Original Research

The Vancouver Lymphadenopathy-AIDS Study: 7. Clinical and laboratory features of 87 cases of primary HIV infection

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In an ongoing prospective study of homosexual men conducted in Vancouver since November 1982, 87 cases of human immunodeficiency virus (HIV) seroconversion have been documented to date. Comparison of laboratory results obtained a mean of 4.9 months before and 5.4 months after the estimated date of seroconversion revealed that a significant increase in the serum IgG level (from 1149 to 1335 mg/dl on average) and in C1q binding (from 8.8% to 14.2% on average) was associated with early HIV infection ($p < 0.001$). A marginally significant decrease in the ratio of helper to suppressor (CD4 to CD8) cells (from 1.55 to 1.29 on average) was also noted ($p = 0.025$). A marked decrease in absolute number of CD4 cells was not seen with seroconversion, which suggests that profound loss of these cells may be a long-term effect of HIV infection. The occurrence of symptoms (including fatigue, fever, night sweats, unintentional weight loss, diarrhea, joint pains, cough unrelated to smoking, shortness of breath, oral thrush, herpes zoster and rash) did not increase with seroconversion. This finding suggests that most cases of HIV seroconversion may be asymptomatic or associated with relatively minor symptoms. On the other hand, generalized lymphadenopathy was found to develop after HIV seroconversion in about 50% of cases.

Chez des homosexuels masculins suivis continuellement à Vancouver depuis novembre 1982, nous enregistrons à ce jour 87 virages sérologiques à l'égard du virus immunodéficitaire humain (VIH). Si on compare les chiffres consignés en moyenne 4,9 mois avant la date présumée du virage à ceux consignés en moyenne 5,4 mois après, on note que l'infection récente par le VIH s'accompagne d'une augmentation significative ($p < 0,001$) de l'IgG sérique (de 1149 à 1335 mg/dl en moyenne) et de la fixation de C1q (de 8,8% à 14,2% en moyenne), de même qu'un abaissement du rapport lymphocytes inducteurs-inhibiteurs (CD4-CD8) (de 1,55 à 1,29 en moyenne), qui est faiblement significatif ($p = 0,025$). Le nombre absolu de CD4 ne s'abaissant pas de façon marquée lors du virage, on peut croire qu'une perte grave de ces lymphocytes est le fait d'une infection à VIH de longue terme. Le virage ne s'accompagne pas d'une augmentation de fréquence de divers symptômes: abattement, fièvre, sueurs nocturnes, perte de poids involontaire, diarrhée, arthralgie, toux non reliée au tabac, dyspnée, muguet buccal, zona et éruptions. Dans la plupart des cas on peut dire qu'il est asymptomatique ou ne se manifeste que par des symptômes relativement légers, hormis la lymphadénopathie généralisée qui survient chez environ la moitié des sujets après virage sérologique.

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Human immunodeficiency virus (HIV) is now accepted as the causative agent of acquired immune deficiency syndrome (AIDS). In two small, recent studies of acute HIV infection, most subjects had an acute febrile illness

resembling influenza or acute infectious mononucleosis.[1,2] Leukopenia, transient lymphopenia, mild thrombocytopenia and inversion of the ratio of helper to suppressor (CD4 to CD8) cells due to an increase in the number of CD8 cells were also observed. However, in some cases asymptomatic seroconversion was noted. The proportion of seroconverters who have clinical or laboratory abnormalities as well as the spectrum of disease associated with acute HIV infection have not been reported from a large series. We describe the clinical and laboratory features of 87 subjects who underwent HIV seroconversion while being followed in our ongoing prospective study.

Methods

As previously described,[3] the Vancouver Lymphadenopathy–AIDS Study is an ongoing prospective study of over 700 homosexual men who were recruited from six general practices in central Vancouver between November 1982 and February 1984. Only patients already enrolled in the practices were asked to participate; approximately 5% refused. Subjects have been returning for follow-up visits approximately every 6 months since enrolment. During each visit a questionnaire is administered, a complete physical examination is performed by the patient's physician, and a blood sample is drawn for immunologic and HIV antibody testing. As well, some subjects undergo HIV antibody testing between study visits, at their physician's discretion.

HIV antibody tests are performed at the Laboratory Centre for Disease Control (LCDC), in Ottawa, by means of the enzyme-linked immunosorbent assay (ELISA). Equivocal results are confirmed by means of the Western blot technique.[4] Seroconversion is defined as the occurrence of a positive result of HIV antibody testing during the observation period in a subject who was seronegative at the time of enrolment. The date of seroconversion is estimated as the midpoint of the interval between the last negative and the first positive test result.

