, 10, 12, and 13) were identified before 1992 from the Transfusion Acquired HIV Registry in New South

TABLE 1. OUTCOME IN 13 PERSONS WHO RECEIVED BLOOD PRODUCTS FROM THE DONOR BETWEEN 1980 AND 1984.*

RECIPIENT No.	DATE OF BIRTH	SEX	DATE OF TRANSFUSION	INDICATION FOR TRANSFUSION	PRODUCT	HIV INFECTION	AGE AT INFECTION (YR)	OTHER INFORMATION
1†	7/10/17	NA	8/20/80	NA	Red cells	No	—	
2†	NA	NA	11/12/80	NA	Red cells	No	—	
3†	NA	NA	11/16/80	NA	Plasma	No	—	
4†	2/23/46	Male	2/11/81	Motor vehicle accident	Red cells	Yes	34	
5†	9/30/17	Female	4/29/81	Coronary-artery bypass	Red cells	Yes	53	Died 10/17/94
6†	1/14/38	Female	7/13/81	Renal dialysis	Red cells	No	—	
7	7/11/37	Male	1/2/82	Coronary-artery bypass	Red cells	Yes	44	Prednisone for asthma since 1/95
8	12/21/64	Female	12/30/82	Renal failure	Platelets	Yes	18	Died 4/24/87
9	3/20/26	Female	5/4/83	Acoustic neuroma	Red cells	Yes	57	
10	10/12/12	Male	8/31/83	Colectomy	Red cells	Yes	71	Died 11/11/95
11	3/19/74	Female	3/9/84	Thalassemia	Red cells (triple-washed)	No	—	
12	6/9/54	Female	6/11/84	Antepartum hemorrhage	Red cells	Yes	30	
13	2/17/28	Male	7/24/84‡	Acute hematemesis	Red cells	Yes	56	Insulin-dependent diabetes, hepatitis C

*NA denotes not available.

†The recipient was identified after October 1992.

‡There were no more transfusions from the donor after this date.

Wales, Australia.¹¹ Two more recipients infected by blood products from the donor (Recipients 5¹⁸ and 4¹⁹) were identified in 1993 and 1996, respectively. A total of 13 persons who received blood components from the donor between August 1980 and the last donation in July 1984 have been identified (Table 1). Three recipients (Recipients 1, 2, and 3) who received blood products between August and December 1980 are HIV-seronegative. The first recipient to become infected (Recipient 4) received the donor's next donated unit in February 1981. Only 2 of the 10 units transfused after February 1981 failed to transmit HIV-1 (in Recipients 6 and 11). Repeated testing of both these recipients by enzyme immunoassay and immunoblotting has failed to detect antibodies to HIV. In addition, peripheral-blood mononuclear cells from Recipient 6 lacked HIV-specific cytotoxic T lymphocytes (Dong T, Rowland-Jones S: personal communication). After reviewing all available medical and transfusion records, we could not confirm that the donated unit, although it had been cross-matched for Recipient 6, had actually been transfused. Recipient 11 received a unit of erythrocytes that had been triple-washed, a procedure known to remove HIV-1 in some instances.²⁰ Additional tracing identified eight deceased recipients who had received units from the donor between February 1981 and late 1984 and who had died from causes clearly related to their original diagnoses.

In two of the three recipients who have died (Recipients 5 and 10), the causes of death were clearly unrelated to HIV infection. Because they were not described in our original report,¹¹ the findings in these two patients as well as those in Recipient 4 are provided below. In addition, further details on the third deceased recipient (Recipient 8) are given.

Findings in Recipients 4, 5, 8, and 10

Recipient 8 was infected with HIV by a blood transfusion on December 30, 1982, and died of combined *Pneumocystis carinii* and pneumococcal pneumonia in April 1987. When systemic lupus erythematosus was diagnosed in August 1982, the patient was given prednisone (60 mg per day, reduced to 20 mg per day). In late 1984, she had a severe exacerbation of systemic lupus erythematosus, with pulmonary vasculitis and marked hemoptysis, while taking prednisone (20 mg per day). She was hospitalized and treated intravenously with prednisone (2 g per day) and cyclophosphamide (200 mg per day). She was discharged with a prescription for oral prednisone (15 mg per day), but compliance was erratic. Her condition worsened, and from May 1986 to February 1987 she was treated with azathioprine (100 mg per day) and prednisone (15 mg per day). In February 1987, the dose of prednisone was increased to 60 mg per day and the dose of azathioprine to 150 mg per day. The patient was hospitalized on March 20, 1987,

