Immunopathogenic Aspects of Infection by the Human Immunodeficiency Virus in Venezuela

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A study of the immunopathogenic characteristics of HIV infection was begun in 1984 at the National Reference Center on Clinical Immunology (CNRIC) in Caracas, Venezuela, on 240 individuals with a variety of clinical manifestations. The most important findings were depletion of the CD4 cells in HIV-infected individuals, including asymptomatic carriers; significant reduction of the CD3⁻, CD16⁺ large granular lymphocytes (LGL) in AIDS cases; decrease in LGL cytotoxic activity in infected persons versus controls, along with increased lytic function induced by stimulation with recombinant interleukin-2 in both groups; and reduction of the CD4 population in AIDS patients, independent of the presence or absence of free serum antigen. Such research is helping to clarify the immunopathogenic mechanisms of HIV and possible geographic and demographic variations.

The physiopathogenesis of human immunodeficiency virus (HIV) infection is largely due to profound changes in the lymphocytic subpopulations. Since acquired immunodeficiency syndrome (AIDS) was first described by Gottleib *et al.* (*1*), studies have established that the lymphocytes of the infected host are victims of a specific tropism of HIV for those cells and particularly for subpopulations of T lymphocytes that express the CD4 antigen (*2–4*). The immunopathogenesis of HIV generally compromises not only CD4 lymphocytes but also the large granular lymphocytes (LGL), which have the $CD3^-$, $CD16^+$ phenotype surface marker and whose principal function is natural cytotoxicity (*5*).

In the last two years, the availability of kits for detecting circulating HIV antigens and anti-HIV antibodies has made possible immunologic and clinical research on the virus-host relationships and their implications for the natural history of HIV infection.

MATERIALS AND METHODS

Since 1984, a prospective study of 240 patients with HIV infection has been carried out at the National Reference Center for Clinical Immunology in Caracas in an attempt to establish the immunopathogenic characteristics of HIV infection in Venezuela. The patients were grouped according to the classification recommended by the U.S. Centers for Disease Control (CDC) in Atlanta, Georgia (*6*), on the basis of their clinical picture when they were first examined at the Center.

Ninety-five patients were asymptomatic HIV carriers (Group II), 34 had persistent chronic lymphadenopathy (Group III), 22 had HIV-related symptoms but did not meet the criteria for

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AIDS (Group IV–without AIDS), and 89 had AIDS (*7*). Regarding the risk factors for HIV infection, 234 (97.5%) of the patients were homosexual or bisexual males, heterosexual transmission was confirmed in five of the six females (known seropositive partners), and transmission occurred via blood transfusion in the remaining female.

The presence of antibodies for HIV was investigated using enzyme-linked immunosorbent assay (ELISA) (Abbott HTLV III EIA and some samples of Abbott Recombinant HIV-1 EIA, Abbott Laboratories, Diagnostic Division, Chicago, IL, USA), and their specificity against the isolated virus proteins was tested by means of Western blot (Biotech/Dupont HIV Western blot, Dupont Company, Wilmington, DE, USA).

The presence of free circulating antigens was confirmed by ELISA. In 49 patients the lymphocytic subpopulations were studied at the same time.

Peripheral blood mononuclear cells (PBMC) were obtained by centrifuge on Ficoll-Hypaque gradients (*8*). Identification of T lymphocytes (CD3) and CD4 and CD8 subpopulations was done by first marking the cell surface with monoclonal antibodies against CD3, CD4, and CD8 antigens (OKT-3, 4, 8, Ortho Diagnostic Systems, Inc., and Leu-2, 3, 4 donated by Dr. E. Engleman of Stanford University, USA). In the second phase, a secondary fluorescein-marked antibody was added, and the samples were then examined with a fluorescence microscope (GAMFIT, Ortho Diagnostic Systems, Inc., USA). All assays included two control samples obtained from healthy volunteer blood donors or laboratory personnel, and a total of 100 controls was accumulated.

The number of CD16 cells was determined by indirect immunofluorescence, using the B73.1 monoclonal antibody (Leu-11a, donated by Dr. Félix Tapia, Instituto de Biomedicina, Caracas) (*9*). Natural cytotoxic activity was evaluated against the K562 cell line in a short-duration (four hours) microcytotoxicity assay by release of radioactive chromium (^{51}Cr) (*10, 11*). In a second set of experiments, the PBMC obtained from patients and controls were treated with recombinant interleukin-2 (rIL-2) before the cytotoxicity assay against K562 cells.

