

STUDIES IN SUBJECTS WITH LONG-TERM NONPROGRESSIVE HUMAN IMMUNODEFICIENCY VIRUS INFECTION

GIUSEPPE PANTALEO, M.D., STEFANO MENZO, M.D., MAURO VACCAREZZA, M.D., CECILIA GRAZIOSI, PH.D., OREN J. COHEN, M.D., JAMES F. DEMAREST, B.S., DAVID MONTEFIORI, PH.D., JAN M. ORENSTEIN, M.D., CECIL FOX, PH.D., LEWIS K. SCHRAGER, M.D., JOSEPH B. MARGOLICK, M.D., PH.D., SUSAN BUCHBINDER, M.D., JANIS V. GIORGI, PH.D., AND ANTHONY S. FAUCI, M.D.

Abstract Background. In a small percentage of persons infected with human immunodeficiency virus type 1 (HIV-1), there is no progression of disease and CD4+ T-cell counts remain stable for many years. Studies of the histopathological, virologic, and immunologic characteristics of these persons may provide insight into the pathogenic mechanisms that lead to HIV disease and the protective mechanisms that prevent progression to overt disease.

Methods and Results. We studied 15 subjects with long-term nonprogressive HIV infection and 18 subjects with progressive HIV disease. Nonprogressive infection was defined as seven or more years of documented HIV infection, with more than 600 CD4+ T cells per cubic millimeter, no antiretroviral therapy, and no HIV-related disease. Lymph nodes from the subjects with nonprogressive infection had significantly fewer of the hyperplastic features, and none of the involuted features, characteristic of nodes from subjects with progressive disease. Plasma levels of HIV-1 RNA and the viral bur-

den in peripheral-blood mononuclear cells were both significantly lower in the subjects with nonprogressive infection than in those with progressive disease ($P=0.003$ and $P=0.015$, respectively). HIV could not be isolated from the plasma of the former, who also had significantly higher titers of neutralizing antibodies than the latter. There was viral replication, however, in the subjects with nonprogressive infection, and virus was consistently cultured from mononuclear cells from the lymph nodes. In the lymph nodes virus "trapping" varied with the degree of formation of germinal centers, and few cells expressing virus were found by *in situ* hybridization. HIV-specific cytotoxic activity was detected in all seven subjects with nonprogressive infection who were tested.

Conclusions. In persons who remain free of disease for many years despite HIV infection the viral load is low, but viral replication persists. Lymph-node architecture and immune function appear to remain intact. (*N Engl J Med* 1995;332:209-16.)

THE typical course of human immunodeficiency virus (HIV) infection includes an acute clinical syndrome of variable severity, a prolonged period of clinical latency, and then a stage of clinically apparent disease characterized by increased susceptibility to opportunistic infections and certain neoplasms.¹ The acute phase of infection progresses to the latent phase in the vast majority of HIV-infected people even though vigorous HIV-specific cell-mediated and humoral immune responses are generally present early in primary infection.¹⁻⁷ These early responses almost invariably curtail replication of the virus, resulting in a marked decrease in plasma viremia, but they usually fail to eliminate HIV from the body.^{2,3,8} The duration of clinical latency varies widely, and the progression to the acquired immunodeficiency syndrome (AIDS) occurs over a median period of 8 to 10 years.⁹⁻¹¹ More than a decade into the AIDS epidemic, it has become clear

that there is a group of infected persons whose HIV disease does not progress over an extended time.¹¹⁻¹⁵

Even during the period of clinically latent HIV infection, there is active viral replication and histologic changes in lymphoid tissue.^{1,16,17} Therefore, we compared the histopathological features of the lymph nodes and virologic characteristics of subjects with long-term nonprogressive infection with those of subjects with progressive disease. In addition, we analyzed both humoral and cellular immune responses to HIV in subjects with long-term nonprogressive infection.

METHODS

Study Subjects

Fifteen subjects with long-term nonprogressive HIV infection were studied (Table 1). The criteria used to define nonprogression included documented HIV infection for more than seven years, stable CD4+ T-cell counts greater than 600 per cubic millimeter, the absence of symptoms, and no antiretroviral therapy. Seven subjects with long-term nonprogressive infection were from the Multicenter AIDS Cohort Study, four were from the San Francisco City Clinic Cohort Study, and four were from the lymph-node study of the National Institute of Allergy and Infectious Diseases. Thirteen of the 15 subjects had been infected with HIV for at least 10 years (Table 1). For Patient 11 the date of seroconversion was determined retrospectively from a serum sample collected in 1980 during a trial of hepatitis B virus vaccine. Analysis of serial CD4+ T-cell counts in six subjects with long-term nonprogressive infection from the Multicenter AIDS Cohort Study showed no decrease (a slope of 0 or above) (Fig. 1). All subjects with long-term nonprogressive HIV infection had high CD8+ T-cell counts (ranging from 527 to 2483 per cubic millimeter). No particular patterns of HLA haplotypes were observed. HIV was transmitted through homosexual contact in all these subjects except Subject 7, in whom it was transmitted heterosexually.