with respiratory symptoms. Her total lymphocyte count was 300 cells per cubic millimeter. A sample of sputum obtained soon after admission was negative for *P. carinii*. Because of severe systemic lupus erythematosus, she was given a single intravenous dose of 850 mg of cyclophosphamide and 400 mg of intravenous hydrocortisone per day, and her total lymphocyte count subsequently decreased to 100 cells per cubic millimeter. She did not receive *P. carinii* prophylaxis. *P. carinii* pneumonia developed on April 3, 1987, and pneumococcal pneumonia coinfection was diagnosed later. On April 15, 1987, nine days before the patient died from respiratory failure, a diagnosis of HIV infection was made, and her first and only lymphocyte-subgroup analysis revealed 90 CD4 lymphocytes per cubic millimeter at a time when she had a total lymphocyte count of 700 per cubic millimeter. Two years before her death, a blood sample had been collected, and genomic DNA from peripheral-blood mononuclear cells had been stored at -20°C . HIV-1 *nef*-LTR sequences were identified in this sample only after a fourth round of nested PCR amplification. Analysis of the PCR products showed deletions and mutations in the *nef*-LTR region that were characteristic of the Sydney Blood Bank Cohort attenuated quasispecies of HIV-1 (data not shown).

Recipient 5, also deceased, was infected in April 1981 from a transfusion of erythrocytes given during coronary-artery bypass surgery. She was identified in 1993 and died on October 17, 1994, at the age of 77 years, from metastatic gastric cancer unrelated to HIV infection. The immediate cause of death was acute hepatorenal syndrome secondary to carcinoma of the stomach, with liver metastases for 18 months. This recipient had no clinical or laboratory signs of HIV progression, and her only CD4 lymphocyte count, obtained 12 years after infection, was 770 cells per cubic millimeter. She also had a host genotype recently identified¹⁴ as being associated with delayed progression to AIDS — a homozygous 3' A mutation in the *SDF-1* β_2 gene (data not shown).

The third deceased recipient, Recipient 10, was infected in August 1983 from a transfusion of erythrocytes given during a colectomy for colon cancer. In October 1995, pneumonia, dementia, and atrial fibrillation developed, and the patient died on November 11, 1995, at the age of 83 years. A full autopsy revealed severe atherosclerosis of the coronary arteries. There was no gross or histologic evidence of HIV infection; lymphoid size and structure were normal for the patient's age; and cultures of spleen, brain, kidney, lymph node, and lung tissue for HIV were negative. The direct cause of death was recorded as bacterial pneumonia.

The last traced recipient, Recipient 4, was identified in 1996. He received a unit of erythrocytes in February 1981. His first HIV serologic result, on Febru-

ary 2, 1996, was weakly positive according to enzyme-linked immunosorbent assay with an indeterminate Western blot, and subsequent results have been similar. HIV-1 DNA sequences consistent with the Sydney Blood Bank Cohort HIV-1 quasispecies have been found in genomic DNA from peripheral-blood mononuclear cells amplified by nested PCR. The patient remains free of signs or symptoms of HIV infection, with a viral load below the limit of detection and a borderline but stable median CD4 lymphocyte count of 480 per cubic millimeter (Table 2). This member of the Sydney Blood Bank Cohort has also been identified as having a host genotype associated with the slow progression of HIV-1 infection⁵ — a heterozygous $\Delta 32$ mutation of the *CCR5* gene (data not shown).

Laboratory Results

The median plasma HIV-1 RNA concentrations in the seven tested members of the Sydney Blood Bank Cohort (Table 2) ranged from below the limit of detection in three members (Recipients 4, 9, and 12) to between 645 and 2850 copies per milliliter in four members (the donor and Recipients 7, 10, and 13); the donor has had an increase in viral RNA in the past year. HIV-1 has been isolated from cultures of peripheral-blood mononuclear cells from five members of the Sydney Blood Bank Cohort, and with the exception of that from the donor, all isolates have had the classic non-syncytium-inducing phenotype, confirmed by coreceptor use in human osteosarcoma (HOS) cells. In contrast, cultures from the donor have consistently yielded HIV-1 isolates capable of productively infecting MT-2 lymphoblastoid cells and with a dual-tropic V3 loop sequence.