The statistical significance of differences between mean results was analyzed by application of the Student-Fisher t-test for unpaired data, using a Hewlett-Packard model 67 calculator.

RESULTS

All the patients studied had anti-HIV antibodies detected by ELISA and confirmed by Western blot, with visible bands for the proteins of at least two viral genes.

Sera of 56 patients at different clinical stages of the infection were studied; free antigen was detected in 22 cases (39%). Table 1 shows the distribution of the results by CDC clinical group. In cases diagnosed as AIDS, 13 of 20 individuals (65%) had measurable levels of serum antigen, whereas circulating HIV antigen was detected in only one-third (seven of 20) of the asymptomatic carriers (Group II).

The CD4 population was lower in all

Table 1. Distribution of free HIV serum antigen in patients grouped according to the U.S. Centers for Disease Control classification, Caracas, Venezuela, 1984–1988.

	Clinical group			AIDS	
Antigen	II[a]	III[b]	IV[c]	patients	Total
Positive	7	0	2	13	22
Negative	13	10	4	7	34
Total	20	10	6	20	56

[a]Asymptomatic infection.
[b]Persistent generalized lymphadenopathy.
[c]Other illness associated with HIV.

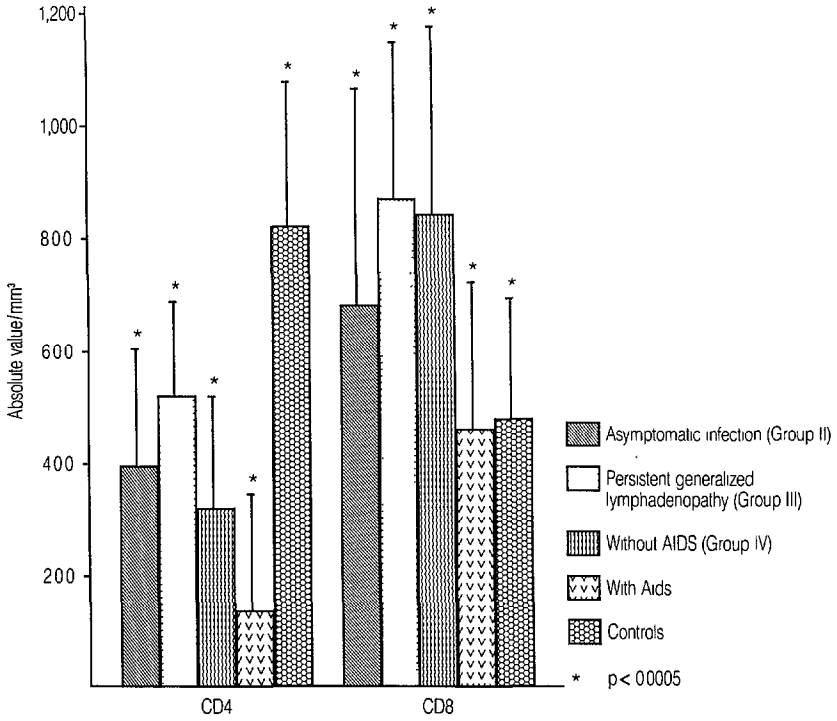

Figure 1. Absolute values ($\bar{x} \pm 1SD$) of CD4 and CD8 antigens in controls and HIV-infected patients grouped according to the U.S. Centers for Disease Control classification, Caracas, Venezuela, 1984–1988.

the clinical groups than in the controls (Figure 1). It is notable that the asymptomatic carriers had absolute values of CD4 below 50% of the values observed in controls (398/mm^3 compared to 825/mm^3), a significant reduction ($p < 0.005$). An increase of the CD8 population was evidenced in all the groups, with the exception of the AIDS patients; the increase was most pronounced in the patients with persistent generalized lymphadenopathy (Group III). A reduced CD4/CD8 ratio in all groups reflects the changes in the CD4 and CD8 populations.