Eighteen HIV-infected subjects who were enrolled in a lymph-node study and who had progression of disease in varying degrees

From the Laboratory of Immunoregulation (G.P., S.M., M.V., C.G., O.J.C., J.F.D., A.S.F.) and the Division of AIDS (L.K.S.), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.; the Department of Medicine, Cellular Immunology, and Cytometry, UCLA School of Medicine, Los Angeles (J.V.G.); the Department of Surgery, Center for AIDS Research, Duke University Medical Center, Durham, N.C. (D.M.); the Department of Pathology, George Washington University, Washington, D.C. (J.M.O.); Molecular Histology, Inc., Gaithersburg, Md. (C.F.); the Department of Environmental Health Sciences, Department of Immunology and Infectious Diseases, Johns Hopkins School of Hygiene and Public Health, Baltimore (J.B.M.); the Research Branch, AIDS Office, Department of Public Health, San Francisco (S.B.); and the Institute of Microbiology, University of Ancona Medical School, Ancona, Italy (S.M.). Address reprint requests to Dr. Pantaleo at the Laboratory of Immunoregulation, Bldg. 10, Rm. 11B13, 10 Center Dr., MSC 1876, Bethesda, MD 20892-1876.

Supported in part by grants (U01-AI-35042, U01-AI-35043, U01-AI-35039, U01-AI-35040, U01-AI-35041) from the Public Health Service and by a grant (5-M01-RR-00722) from the National Institute of Allergy and Infectious Diseases.

Table 1. Clinical Characteristics of 15 Subjects with Long-Term Nonprogressive HIV Infection.*

SUBJECT NO.	AGE (YR)/SEX	YEAR OF SEROCONVERSION	LYMPH-NODE BIOPSY	CD4+ CELLS		CD8+ CELLS		HLA HAPLOTYPE	
				no./mm ³	percent	no./mm ³	percent	CLASS I	CLASS II
1	46/M	1983	Inguinal	685	32	1178	55	A31,3; B8; C7; B6	DQ2; DR3; DR52
2	34/M	1984	Inguinal	817	38	1010	47	A11; B35,53; C4; B4,6	DQ1,3; DR15,4; DR53
3	41/M	1985	Axillary	1238	32	2090	54	A29,30; B44,14; C4; B4,6	DQ2,3; DR7,11; DR52,53
4	36/M	1985	Axillary	637	27	1274	54	A3,24; B27,63; C2; B4	DQ1,3; DR6,4; DR52,53
5	32/M	1984	Cervical	1287	44	1141	39	A2,25; B51,18; Cx; B4,6	DQ3; DR4,11; DR52,53
6	42/M	1984	Axillary	720	20	2483	69	A24,33; B60,63; C3,x; B4,6	DQ1,3; DR15,11; DR52
7	34/F	1985	Cervical	714	38	742	40	A2,3; B27,70; C2,7; B4,6	DQ1; DR15,6; DR52
8	45/M	1987	Inguinal	1038	37	1291	46	A2; B52,44; C5; B4	DQ1,3; DR15,11; DR52
9	40/M	1985	Inguinal	1053	27	2379	61	A2,3; B44,14; C5; B4,6	DQ1,3; DR15,6; DR52,53
10	36/M	1984	Inguinal	702	38	979	53	A1,24; B57,18; C5,6; B4,6	DQ2,3; DR3,7; DR52,53
11	41/M	1980	Inguinal	773	38	855	42	A2,28; B7,13; C6,7; B4,6	DQ21,2; DR15,7; DR53
12	39/M	1984	Axillary	810	40	878	43	A2,25; B27,18; C2; B4,6	DQ1,3; DR1,4; DR53
13	41/M	1984	ND	1142	44	961	37	A2,3; B44,61; C2,5; B4,6	DQ1,3; DR6,11; DR52
14	33/M	1986	Inguinal	674	41	527	32	A25,32; B44,18; C5; B4,6	DQ1,3; DR15,11; DR52
15	40/M	1983	Inguinal	690	41	858	51	A2,24; B63,50; C3,7; B4,6	DQ1,3; DR4,6; DR52,53

*CD4+ and CD8+ cell counts shown were determined by cytofluorometry at the time of the lymph-node biopsy. Percentages shown are percentages of the total number of lymphocytes. ND denotes not done, and x haplotype of undetermined specificity.

were studied similarly, as controls. These control subjects with progressive HIV disease were randomly selected from among patients for whom clinical specimens (i.e., plasma and mononuclear cells from peripheral blood and lymph nodes) were available. Lymph-node biopsies were performed in control subjects with palpable nodes. In

6 of the 18 controls, biopsies of inguinal lymph nodes were performed, because lymph nodes in other sites were not palpable. Biopsies of axillary lymph nodes were performed in the 12 remaining controls. Seropositivity was documented in 5 of the 18 controls between 1989 and 1991 and in the remaining 13 between 1984 and 1987. This group of HIV-infected persons with progressive disease included 3 with CD4+ T-cell counts above 500 cells per cubic millimeter, 11 with counts from 200 to 500 per cubic millimeter, and 4 with counts below 200 per cubic millimeter. In the three control subjects with CD4+ T-cell counts above 500 cells per cubic millimeter, the status of disease progression was determined on the basis of declining CD4+ T-cell counts over time. In one subject the count was 670 cells per cubic millimeter at the time of the lymph-node biopsy (April 1993), but at the time of seroconversion (1986) it was 1100 cells per cubic millimeter. In the second control subject, the count was 550 cells per cubic millimeter at the time of the lymph-node biopsy (April 1993), but it had been 1920 in 1988, and it dropped to 351 in 1994. In the third subject the CD4+ T-cell count was 600 cells per cubic millimeter at the time of the biopsy (September 1993), but since the time of seroconversion (1986) this subject has had progressive constitutional symptoms during the development of which his CD4+ T-cell count ranged from 200 to 500 cells per cubic millimeter.