When first tested five years after infection, the donor had CD4 lymphocyte counts that were low but within the normal range.* There has subsequently been a gradual, but definite, downward trend, with an average decrease of 16 cells per cubic millimeter per year. From 1996 to 1998, six of seven determinations revealed 500 or fewer cells per cubic millimeter, with the lowest value being 282 (in September 1998). The donor declined antiretroviral therapy until February 1999. He then commenced antiretroviral therapy because of a further decrease in his CD4 lymphocyte cell count, to 160 per cubic millimeter, in January 1999 and the development of HIV-related meningoencephalitis. Two other cohort members, Recipients 7 and 13, have also had significant decreases in CD4 lymphocyte counts, averaging 73 and 58 cells per cubic millimeter per year, respectively ($P < 0.001$) (Table 2 and Fig. 1). The proportion of CD4 lymphocytes has declined significantly in five cohort

members ($P < 0.01$) (Table 2).* Three members of the cohort had significant ($P < 0.001$) increases in CD8 lymphocyte counts, and two other members had increases in CD8 lymphocyte counts that were of borderline statistical significance (Table 2). In Recipient 13, the high values for total lymphocyte counts (data not shown) and for the CD4 and CD8 subgroups were attributed to an earlier splenectomy.²¹ The three surviving members of the Sydney Blood Bank Cohort with detectable viral loads have declining CD4 counts, whereas none of those with undetectable viral loads have declining values. Recipient 7, the recipient with the lowest detectable viral load, had the most rapid decline in CD4 numbers, whereas the donor, who had the highest viral load of any member of the cohort (and a dual-tropic isolate), had the slowest CD4 lymphocyte decline, albeit from a low base line. The deceased Recipient 10, with a relatively low viral load, had the greatest increases in CD8 lymphocyte counts. The donor, over the past year, had rapidly rising CD8 lymphocyte counts. Scores for cutaneous delayed-type hypersensitivity testing with the Multitest CMI (Pasteur-Mérieux, Lyons, France) were normal for all surviving Sydney Blood Bank Cohort recipients, whereas the donor was anergic.²²

DISCUSSION

The attenuation of the Sydney Blood Bank Cohort strain of HIV is substantiated by the prolonged AIDS-free survival without therapy of the recipients of infected blood, as compared with that of other cohorts of HIV-1-infected subjects, in which, regardless of the age of the subjects or the mode of transmission, the median time of progression to AIDS has ranged from 7.2 to 11 years.^{1,4,23,24} After having been infected with HIV-1 for 12 to 13 years, two members of the Sydney Blood Bank Cohort died at advanced ages of conditions almost certainly unrelated to HIV-1 infection. However, the cause of death of the recipient with severe systemic lupus erythematosus, Recipient 8, will never be entirely clarified. Although this patient had *P. carinii* pneumonia and a single low CD4 count several days before death, it is well recognized that patients with systemic lupus erythematosus treated with high-dose glucocorticoids or other immunosuppressive medications may have low CD4 counts and inverted CD4:CD8 ratios, and may have pneumocystis pneumonia.²⁵⁻²⁷ Alternatively, it is possible that immunosuppressive therapy for systemic lupus erythematosus augmented replication of the attenuated strain of HIV-1 that infected this patient, with additive or synergistic immunosuppressive effects. The difficulty in amplifying viral sequences from DNA from this patient's peripheral-blood mononuclear cells, which could be accomplished only after quadruple-nested PCR, argues against the latter possibility.

The Sydney Blood Bank Cohort strain of HIV

*See NAPS document no. 05523 for 4 pages of supplementary material. To order, contact NAPS, c/o Microfiche Publications, 248 Hempstead Tpke., West Hempstead, NY 11552.