The population of large granular lymphocytes (LGL, natural cytotoxic cells) was evaluated both in terms of variations in the $CD3^-$, $CD16^+$ subpopulation and in their lytic activity against the K562 cell line. A significant depletion of these LGL was detected in AIDS patients compared to controls, but the reduced values in seropositive patients without AIDS were not statistically significant. While the cytotoxic function against K562 cells was diminished in both groups, in neither case was the reduction significant (Table 2). The response of this subpopulation after stimulation with rIL-2 was also investigated in both infected patients and controls. In both groups, a considerable increase of lytic function was observed (Table 3).

In 49 patients with HIV infection, the presence or absence of serum antigen was investigated along with the values for subpopulations of T lymphocytes. The number of CD4 cells in HIV-infected patients without AIDS but with detect-

Table 2. Functional activity of natural cytotoxic lymphocytes in 22 HIV-infected patients and 11 controls, Caracas, Venezuela, 1984–1988.

	$CD16^+$ lymphocytes[a]		
Clinical group	% (+ SD)	Absolute value per mm^3 ($\bar{x} \pm$ SD)	Lysis[b] (%)
AIDS (n=7)	8 ± 1	139 ± 112[c]	28 ± 13
Without AIDS (n=15)	11 ±3	280 ± 150	28 ± 13
Controls (n=11)	11 ± 2	327 ± 77	36 ± 11

[a] $CD3^-$, $CD16^+$ (Leu-11c)
[b] Release of ^{51}Cr, K562 cell line
[c] $p < 0.005$ vs. controls

able levels of serum antigen was significantly less ($p < 0.05$) than in patients in the same clinical group but negative for antigen. However, among patients with AIDS, depletion of the CD4 population was not correlated with the presence or absence of HIV antigen (Table 4).

DISCUSSION

One of the most notable findings in the initial report of AIDS (*1*) was the depletion of CD4 lymphocytes. Since then, the specific tropism of HIV for lymphocytes and glial cells has been confirmed. Recently, alterations of the LGL that carry the phenotype $CD3^-$, $CD16^+$ have also been found to be among the profound immunopathogenic effects of HIV infection on the immune system (*5*).

CD4 lymphocytes are particularly susceptible to the cytopathic action of HIV (*2–4*), as are other cells that have the CD4 molecule on their surface (macrophages, Langerhans cells, and glial cells). CD4 appears to be the receptor molecule for HIV, and the HIV-CD4 interaction is mediated by the gp120 and gp41 proteins of the virus (*12*). Moreover, monoclonal antibodies directed against epitopes of the CD4 molecule can inhibit the cytopathic effect of HIV in vitro (*13*).

The CD4 subset is made up of lymphocytic subpopulations that activate CD8 suppressor lymphocytes and of subpopulations of helper cells that cooperate

Table 3. Response of natural cytotoxic lymphocytes to recombinant interleukin-2 (rIL-2) in HIV-infected patients and controls in Caracas, Venezuela, 1984–1988.

	Baseline (% cytotoxicity)	After rIL-2 (500 u/ml)
Patients		
1	20	30
2	8	30
3	74	71
4	34	37
5	33	53
6	14	58
7	32	71
Controls		
1	52	71
2	28	71
3	34	73

Table 4. Absolute values of CD4 and detection of HIV antigen in serum in patients in Caracas, Venezuela, 1984–1988.

	CD4 per mm^3	
Clinical group	Positive for antigen (n) $\bar{x} \pm$ SD	Negative for antigen (n) $\bar{x} \pm$ SD
With AIDS (n=16)	(10) 142 ± 220	(6) 110 ± 102
Without AIDS (n=33)	(8) 219 ± 172	(25) 436 ± 342[a]

[a] Difference significant ($p < 0.05$)

with B lymphocytes in the synthesis of specific antibodies. The phenotype of the first subpopulation is $CD4^+$, $CD45R^+$, and that of the second is $CD4^+$, $CDW29^+$ (*14, 15, 16*). Recent research has yielded preliminary information on the immunopathogenic effects of HIV on these subpopulations. Vuillier *et al.* (*17*) evaluated 352 patients at different clinical stages of HIV infection, and compared them with 16 high-risk seronegative homosexuals and 61 controls. The findings reveal a decrease in the number of CD4 lymphocytes from the initial stages of the infection, with reduction of the $CD4^+$, $CDW29^+$ and $CD4^+$, $CD45R^+$ subpopulations in asymptomatic carriers, persons with AIDS-related complex, and AIDS patients. However, in Group III patients the $CD4^+$, $CD45R^+$ subpopulation remained intact, while in high-risk seronegatives the $CD4^+$, $CDW29^+$ subpopulation showed a significant increase.