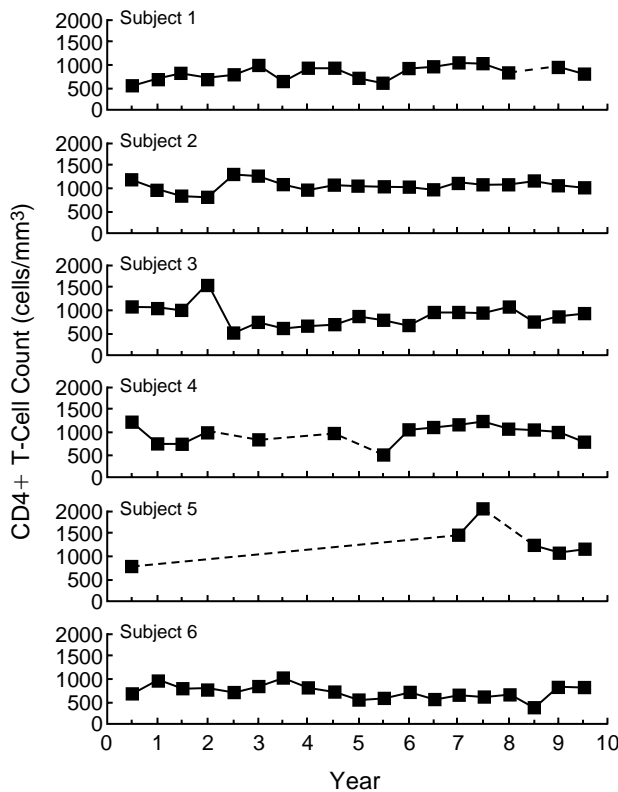


Figure 1. Serial CD4+ T-Cell Counts over a 10-Year Period in Six Subjects from the Multicenter AIDS Cohort Study Who Had Long-Term Nonprogressive HIV Infection.

The points represent the CD4+ T-cell counts obtained at six-month intervals. The broken lines indicate clinic visits that were missed.

Clinical Specimens

All excisional lymph-node biopsies were performed at the National Institutes of Health Clinical Center under an approved protocol. The tissue specimens were processed immediately after their removal.¹⁶ Two small tissue specimens obtained from each lymph node were fixed in formaldehyde and glutaraldehyde for routine histologic analysis, in situ hybridization, and electron microscopy. The remaining specimens were minced with a scalpel, and cells were teased out. A small aliquot was fixed in glutaraldehyde for electron microscopy, and cell pellets were immediately prepared for study by the polymerase chain reaction (PCR) and stored at -80°C.

Quantitation of Proviral HIV-1 DNA

Proviral HIV type 1 (HIV-1) DNA was quantitated by a semiquantitative PCR assay with a primer pair specific for the *gag* (SK145/101) gene segment.^{16,17} The results were expressed as the number of copies of DNA per million cells.

Quantitation of HIV-1 Genomic RNA

Whole blood was centrifuged at 200 ×g for 10 minutes. The plasma fraction was then centrifuged twice at 1000 ×g for 10 minutes to en-