TABLE 2. LABORATORY RESULTS FOR THE DONOR AND SIX OF THE RECIPIENTS.*

VARIABLE	DONOR	RECIPIENT 4	RECIPIENT 7	RECIPIENT 9	RECIPIENT 12	RECIPIENT 13	RECIPIENT 10 (DECEASED 1995)
Length of infection (yr)†	17.8	17.6	16.7	15.4	14.3	14.2	12.0
Length of follow-up (yr)‡	13.8	2.6	10.3	10.8	11.3	11.3	8
HIV RNA							
Median (copies/ml)	2850	<200	645	<200	<200	2300	1400
Range (copies/ml)	500–20,812	<200	<200–1000	<200	<200	400–3500	1200–1600
Slope (copies/ml/yr)	3819	—	-114	—	—	225	-291
P value	<0.001	—	0.305	—	—	0.409	0.511
95% CI (copies/ml/yr)	2251 to 5387	—	-354 to 126	—	—	-353 to 804	-1873 to 1290
Virus isolated in culture	Yes	No	Yes	Yes	No	Yes	Yes
CD4 count§							
Median (cells/mm ³)	504	480	693	900	1008	1466	769
Range (cells/mm ³)	282–713	392–646	391–1225	601–1240	450–1458	882–2088	394–1080
Slope (cells/mm ³ /yr)	-15.94	-29.96	-73.33	-8.70	14.37	-58.06	-16.88
P value	<0.001	0.455	<0.001	0.279	0.354	<0.001	0.189
95% CI (cells/mm ³ /yr)	-22.85 to -9.02	-116.80 to 56.89	-91.08 to -55.58	-24.83 to 7.44	-17.67 to 46.41	-83.32 to -32.79	-42.47 to 8.81
Proportion CD4 cells¶							
Median (%)	24	30	37	36	53	31	26
Range (%)	11–36	27–38	29–50	21–45	42–59	25–42	14–36
Slope (%/yr)	-1.31	-0.27	-2.03	-0.72	0.96	0.53	-1.17
P value	<0.001	0.843	<0.001	0.014	0.001	0.004	0.002
95% CI (%/yr)	-1.60 to -1.02	-3.22 to 2.69	-2.46 to -1.6	-1.28 to -0.15	0.47 to 1.46	0.18 to 0.88	-1.85 to -0.48
CD8 count							
Median (cells/mm ³)	969	462	722	874	459	1917.5	987
Range (cells/mm ³)	432–1802	434–693	377–1008	505–1523	260–729	756–3024	208–1548
Slope (cells/mm ³ /yr)	60.33	-29.17	40.66	26.04	15.17	-22.11	121.19
P value	<0.001	0.383	<0.001	0.053	0.085	0.396	<0.001
95% CI (cells/mm ³ /yr)	43.93 to 76.73	-101.11 to 42.78	27.19 to 54.13	-0.36 to 52.45	-2.38 to 32.72	-74.51 to 30.28	74.39 to 167.99
Proportion CD8 cells**							
Median (%)	45	31	45	35	26	44	36
Range (%)	24–64	30–33	18–54	21–43	18–33	15–51	8–48
Slope (%/yr)	1.74	-0.23	3.65	0.73	0.84	1.70	2.74
P value	<0.001	0.642	<0.001	0.006	<0.001	<0.001	<0.001
95% CI (%/yr)	1.31 to 2.18	-1.31 to 0.85	3.27 to 4.02	0.23 to 1.22	0.45 to 1.22	1.13 to 2.26	1.17 to 4.32

*There are no follow-up data available for two deceased recipients, Recipients 5 and 8. The slope is the annual change. P values of less than 0.01 were considered to indicate statistical significance. CI denotes confidence interval.

†The time is from infection to September 30, 1998 (to October 1995 for Recipient 10).

‡The period of follow-up is from the first testing to September 30, 1998 (to October 1995 for Recipient 10).

§The reference range is 500 to 1650 cells per cubic millimeter.

¶The reference range is 30 to 57 percent.

||The reference range is 210 to 1000 cells per cubic millimeter.