Although the pattern of progressive reduction of CD4 in infected patients found in the study reported here was similar to that described in other studies, the CD4 values in Group II patients (asymptomatic carriers) in the present study showed a greater depletion of that subset than was reported by Vuillier *et al.* (*17*) and Andrieu *et al.* (*18*).

This finding is important considering that the more depleted CD4 lymphocytes become, the greater the likelihood that AIDS will develop within a shorter time period (*18, 19, 20*), which could imply a more accentuated cytopathic effect of HIV in the patients studied. The seven (35%) of 20 Group II patients who had circulating HIV antigen experienced a loss of specific immune response to tetanus toxoid-type soluble antigens (results not presented in detail here) similar to that reported by Fauci *et al.* (*12*).

In contrast to $CD4^+$ lymphocytes, the numbers of $CD3^+$, $CD4^-$, $CD8^+$ lymphocytes (responsible for suppressor and cytotoxic functions) are generally significantly increased in peripheral blood. The apparent resistance of CD8 lymphocytes to HIV infection was suggested by C. M. Walker *et al.* (*21*). Moreover, evidence of the existence of cytotoxic T lymphocytes (CTL) of phenotypes $CD3^+$, $CD8^+$, $CD11^-$ that are specific against components of HIV was found by B. D. Walker *et al.* (*22*) using recombinant vaccinia virus transfected with the different HIV genes, which induces the expression of the virus proteins in B lymphocytes previously transformed by the Epstein-Barr virus. Furthermore, in bronchoalveolar washings from patients infected by HIV, Plata *et al.* (*23*) found CTL directed against autologous alveolar macrophages previously hybridized with DNA probes that contained the complete HIV genome. It has not yet been determined for certain whether the elevated number of $CD8^+$ lymphocytes observed even in critical stages of HIV infection implies an active state of specific in vitro defense against HIV. Moreover, studies of the $CD3^+$, $CD4^-$, $CD8^+$, $CD11^+$ subpopulation, which is basically formed by suppressor T lymphocytes, have not clarified the situation. This subpopulation does not generally show changes during different clinical stages of the infection, but Vuillier *et al.* (*5*) reported a significant increase in these lymphocytes in high-risk seronegative homosexuals. Thus, the immunopathogenic role that might be played by suppressor lymphocytes in patients with HIV infection is unknown.

In the present study, the $CD3^+$, $CD4^-$, $CD8^+$ subpopulation appears to have increased significantly in groups II, III, and IV (without AIDS) HIV-infected patients; however, the absolute value of this subpopulation in AIDS patients was similar to that in controls.

Initial observations based on the in vitro testing of the LGL cells' activity against tumor cell lines such as the K562

indicated diminished lytic activity in AIDS patients (*24–26*). Later, Ruscetti *et al.* (*27*) reported that LGL with the $CD16^+$, Leu-19^+ phenotype from healthy donors, activated in vitro with rIL-2, had optimal lytic activity against white blood cells infected by HTLV-I or HIV. Studies of $CD3^+$, $CD16^+$ subpopulations (*28, 29*) found that the LGL were capable of serving as effectors for antibody-dependent cellular cytotoxicity against P-815 cell lines, concomitant with a considerable decrease in natural cytotoxic action against the K562 line. The recent studies of Vuillier *et al.* (*5*) have provided more concrete information about the LGL complex ($CD3^-$, $CD8^+$, $CD16^+$, Leu-19^+) during the course of HIV infection. When this population is analyzed as a whole, a decline of the $CD3^-$, $CD16^+$ lymphocytes is observed, probably associated with lymphopenia induced by HIV. Nonetheless, on investigating the same population using two-color flow cytometry (simultaneous use of two monoclonal antibodies), a significant reduction of the number of $CD3^-$, $CD8^+$, $CD16^+$ lymphocytes was found, particularly those that expressed low-density $CD8^+$ ($CD3^-$, $CD8BD^+$, $CD16^+$), which account for 95% of this population in peripheral blood under normal conditions. Moreover, in high-risk seronegative individuals, a similar but less marked reduction of the $CD3^-$, $CD8BD^+$, $CD16^+$ lymphocytes was demonstrated.