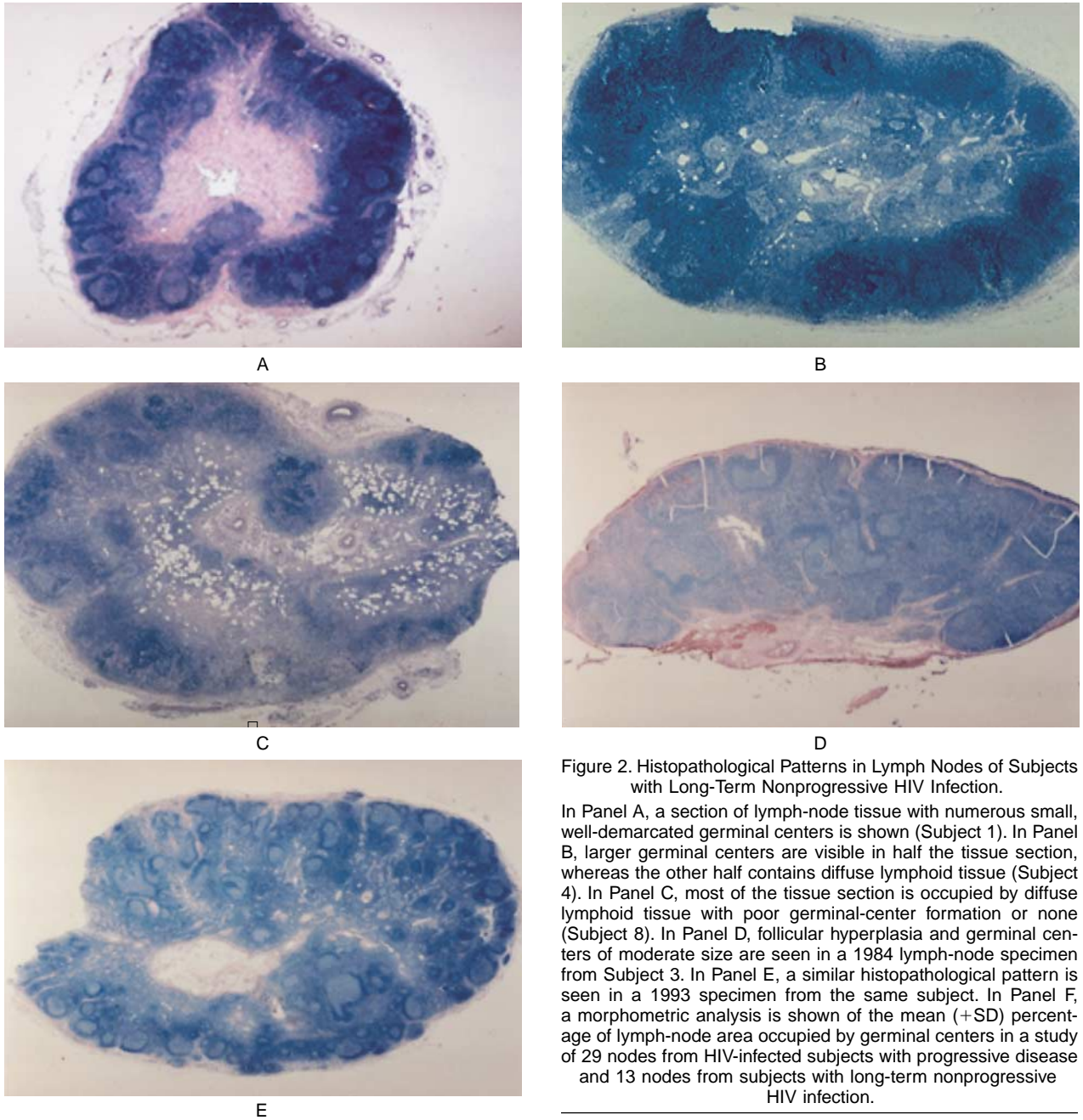
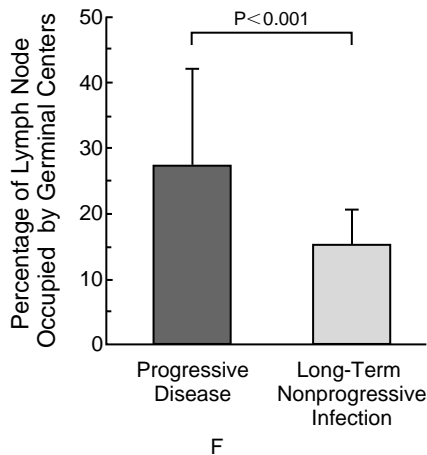


Figure 2. Histopathological Patterns in Lymph Nodes of Subjects with Long-Term Nonprogressive HIV Infection.

In Panel A, a section of lymph-node tissue with numerous small, well-demarcated germinal centers is shown (Subject 1). In Panel B, larger germinal centers are visible in half the tissue section, whereas the other half contains diffuse lymphoid tissue (Subject 4). In Panel C, most of the tissue section is occupied by diffuse lymphoid tissue with poor germinal-center formation or none (Subject 8). In Panel D, follicular hyperplasia and germinal centers of moderate size are seen in a 1984 lymph-node specimen from Subject 3. In Panel E, a similar histopathological pattern is seen in a 1993 specimen from the same subject. In Panel F, a morphometric analysis is shown of the mean (+SD) percentage of lymph-node area occupied by germinal centers in a study of 29 nodes from HIV-infected subjects with progressive disease and 13 nodes from subjects with long-term nonprogressive HIV infection.



sure the removal of residual cellular or platelet debris. RNA was extracted from both plasma and mononuclear cells by the guanidium thiocyanate method. In the case of plasma, RNA was extracted either from the plasma virion pellet after ultracentrifugation (at 30,000 rpm) of 1 ml of plasma diluted in 9 ml of RPMI-1640 or from 20 μ l of undiluted plasma. In the case of mononuclear cells, RNA was extracted from a sample containing 1 million cells. Reverse transcription, amplification, and quantitation of HIV-1 genomic RNA were performed as described elsewhere.^{18,19}

In Situ Hybridization and Immunohistochemical Analysis

In situ hybridization was performed with a mixture of RNA probes synthesized by five DNA templates that represent 90 percent of the HIV-1 genome.²⁰ In experiments using double-labeling (immunohistochemical analysis plus in situ hybridization), slides were first stained with anti-CD21 antibody (Dako, Carpinteria, Calif.), which

number of copies of HIV-1 RNA per milliliter of plasma was $70,818 \pm 67,931$ in the subjects with long-term nonprogressive infection, as compared with $1,586,967 \pm 2,200,198$ in the controls ($P = 0.003$). A retrospective analysis of five subjects with nonprogressive infection from the Multicenter AIDS Cohort Study, involving specimens collected over a period of five years (from 1988 to 1993), demonstrated that plasma viremia and CD4+ T-cell counts remained relatively stable (Fig. 4). Only in Subject 2 did plasma viremia increase greatly (10-fold between 1988 and 1993) (Fig. 4); the subject's CD4+ T-cell count dropped slightly, to 817 cells per cubic millimeter, but remained essentially unchanged after one year (798 cells per cubic millimeter in 1994).