**The reference range is 14 to 35 percent.

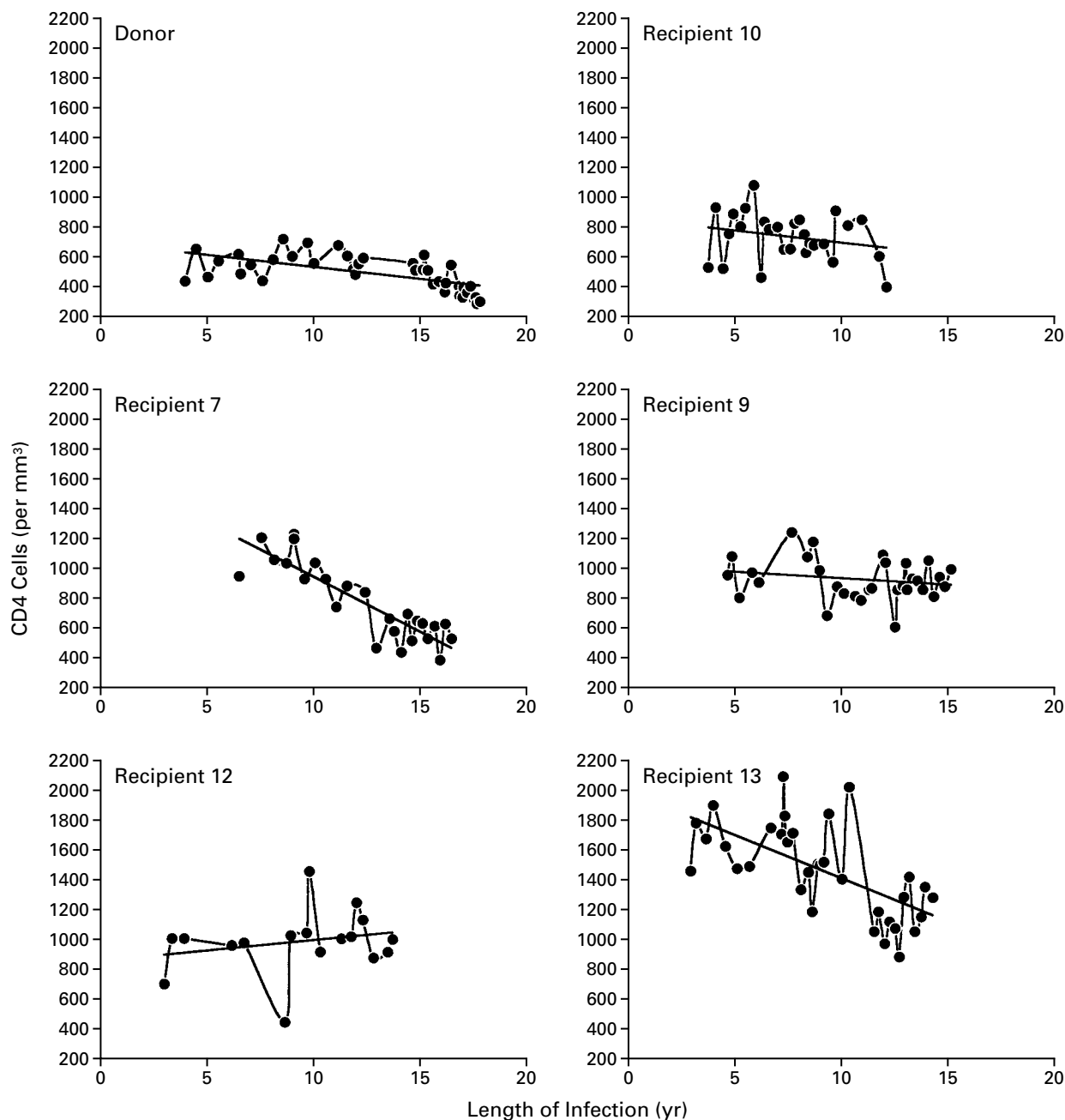


Figure 1. Temporal Changes in CD4 Lymphocyte Counts in the Donor and Five of the Recipients in the Sydney Blood Bank Cohort. The slope of the change in CD4 counts with time (determined by least-squares analysis) is shown by a solid line. Results from Recipient 4 have been excluded because of unavailable or insufficient data.

lacks a functional *nef* gene and also has an unusual LTR.¹² Full-length proviral sequences are available from four members of this cohort (the donor and Recipients 7, 10, and 13),²⁸ and the *nef*-LTR mutations are the only unusual features of these viruses. Furthermore, the results of serologic tests for Nef peptide²⁹ and the failure to amplify wild-type se-

quences with PCR primers in the conserved deletion (unpublished data) provide evidence that no members of the cohort were infected with wild-type HIV-1. The clinical and laboratory features of this cohort can thus be attributed solely to infection with a virus with a *nef*-LTR mutation and not to reversion to or superinfection by a wild-type strain of

HIV. Since the *nef* gene has been clearly established as a cause of slowly progressive SIV infection in macaques,^{9,30} it seems likely that the *nef* gene deletion is the principal cause of the attenuation of the Sydney Blood Bank Cohort HIV. The mechanism by which *nef* enhances HIV and SIV replication in vivo is complex, probably multifactorial, and still under investigation.³¹⁻³³

However, these data support previous suggestions^{13,31} that a drug inhibiting the action or actions of the Nef protein might substantially ameliorate the progression of HIV-1 infection. Although some studies have suggested that LTR sequences have no role in pathogenesis, a role of the mutant Sydney Blood Bank Cohort HIV-1 LTR cannot be wholly ruled out.³⁴