The present study, in contrast to other studies (*12, 18*), recorded changes in the LGL population ($CD3^-$, $CD16^+$) that confirm the depletion of these natural cytotoxic cells originally reported by Vuillier *et al.* (*5*). The decrease was significant ($p < 0.05$) only in patients with AIDS. Furthermore, controverting the idea that the cytotoxic capacity of $CD3^-$, $CD16^+$ cells against K562 lines declines (*12, 25, 26, 29*), patients in the present study showed an insignificant decline, compared to controls, in the lytic capacity, and unconfirmed results indicate that it can be maintained within the normal range in patients with terminal AIDS, in whom more than 80% of the total volume of LGL is depleted. The increase in lytic action against the K562 cell line after its incubation with rIL-2 was similar to previously reported results (*12, 25*). The cause of the progressive depletion of the $CD3^-$, $CD16^+$ lymphocytic subpopulation remains obscure. Moreover, reduction of the cell volume of this subset appears to be similar to that observed for the CD4 lymphocytes, which suggests the need to carry out prospective investigations to explain both the depletion mechanism and its implications for the natural history of HIV infection.

Even though HIV antigens were more commonly detected in the blood of AIDS patients (65%), our results show significant depletion of CD4 lymphocytes in those patients, independent of the presence or absence of circulating antigen. The absolute values of CD4 in the HIV-infected patients without AIDS appear to be lower. Finally, the free antigen was detectable in only 35% of the asymptomatic carriers.

These findings have made it possible to begin to define the immunopathogeny of HIV infection and possible geographic and demographic variations. Nonetheless, many questions have yet to be resolved and will require the application of new immunologic, clinical, and therapeutic approaches.

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REFERENCES

1. Gottlieb, M. S., R. Schroff, H. M. Schanker, *et al. Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. *N Engl J Med* 305:1425–1431, 1981.
2. Dalgleish, A. G., P. C. Beverly, P. R. Clapham, *et al.* The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* 312:763, 1984.
3. Klatzmann, D., E. Champagne, S. Chamaret, *et al.* T lymphocyte T4 molecule behaves as the receptor for human retrovirus. *Nature* 312:767, 1984.
4. McDougal, J. S., A. Mawle, S. P. Cort, *et al.* Cellular tropism of the human retrovirus HTLV-III/LAV. I. Role of T cell activation and expression of the T4 antigen. *J Immunol* 135: 3151–3162, 1985.
5. Vuillier, F., N. E. Bianco, L. Montagnier, *et al.* Selective depletion of low density $CD8^+$, $CD16^+$ lymphocytes during HIV infection. *AIDS Research and Human Retroviruses* 4:121–129, 1988.
6. Centers for Disease Control. Classification system for human T-lymphotropic virus type III. Lymphadenopathy-associated virus infection. *MMWR* 35:334, 1986.
7. Centers for Disease Control. Update on acquired immune-deficiency syndrome (AIDS). *MMWR* 31:507–514, 1982.
8. Boyum, A. J. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 97:1–10, 1968.
9. Rodríguez, M., I. Blanca, M. L. Baroja, *et al.* Helper activity by human large granular lymphocytes in "in vitro" immunoglobin synthesis. *J Clin Immunol* 7(5):356–364, 1987.
10. Bloom, E. T., and E. L. Korn. Quantification of natural cytotoxicity by human lymphocyte subpopulations isolated by density: Heterogeneity of the effector cells. *J Immunol Methods* 58:323–325, 1983.
11. Lozzio, C. B., and B. B. Lozzio. Human chronic myelogenous leukaemia cell line with positive philadelphia chromosome. *Blood* 45:321–334, 1975.
12. Fauci, A. S. The human immunodeficiency virus: Infectivity and mechanisms of pathogenesis. *Science* 239:617–622, 1988.
13. Ho, D. D., R. J. Pomerantz, and J. C. Kaplan. Pathogenesis of infection with human immunodeficiency virus. *N Engl J Med* 317:278–286, 1987.
14. Morimoto, C., N. L. Letvin, A. W. Boyd, *et al.* The isolation and characterization of the human helper inducer T cell subset. *J Immunol* 134:3762–3768, 1985.
15. Morimoto, C., N. L. Letvin, J. A. Distaso, *et al.* The isolation and characterization of the human suppressor inducer T cell subset. *J Immunol* 134:1508–1515, 1985.
16. Takeuchi, T., M. Dimaggio, H. Levine, *et al.* CD11 molecule defines the two types of suppressor cells within the $T8^+$ population. *Cell Immunol* 111:398–409, 1988.
17. Vuillier, F., C. Lapresle, and G. Dighiero. Comparative analysis of CD4-4B4 and CD4-2H4 lymphocyte subpopulations in HIV negative homosexual, HIV seropositive and healthy subjects. *Clin Exp Immunol* 71:8–12, 1988.
18. Andrieu, J. M., D. Eme, A. Venet, *et al.* Serum HIV antigen and anti-p24 antibodies in 200 HIV seropositive patients: Correlation with CD4 and CD8 lymphocyte subsets. *Clin Exp Immunol* 73:1–5, 1988.
19. Polk, B. F., R. Fox, R. Brookmeyer, *et al.* Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. *N Engl J Med* 316:61–67, 1987.
20. Fahey, J. L., J. Giorgi, O. Martinez-Maza, *et al.* Immune pathogenesis of AIDS and related syndromes. *In: Acquired Immunodeficiency Syndrome* (eds. J. C. Gluckman and E. Vilmer), Paris, Elsevier, 1987, p. 107.
21. Walker, C. M., D. J. Moody, D. P. Stites, and J. A. Levy. $CD8^+$ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* 234:1563–1566, 1986.
22. Walker, B. D., S. Chakrabarti, B. Moss, *et al.* HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature* 328:345–348, 1987.
23. Plata, F., B. Autran, L. Pedroza Martins, *et al.* AIDS virus-specific cytotoxic T lymphocytes in lung disorders. *Nature* 328:348–351, 1987.
24. Poli, G., M. Introna, F. Zariaboni, *et al.* Natural killer cells in intravenous drug abusers with lymphadenopathy syndrome. *Clin Exp Immunol* 62:128, 1985.