In Situ Hybridization and Electron Microscopy

Variable levels of virus "trapping" were observed in the follicular-dendritic-cell networks of the germinal centers by in situ hybridization. Virus was detected in 9 of 14 lymph nodes, and its presence was strictly dependent on the presence of germinal centers. In fact,

virtually no virus was detected in the five lymph nodes in which few germinal centers, or none, had formed (Subjects 8, 9, 11, 14, and 15). Typical examples of the patterns of virus trapping in the lymph nodes are shown in Figures 5A and 5B. The absence of trapped virus did not result from the lack of follicular dendritic cells, since such cells were still detected in lymph nodes that had poor or nonexistent formation of germinal centers, as indicated by immunohistochemical analysis (Fig. 5C). Electron-microscopical analysis showed that follicular dendritic cells in the lymph nodes of subjects with long-term nonprogressive HIV infection were generally healthy. In addition, virus particles were rarely detected (in only 3 of 12 cases) by electron microscopy in either tissue or cell suspensions, and cells expressing virus were rarely observed (Fig. 5D) by in situ hybridization.

Isolation of Virus

HIV could not be isolated from the plasma of subjects with long-term nonprogressive HIV infection, but it could be isolated from lymph-node mononuclear cells

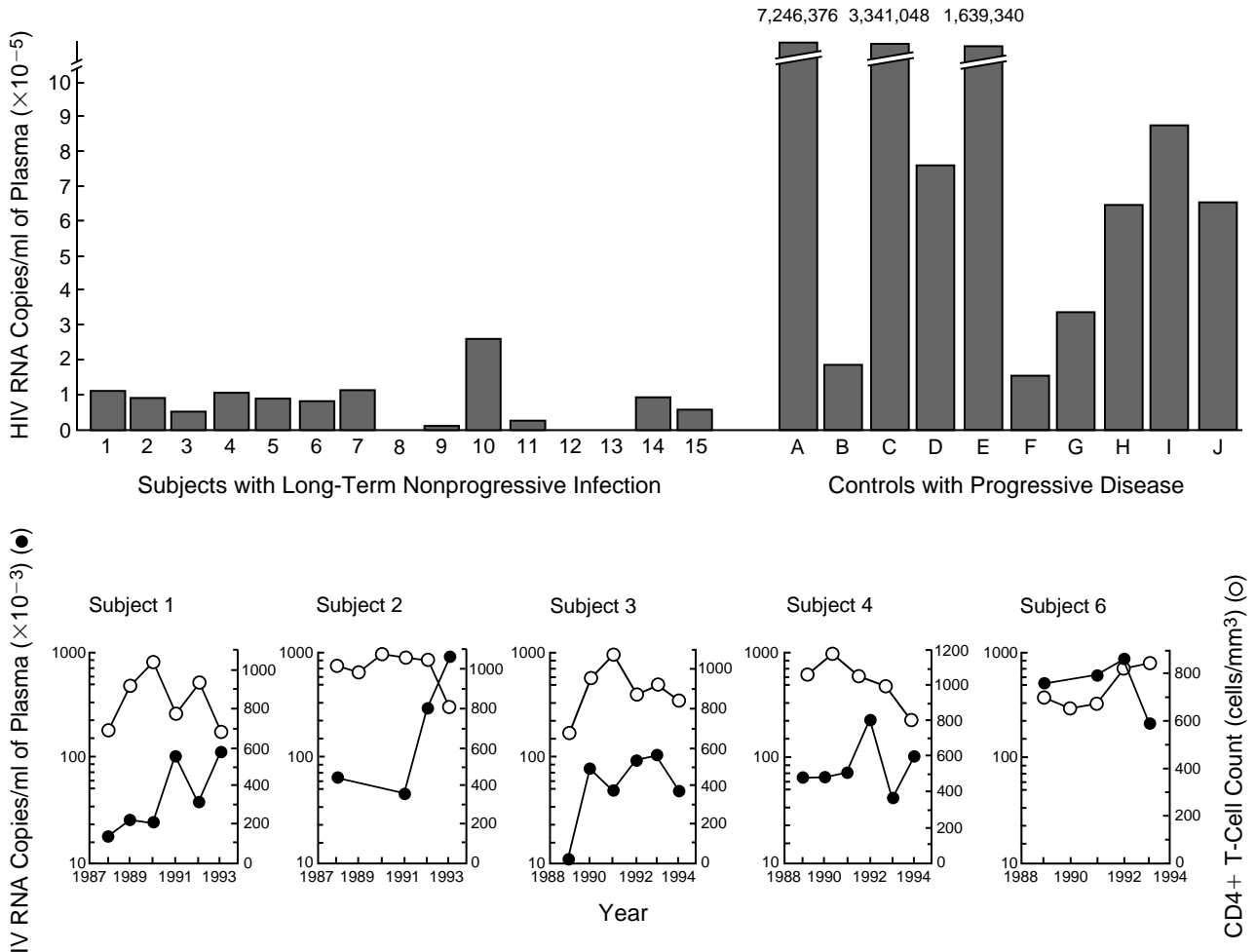


Figure 4. Plasma Viremia in the Study Subjects.

The upper panel shows levels of plasma viremia in 15 subjects with long-term nonprogressive HIV infection and 10 controls with progressive disease. There were 270 copies of HIV RNA per milliliter of plasma in Subject 8, fewer than 500 copies in Subject 12, and 2380 copies in Subject 13. The lower panels show a retrospective analysis of plasma viremia and CD4+ T-cell counts in five subjects with long-term nonprogressive infection.