Despite the attenuated phenotype of the Sydney Blood Bank Cohort HIV, the collective data presented here strongly suggest that it can cause immunologic damage. Three members of the cohort had significant declines in CD4 lymphocyte numbers over periods of observation ranging from 8 to 14 years, and there is a strong and biologically plausible relation between the extent of HIV replication (as indicated by the plasma viral load) and the decline in the CD4 count. Three of the four subjects with detectable HIV RNA in plasma had significantly declining CD4 lymphocyte counts, as compared with none of the three with undetectable RNA. In contrast, Greenough et al.³⁵ have presented recent data from a previously described subject¹⁰ infected with a *nef*-deleted strain of HIV-1 whose CD4 counts are declining despite an undetectable viral load. The concept of a relation between HIV infection and falling CD4 counts in the Sydney Blood Bank Cohort was also reinforced by studies showing that three subjects with falling CD4 counts had poor CD4 proliferative responses to p24 antigen³⁶ (a test shown to be of prognostic value in studies of other HIV-infected patients),³⁷ whereas all the subjects with stable CD4 counts had strong proliferative responses (unpublished data). Reduced expression of activation markers such as CD38 and HLA-DR on CD8 cells suggests that CD8 T cells in members of the Sydney Blood Bank Cohort are less activated than those in other long-term survivors. However, those with detectable viral loads have some evidence of activation (Zaunders JJ: personal communication). To our knowledge, the only subject with other factors that might have contributed to the decline in the CD4 count is Recipient 7, who has used inhaled glucocorticoids since 1995 for the treatment of asthma. This may have exacerbated the CD4 lymphocyte decline, since such therapy has been reported to influence immune function adversely.³⁸

The findings in the Sydney Blood Bank Cohort suggest that *nef*-deleted HIV-1 must have highly potent mechanisms for eliminating CD4 lymphocytes,³⁵

since the depletion of CD4 lymphocytes in these subjects occurred at much lower levels of plasma HIV RNA than in other HIV-infected subjects.³⁹ Ruprecht and her colleagues have hypothesized that the role of HIV-1 Nef in pathogenesis is only to augment the level of HIV-1 replication and that it has no separate immunosuppressive functions,⁴⁰ despite in vitro evidence to the contrary.^{41,42} The present data suggest that whether or not the *nef* gene has an independent role in inducing immunologic abnormalities over the long term, *nef*-deleted strains of HIV-1 caused substantial declines in CD4 counts in all members of the Sydney Blood Bank Cohort who had low but detectable viral loads.

Strains of HIV-1 such as those seen in the Sydney Blood Bank Cohort, or other strains with further mutations, have been suggested as the basis of live attenuated vaccines to prevent infection with wild-type strains of HIV.^{13,43,44} The problem facing researchers is that some low-level continuing replication of the virus is required to develop and sustain a protective immune response. In the case of live attenuated SIV vaccines studied in macaques, the available evidence suggests that even with low-level replication, substantial immunodeficiency developed in a number of infected animals.⁴⁵⁻⁴⁷ Our data reveal a similar pattern in the Sydney Blood Bank Cohort. It appears that even low-level replication of HIV-1 correlates with declining CD4 counts, although the decline may take many years to become evident. Finding a balance between replication of the virus and protection is a critical issue if attenuated strains of HIV-1 are to be considered as the basis of a live attenuated vaccine.

Supported by the HIV Research and Development Syndicate, the Macfarlane Burnet Centre Research Fund, and a grant from the Australian National Council on AIDS through the National Centre in HIV Virology Research.

We are indebted to the members of the Sydney Blood Bank Cohort and their physicians for their continued cooperation; to Gillian Hales, Community HIV Research Network, Sydney, for CMI testing; to John Zaunders, Centre for Immunology, St. Vincent's Hospital, Sydney, for flow cytometry; to Antoniette Violo and Vicky Lawson, Macfarlane Burnet Centre, for HIV culture and V3 sequencing; to Damien Jolley, Computing and Statistical Services, Victoria, for assistance with data analysis; to Sue Serjeantson, Australian National University, for providing the DNA sample from Recipient 8; to Jeanette Wood and Jacquie Murphy, Australian Red Cross Blood Service—New South Wales, for assistance in data collection; to Shalini Saverimuttu, Michelle Walls, and Mary-Rose Birch, Australian Red Cross Blood Service—New South Wales, for manuscript preparation; to Ian Bickerton, University of New South Wales, for editorial assistance; and to the nursing staff at the Australian Red Cross Blood Service—New South Wales for the collection of samples.