Between January 1983 and September 1986, 87 cases of seroconversion were observed among the 345 men who were seronegative at the time of enrolment. For each seroconverter we obtained data on laboratory variables, symptoms and physical findings from the visits that corresponded to the last negative (preseroconversion) and the first positive (postseroconversion) result of testing for HIV antibody. Because of limited facilities during the first two cycles of our study, which were conducted during 1983–84, certain laboratory tests (particularly lymphocyte subset analyses) were carried out only in random subsamples of the cohort. Thus, for some seroconverters whose preseroconversion visit occurred during one of the first two cycles the laboratory result was not available. This accounts for the differing numbers of subjects for whom complete paired sets of data were available. Data on lymphocyte subsets were most affected: results from both visits were available for only 33 of the 87 seroconverters (38%). However, because random subsets were used, no bias was introduced, although the statistical power was reduced.

For purposes of comparison, a seronegative control group was selected. Two seronegative subjects were matched to each seroconverter according to the following criteria: year of birth (within 2 years), a visit within 3 months of the preseroconversion visit by the seroconverter and a subsequent visit within 3 months of the postseroconversion visit by the seroconverter. (For convenience, the visits by the control subjects will also be referred to as preseroconversion and postseroconversion visits.) Data on symptoms and physical findings for the seronegative control subjects were obtained from these two visits.

We carried out statistical analysis of the data using nonparametric methods suitable for paired comparisons because inspection revealed many of the variables not to be normally distributed. Laboratory data were analysed with the Wilcoxon signed-rank test.[5] Data on symptoms and physical findings were analysed by means of Pearson's chi-square statistic and McNemar's test.[6] All p values are two-sided. For all paired comparisons subjects with incomplete or missing data for the variable of interest were excluded from the analysis. All statistical analyses were carried out by means of the SPSS statistical software package.[7]

Because multiple comparisons were made, including consideration of 10 laboratory variables, we adopted the following convention for interpreting statistical significance: p values below 0.005 were considered statistically significant, values above 0.05 were considered nonsignificant, and values between 0.005 and 0.05 were considered to indicate associations that were of marginal statistical significance and worth further consideration.

Results

For the 87 seroconverters the data from the preseroconversion and postconversion visits were obtained a mean of 4.9 months before and 5.4 months after the estimated date of seroconversion; the mean interval between visits was thus 10.3 months. The mean age at the time of the preseroconversion visit was 30.5 (extremes 19 and 47) years. For the seronegative controls the mean interval between visits was 10.1 months, and the mean age at the preseroconversion visit was 30.7 (extremes 18 and 47) years.

The mean laboratory results obtained before and after seroconversion for the seroconverters are shown in Table I. Significant differences were detected before and after seroconversion in the serum IgG level (1149 v. 1335 mg/dl) and in C1q binding (8.8% v. 14.2%). As well, marginally significant declines in the hemoglobin level (from 15.3 to 15.1 g/L) and in the leukocyte count (from 6.3 to 5.8 $\times$ 10^9/L) were noted. Although the mean

number of CD4 cells fell and the mean number of CD8 cells rose, these changes were not statistically significant. However, there was a marginally significant decline in the ratio of CD4 to CD8 cells (from 1.55 to 1.29). No significant difference was found in the lymphocyte count, IgA level or IgM level.

Table II shows the number of subjects with two or more symptoms at the preseroconversion and postseroconversion visits. Overall, 16% of the seroconverters and 11% of the control subjects had two or more symptoms at one visit or more. Among the subjects who were symptom free at the preseroconversion visit 5 of 57 seroconverters and 4 of 112 control subjects had two or more symptoms at the postseroconversion visit, a nonsignificant difference.

No significant differences were detected in the occurrence of the following self-reported symptoms before and after seroconversion: fatigue, fever, night sweats, unintentional weight loss, diarrhea, joint pains, cough unrelated to smoking, shortness of breath, oral thrush and herpes zoster. A total of 55 subjects seroconverted in the interval between completing two successive questionnaires, the mean interval between seroconversion and completing the second questionnaire being 3.5 months. Analysis of the data for this subset also failed to reveal a significant increase in symptoms around the time of seroconversion.