(in seven patients) after coculture with phytohemagglutinin-activated mononuclear cells from an HIV-negative donor (data not shown). In contrast, in the control subjects with progressive disease, HIV could be cultured readily, either from plasma or directly from phytohemagglutinin-stimulated lymph-node mononuclear cells. Levels of viral replication were higher after coculture with phytohemagglutinin-activated mononuclear cells from an HIV-negative donor (data not shown).

Neutralizing Antibodies

Neutralizing antibodies were detected²¹ with two genetically diverse strains of HIV-1 (HIV-1_{III_B} and HIV-1_{MN}). Titers of neutralizing antibodies were significantly higher against both HIV-1_{III_B} (552 ± 324 in the subjects with long-term nonprogressive infection vs. 345 ± 297 in the controls with progressive disease; $P=0.037$) and HIV-1_{MN} (1458 ± 925 vs. 680 ± 408 , respectively; $P=0.02$).

HIV-Specific Cytotoxicity

HIV-specific cytotoxic activity was assayed in 7 of 15 subjects with long-term nonprogressive HIV infection

with freshly isolated or anti-CD3-stimulated unfractionated peripheral-blood mononuclear cells and sorted CD8+ T lymphocytes used as effector cells.²² HIV-specific cytotoxicity against HIV *env* was consistently detected in all seven subjects, whereas HIV-specific cytotoxicity against *gag* was detected in three of six subjects studied (data not shown).

DISCUSSION

We studied histopathological, virologic, and immunologic measures in 15 HIV-infected subjects in whom there has been no clinical or immunologic progression of HIV disease. The study was designed to examine the lymph nodes of these subjects, since our previous studies^{1,17} of subjects with progressive disease had demonstrated active viral replication in lymphoid tissue and histopathological abnormalities even during the clinically latent stage of HIV disease. Although the histopathological analysis was generally limited to a single lymph node, recent studies have demonstrated good concordance of the histopathological findings in lymph nodes from multiple sites in the same HIV-infected person.²³ The histopathological patterns in the subjects

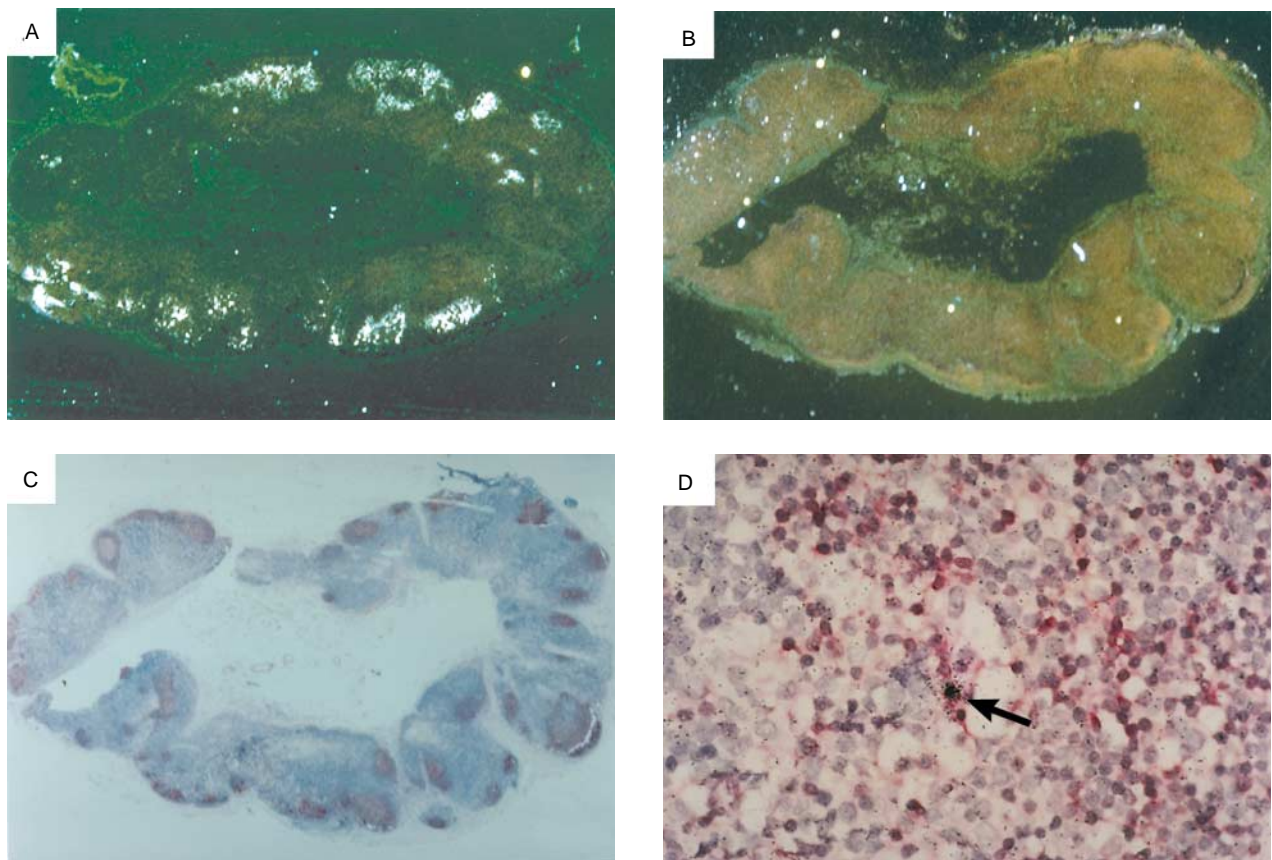


Figure 5. Distribution of Virus in Lymph Nodes of Subjects with Long-Term Nonprogressive Infection.