REFERENCES

1. Kopec-Schrader E, Tindall B, Learmont J, Wylie B, Kaldor JM. Development of AIDS in people with transfusion-acquired HIV infection. *AIDS* 1993;7:1009-13.
2. Ashton LJ, Learmont J, Luo K, Wylie B, Stewart G, Kaldor JM. HIV

- infection in recipients of blood products from donors with known duration of infection. *Lancet* 1994;344:718-20.
3. Operskalski EA, Stram DO, Lee H, et al. Human immunodeficiency virus type 1 infection: relationship of risk group and age to rate of progression to AIDS. *J Infect Dis* 1995;172:648-55.
 4. Muñoz A, Xu J. Models for the incubation of AIDS and variations according to age and period. *Stat Med* 1996;15:2459-73.
 5. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CCR5* structural gene. *Science* 1996;273:1856-62. [Erratum, *Science* 1996;274:1069.]
 6. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996;381:661-6.
 7. Kaslow RA, Carrington M, Apple R, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat Med* 1996;2:405-11.
 8. Martin MP, Dean M, Smith MW, et al. Genetic acceleration of AIDS progression by a promoter variant of *CCR5*. *Science* 1998;282:1907-11.
 9. Kestler HW III, Ringler DJ, Mori K, et al. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. *Cell* 1991;65:651-62.
 10. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC. Absence of intact *nef* sequences in a long-term survivor with nonprogressive HIV-1 infection. *N Engl J Med* 1995;332:228-32.
 11. Learnmont J, Tindall B, Evans L, et al. Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. *Lancet* 1992;340:863-7.
 12. Gurusingham AD, Land SA, Birch C, et al. Reverse transcriptase mutants in sequential HIV isolates in a patient with AIDS. *J Med Virol* 1995;46:238-43.
 13. Deacon NJ, Tsykin A, Solomon A, et al. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science* 1995;270:988-91.
 14. Winkler C, Modi W, Smith MW, et al. Genetic restriction of AIDS pathogenesis by an *SDF-1* chemokine gene variant. *Science* 1998;279:389-93.
 15. Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of *CCR2* and *CCR5* variants on HIV-1 infection and disease progression. *Science* 1997;227:959-65.
 16. Neate EV, Pringle RC, Jowett JBM, Healey DS, Gust ID. Isolation of HIV from Australian patients with AIDS, AIDS related conditions and healthy antibody positive individuals. *Aust N Z J Med* 1987;17:461-6.
 17. Valerie K, Delers A, Bruck C, et al. Activation of human immunodeficiency virus type 1 by DNA damage in human cells. *Nature* 1988;333:78-81.
 18. Learnmont J, Cook L, Dunckley H, Sullivan JS. Update on long-term symptomless HIV type 1 infection in recipients of blood products from a single donor. *AIDS Res Hum Retroviruses* 1995;11:1.
 19. Learnmont J, Rhodes D, Solomon A, et al. Sydney Blood Bank Cohort (SBBC): additional long term non progressor with HIV (LTNP) identified: immunological update on SBBC 12-15 years post infection. In: Program supplement of the 11th International Conference on AIDS, Vancouver, B.C., July 7-12, 1996:33. abstract.
 20. Archer GT, Bolton WV, Cook LA, Learnmont JC, Berdoukas V. The apparent failure of triple-washed cell preparations to transmit HIV infection. In: Book 2 of the Proceedings of the Fourth International Conference on Acquired Immune Deficiency Syndrome (AIDS), Stockholm, Sweden, June 12-15, 1988:354. abstract.
 21. Fairley CK, Spelman D, Street A, Jennens ID, Spicer WJ, Crowe S. CD4 lymphocyte numbers after splenectomy in patients infected with the human immunodeficiency virus. *Int J STD AIDS* 1994;5:177-81.
 22. Dyer WB, Geczy AF, Kent SJ, et al. Lymphoproliferative immune function in the Sydney Blood Bank Cohort, infected with natural *nef*/long terminal repeat mutants, and in other long-term survivors of transfusion-acquired HIV-1 infection. *AIDS* 1997;11:1565-74.
 23. Muñoz A, Sabin CA, Phillips AN. The incubation period of AIDS. *AIDS* 1997;11:Suppl A:S69-S76.