25. Alcocer-Varela, J., D. Alarcón-Segovia, and C. Abid-Mendoza. Immunoregulatory circuits in the acquired immune deficiency syndrome and related complex. Production of and response to interleukin 1 and 2, NK function and its enhancement by interleukin 2 and kinetics of the autologous mixed lymphocyte reaction. *Clin Exp Immunol* 60:31:, 1985.
26. Spicuett, G. P., and A. G. Dalgleish. Cellular immunology of HIV infection. *Clin Exp Immunol* 71:1–7, 1988.
27. Ruscetti, F. W., J. A. Mikovits, V. S. Kalyanaraman, *et al.* Analysis of effector mechanisms against HTLV-I and HTLV-III/LAV-infected lymphoid cells. *J Immunol* 136:3619–3624, 1986.
28. Bonavida, B., J. Katz, and M. Gottlieb. Mechanism of defective NK cell activity in patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex. I. Defective trigger on NK cells for NKCF production by target cells, and partial restoration by IL 2. *J Immunol* 137:1157–1164, 1986.
29. Katz, J. D., R. Mitsuyasu, M. S. Gottlieb, *et al.* Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. II. Normal antibody-dependent cellular cytotoxicity (ADCC) mediated by effector cells defective in natural killer (NK) cytotoxicity. *J Immunol* 139:55–60, 1987.

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HIV and Common Social Situations

Statements have been issued by the WHO Collaborating Centers on AIDS regarding certain common social situations in which HIV-infected individuals may be involved. The statements take into account (1) knowledge obtained through numerous studies conducted in many parts of the world; (2) the statement entitled "Transmission of HIV" from the Third Meeting of Participating Parties in June 1987 (unpublished WHO document SPA/INF/87.5); and (3) the World Health Assembly resolution on "Avoidance of Discrimination in Relation to HIV-infected People and People with AIDS" (WHA41.24).

The areas discussed were housing, employment, and schooling. With regard to housing, there is no public health rationale for subjecting HIV-infected persons to housing restrictions. When an HIV-infected person has occupied and then left a dwelling, no special cleaning or other procedures are needed prior to occupancy by another person. Likewise, there is no public health rationale for restricting healthy HIV-infected persons from employment. Access to and full participation in school activities at the primary or higher level should be ensured for healthy HIV-infected students, since there is no public health rationale for denying such access.

Source: World Health Organization. *Weekly Epidemiological Record* 64:3, 1989.