Panel A shows a dark-field image of in situ hybridization of a lymph-node section from Subject 5 after protease digestion. The location of HIV RNA is indicated by the silver grains, which appear as white dots. There is an intense, granular, diffuse signal, restricted predominantly to the area of the germinal centers. Panel B shows a dark-field image of a lymph-node section from Subject 11 after protease digestion. No diffuse hybridization signal is detected. Panel C shows immunohistochemical staining (new fuchsin red) of the network of follicular dendritic cells from Subject 11 after staining with anti-CD21 antibody. Panel D shows a bright-field image of a lymph-node section from Subject 11 after protease digestion and subsequent immunohistochemical analysis (with OPD-4 antibody, which stains a subgroup of CD45RO+ lymphocytes) plus in situ hybridization. The location of HIV RNA is indicated by the silver grains, which appear as black dots. An isolated cell expresses HIV RNA (arrow).

with long-term nonprogressive HIV infection were heterogeneous, and the degree of lymph-node activation (germinal-center formation) was significantly less than in control subjects with progressive disease. One cannot exclude the possibility that some degree of germinal-center activation occurred early in the course of infection and that because of a lower viral load the lymphoid tissue returned to a nonreactive state. However, the critical difference between the lymph nodes of subjects with nonprogressive infection and those of subjects with progressive disease is that the lymph nodes of the former do not show involution and lymphocyte depletion, typical features of lymph nodes in the latter after HIV infection has lasted 8 to 10 years.²⁴

Viral replication persisted in subjects with long-term nonprogressive HIV infection, but plasma viremia, as well as viral burden and viral replication in mononuclear cells in peripheral blood and lymph nodes, was significantly (4 to 20 times) lower than in control subjects with progressive disease.

The degree of virus trapping in the follicular-dendritic-cell network in the lymph nodes of subjects with long-term nonprogressive HIV infection paralleled the extent of germinal-center formation.^{17,20,24-31} Apparently, this was not the result of lymph-node involution and concomitant loss of the ability to trap virus, which is characteristic of the lymph nodes of persons with progressive disease¹⁷; rather, it is more likely to reflect the relatively nonreactive state of the lymphoid tissue. This lower degree of virus trapping may contribute both to the lower viral load and to the lower rate of tissue activation observed in subjects with long-term nonprogressive infection.

Virus was cultured consistently from the lymph-node mononuclear cells of these subjects, indicating that HIV is infectious and competent to replicate in such persons. The lower efficiency with which virus was isolated from the subjects with long-term nonprogressive HIV infection as compared with the controls with progressive disease may be consistent with the lower viral load in the former. However, one cannot exclude the possibility that genetic defects in the virus in the subjects with long-term nonprogressive HIV infection may account for the low efficiency of virus isolation. In this regard, the V3 region of particle-associated RNA amplified from plasma was directly sequenced in four such subjects. We found no peculiar patterns or major rearrangements with respect to known V3 sequences; the sequences studied had macrophage-tropic genotypes³²⁻³⁴ such as are generally observed in HIV-infected persons during the clinically latent period of infection.^{34,35} The presence of high titers of neutralizing antibodies, together with the consistent detection of HIV-specific cytotoxicity, indicates that both humoral and cell-mediated immune responses are preserved in subjects with long-term nonprogressive infection and strongly suggests that these persons are constantly exposed to HIV antigens.

In conclusion, subjects with long-term nonprogressive HIV infection have preserved lymphoid tissue with reduced formation of germinal centers and reduced HIV trapping, despite a low but persistent level of viral

replication. This finding suggests that persistent, low-level viral replication is not necessarily associated with the progression of disease if it is efficiently controlled over time. It remains unclear what are the relative contributions of host factors, such as the immune system, and virologic factors, such as a defective virus, in determining the lack of progression of HIV disease in these subjects. Understanding the importance of these factors may prove to be critical to the development and testing of vaccines and therapeutic strategies.

We are indebted to the HIV-infected persons who participated in this study; to all the investigators associated with the Multicenter AIDS Cohort Study, particularly Drs. Roger Detels, John Phair, and Charles Rinaldo, and their staffs, who cooperated in recruiting the study subjects; to Drs. William Biddison and Louis DePalma for helpful discussions; and to Mary Rust for expert editorial assistance.