 24. Hessel NA, Palacio H, Gender, ethnicity and transmission category variation in HIV disease progression. *AIDS* 1996;10:Suppl A:S69-S74.
 25. Leong KH, Boey ML, Feng PH. Coexisting *Pneumocystis carinii* pneumonia, cytomegalovirus pneumonitis and salmonellosis in systemic lupus erythematosus. *Ann Rheum Dis* 1991;50:811-2.
 26. Porges AJ, Beattie SL, Ritchlin C, Kimberly RP, Christian CL. Patients with systemic lupus erythematosus at risk for *Pneumocystis carinii* pneumonia. *J Rheumatol* 1992;19:1191-4.
 27. Learnmont J, Sullivan J, Gerrard J. Long term symptomless HIV positive blood transfusion recipients. In: Vol. 2 of the Proceedings of the 10th International Conference on AIDS, Yokohama, Japan, August 7-12, 1994: 254. abstract.
 28. Oelrichs R, Tsykin A, Rhodes D, et al. Genomic sequence of HIV type 1 from four members of the Sydney Blood Bank Cohort of long-term nonprogressors. *AIDS Res Hum Retroviruses* 1998;14:811-4.
 29. Greenway AL, Mills J, Rhodes D, Deacon NJ, McPhee D. Serological detection of attenuated HIV-1 variance with *nef* gene deletions. *AIDS* 1998;12:555-61.
 30. Gibbs JS, Regier DA, Desrosiers RC. Construction and in vitro properties of SIVmac mutants with deletions in "nonessential" genes. *AIDS Res Hum Retroviruses* 1994;10:607-16. [Correction of *AIDS Res Hum Retroviruses* 1994;10:333-42.]
 31. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* 1998;391:397-401.
 32. Miller MD, Warmerdam MT, Gaston I, Greene WC, Feinberg MB. The human immunodeficiency virus-1 *nef* gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. *J Exp Med* 1994;179:101-13.
 33. Spina CA, Kwok TJ, Chowder MY, Guatelli JC, Richman DD. The importance of *nef* in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. *J Exp Med* 1994;179:115-23.
 34. Ilyinskii PO, Daniel MD, Simon MA, Lackner AA, Desrosiers RC. The role of upstream U3 sequences in the pathogenesis of simian immunodeficiency virus-induced AIDS in rhesus monkeys. *J Virol* 1994;68:5933-44.
 35. Greenough TC, Sullivan JL, Desrosiers RC. Declining CD4 T-cell counts in a person infected with *nef*-deleted HIV-1. *N Engl J Med* 1999;340:236-7.
 36. Dyer WB, Ogg GS, Demoitte MA, et al. Strong human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte activity in Sydney Blood Bank Cohort patients infected with *nef*-defective HIV type 1. *J Virol* 1999;73:436-43.
 37. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997;278:1447-50.
 38. Cameron RG, Black PN, Braan C, Browett PJ. A comparison of the effects of oral prednisone and inhaled beclomethasone dipropionate on circulating leukocytes. *Aust N Z J Med* 1996;26:800-5.
 39. Mellors JW, Muñoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126:946-54.
 40. Ruprecht RM, Baba TW, Liska V. Attenuated HIV vaccine: caveats. *Science* 1996;271:1790-2.
 41. Greenway AL, McPhee DA, Grgacic E, et al. Nef 27, but not the Nef 25 isoform of human immunodeficiency virus-type 1 pNL4.3 down-regulates surface CD4 and IL-2R expression in peripheral blood mononuclear cells and transformed T cells. *Virology* 1994;198:245-56.
 42. Greenway A, Azad A, McPhee D. Human immunodeficiency virus type 1 Nef protein inhibits activation pathways in peripheral blood mononuclear cells and T-cell lines. *J Virol* 1995;69:1842-50.
 43. Desrosiers RC. HIV with multiple gene deletions as a live attenuated vaccine for AIDS. *AIDS Res Hum Retroviruses* 1992;8:411-21.
 44. *Idem*. Yes, it is time to consider use of a live-attenuated virus vaccine against HIV-1. In: Controversies in science: a live-virus AIDS vaccine? *J NIH Res* 1994;6:54, 56-9.
 45. Proposed live HIV vaccine trials face many problems. *Antiviral Agents Bull* 1997;10:353-5.
 46. Cohen J. Weakened SIV vaccine still kills. *Science* 1997;278:24-5.
 47. Baba TW, Liska V, Khimani AH, et al. Live-attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. *Nat Med* 1999;5:194-203.