REFERENCES

- Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* 1993;328:327-35.
- Clark SJ, Saag MS, Decker WD, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med* 1991;324:954-60.
- Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med* 1991;324:961-4.
- Safrit JT, Andrews CA, Zhu T, Ho DD, Koup RA. Characterization of human immunodeficiency virus type 1-specific cytotoxic T lymphocyte clones isolated during acute seroconversion: recognition of autologous virus sequences within a conserved immunodominant epitope. *J Exp Med* 1994;179:463-72.
- Reimann KA, Tenner-Racz K, Racz P, et al. Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. *J Virol* 1994;68:2362-70.
- Pantaleo G, Demarest JF, Soudeyns H, et al. Major expansion of CD8+ T cells with a predominant V β usage during the primary immune response to HIV. *Nature* 1994;370:463-7.
- Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 1994;68:6103-10.
- Graziosi C, Pantaleo G, Butini L, et al. Kinetics of human immunodeficiency virus type 1 (HIV-1) DNA and RNA synthesis during primary HIV-1 infection. *Proc Natl Acad Sci U S A* 1993;90:6405-9.
- Fauci AS, Schnittman SM, Poli G, Koenig S, Pantaleo G. Immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection. *Ann Intern Med* 1991;114:678-93.
- Lifson AR, Buchbinder SP, Sheppard HW, et al. Long-term human immunodeficiency virus infection in asymptomatic homosexual and bisexual men with normal CD4+ lymphocyte counts: immunologic and virologic characteristics. *J Infect Dis* 1991;163:959-65.
- Buchbinder SP, Katz MH, Hessel NA, O'Malley PM, Holmberg SD. Long-term HIV-1 infection without immunologic progression. *AIDS* 1994;8:1123-8.
- Sheppard HW, Lang W, Ascher MS, Vittinghoff E, Winkelstein W. The characterization of non-progressors: long-term HIV-1 infection with stable CD4+ T-cell levels. *AIDS* 1993;7:1159-66.
- Levy JA. HIV pathogenesis and long-term survival. *AIDS* 1993;7:1401-10.
- Easterbrook PJ. Non-progression in HIV infection. *AIDS* 1994;8:1179-82.
- Schrager LK, Young JM, Fowler MG, Mathieson BJ, Vermund SH. Long-term survivors of HIV-1 infection: definitions and research challenges. *AIDS* 1994;8:Suppl:S95-S108.
- Pantaleo G, Graziosi C, Butini L, et al. Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc Natl Acad Sci U S A* 1991;88:9838-42.
- Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 1993;362:355-8.
- Menzo S, Bagnarelli P, Giacca M, Manzin A, Valardo PE, Clementi M. Absolute quantitation of viremia in human immunodeficiency virus infection by competitive reverse transcription and polymerase chain reaction. *J Clin Microbiol* 1992;30:1752-7.
- Bagnarelli P, Menzo S, Valenza A, et al. Molecular profile of human immunodeficiency virus type 1 infection in symptomless patients and in patients with AIDS. *J Virol* 1992;66:7328-35.
- Fox CH, Tenner-Racz K, Racz P, Firpo A, Pizzo PA, Fauci AS. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. *J Infect Dis* 1991;164:1051-7.

21. Montefiori DC, Robinson WE Jr, Schuffman SS, Mitchell WM. Evaluation of antiviral drugs and neutralizing antibodies to human immunodeficiency virus by a rapid and sensitive microtiter infection assay. *J Clin Microbiol* 1988;26:231-5.
22. Pantaleo G, DeMaria A, Koenig S, et al. CD8+ T lymphocytes of patients with AIDS maintain normal broad cytolytic function despite the loss of human immunodeficiency virus-specific cytotoxicity. *Proc Natl Acad Sci U S A* 1990;87:4818-22.
23. Burke AP, Anderson D, Mannan P, et al. Systemic lymphadenopathic histology in human immunodeficiency virus-1-seropositive drug addicts without apparent acquired immunodeficiency syndrome. *Hum Pathol* 1994;25:248-56.
24. Biberfeld P, Ost A, Porwit A, et al. Histopathology and immunohistology of HTLV-III/LAV related lymphadenopathy and AIDS. *Acta Pathol Microbiol Immunol Scand* 1987;95:47-65.
25. Armstrong JA. Ultrastructure and significance of the lymphoid tissue lesions in HIV infection. In: Racz P, Dijkstra CD, Gluckman J-C, eds. Accessory cells in HIV and other retroviral infections: morphological and functional aspects. Basel, Switzerland: Karger, 1991:69-82.
26. Tenner-Racz K, Racz P, Dietrich M, Karin P. Altered follicular dendritic cells and virus-like particles in AIDS and AIDS-related lymphadenopathy. *Lancet* 1985;1:105-6.
27. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 1993;362:359-62.
28. Armstrong JA, Dawkins RL, Horne R. Retroviral infection of accessory cells and the immunological paradox in AIDS. *Immunol Today* 1985;6:121-2.
29. Emilie D, Peuchmaur M, Maillot M, et al. Production of interleukins in human immunodeficiency virus-1-replicating lymph nodes. *J Clin Invest* 1990; 86:148-59.
30. Spiegel H, Herbst H, Niedobitek G, Foss HD, Stein H. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells. *Am J Pathol* 1992;140:15-22.
31. Tenner-Racz K, Racz P, Bofill M, et al. HTLV-III/LAV viral antigens in lymph nodes of homosexual men with persistent generalized lymphadenopathy and AIDS. *Am J Pathol* 1986;123:9-15.
32. Shioda T, Levy JA, Cheng-Mayer C. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. *Nature* 1991;349:167-9.
33. Westervelt P, Gendelman HE, Ratner L. Identification of a determinant within the human immunodeficiency virus 1 surface envelope glycoprotein critical for productive infection of primary monocytes. *Proc Natl Acad Sci U S A* 1991;88:3097-101.
34. Donaldson YK, Bell JE, Holmes EC, Sughes ES, Brown HK, Simmonds P. In vivo distribution and cytopathology of variants of human immunodeficiency virus type 1 showing restricted sequence variability in the V3 loop. *J Virol* 1994;68:5991-6005.
35. Zhu T, Mo H, Wang N, et al. Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. *Science* 1993;261:1179-81.