Table III shows the number of subjects with generalized lymphadenopathy (defined as the presence of lymph nodes greater than 1 cm in diameter at two or more extrainguinal sites). A significant association was observed between the appearance of generalized lymphadenopathy and seroconversion. Of the subjects who were free of the condition at the preseroconversion visit 20 of 40 seroconverters (50%) and 7 of 110 control subjects (6%) showed it at the postseroconversion visit ($p < 0.0001$). It is noteworthy that 46% of the seroconverters had generalized lymphadenopathy before the estimated date of seroconversion. A marginally significant trend toward resolution of

Table I — Laboratory results obtained before and after human immunodeficiency virus seroconversion for 87 seroconverters in a cohort of homosexual men

Variable	No. of subjects*	Mean result (and standard deviation) Before seroconversion	After seroconversion	p†
Hemoglobin level, g/L	70	15.3 (0.89)	15.1 (0.93)	0.035
Leukocyte count, $\times 10^9$/L	70	6.3 (1.8)	5.8 (1.6)	0.024
Lymphocyte count, $\times 10^9$/L	66	2.1 (0.8)	2.1 (0.8)	NS
IgG level, mg/dl	64	1149 (346)	1335 (385)	< 0.001
IgA level, mg/dl	64	197 (105)	202 (125)	NS
IgM level, mg/dl	64	135 (53)	142 (62)	NS
C1q binding, %	49	8.8 (5.6)	14.2 (11.1)	< 0.001
Absolute no. of CD4 cells per millilitre	33	863 (325)	799 (323)	NS
Absolute no. of CD8 cells per millilitre	33	598 (207)	670 (305)	NS
Ratio of CD4 to CD8 cell counts	33	1.55 (0.66)	1.29 (0.514)	0.025

*Number for whom both sets of results were available.
†Based on Wilcoxon test for paired samples; NS = not significant.

Table II — Number of subjects with two or more symptoms* at the preseroconversion and postseroconversion visits

Group	No. (and %) of subjects Neither visit	Preseroconversion visit only	Postseroconversion visit only	Both visits
Seroconverters (n = 62)	52 (84)	3 (5)	5 (8)	2 (3)
Seronegative control subjects (n = 122)	108 (88)	9 (7)	4 (3)	1 (1)

*Includes fatigue, fever, night sweats, unintentional weight loss, diarrhea, joint pains, cough unrelated to smoking, shortness of breath, oral thrush and herpes zoster.

Table III — Number of subjects with generalized lymphadenopathy at the two visits

Group	No. (and %) of subjects Neither visit	Preseroconversion visit only	Postseroconversion visit only	Both visits
Seroconverters (n = 74)	20 (27)	5 (7)	20 (27)	29 (39)
Seronegative control subjects (n = 144)	103 (72)	18 (12)	7 (5)	16 (11)

generalized lymphadenopathy between the visits was observed for the control subjects (McNemar's test for discordant pairs 18/7, $p = 0.043$). The rates of rash, hepatomegaly and splenomegaly did not differ between the two groups.

Discussion

Comparison of laboratory results obtained before and after seroconversion for the 87 seroconverters in our cohort revealed that a marked increase in C1q binding, which represents an increase in immune complex levels, was associated with acute HIV infection. Increased immune complex levels have previously been noted in patients with AIDS or other HIV-related syndromes.[8-10] While the complete composition of these immune complexes is not known, recent reports suggest that HIV and other viruses may form one component.[11] The early rise in immune complex levels that we observed is consistent with the presence of HIV–anti-HIV complexes.[11] Alternatively, antilymphocyte autoantibody,[12] other tissue-specific antibodies, antibodies to drug protein complexes and antisperm antibodies[13] could also contribute to the C1q-binding activity. The importance of elevation of immune complex levels in the development of progressive immune dysfunction has not been systematically studied; however, in a previous, longitudinal study we did not identify elevated immune complex levels as a significant antecedent laboratory marker in a group of seropositive men in whom AIDS developed who were compared with a group of AIDS-free seropositive control subjects.[14] In the present study the increase in immune complex levels was paralleled by an increase in serum IgG levels. The polyclonal increase in the serum IgG level could have been a response to HIV, a direct mitogenic effect of HIV on B-cells, a response to reactivation of other viruses or a result of loss of immune regulatory control.[15-17]

A profound decrease in the absolute number of CD4 cells did not occur during the observation period. We also did not detect a marked rise in the number of CD8 cells, although there was some evidence of a mild change in both variables with seroconversion. Because the number of paired observations was lowest for lymphocyte subsets, a type II error in this regard was possible. Although acute lymphopenia with a short-term decrease in the absolute number of CD4 cells has been noted in a few cases after seroconversion,[2] our data suggest that any initial depletion is not persistent; thus, the marked decrease in number of CD4 cells among seropositive subjects appears to be a longer-term effect of HIV infection. The lack of marked early depletion of CD4 cells suggests that the virus is in a latent, nonlympholytic phase in most subjects. Subsequent depletion of CD4 cells may be due to antigen stimulation or to reactivation of Epstein–Barr virus (EBV) or cytomegalovirus (CMV),[18,19] which could give rise to lymphocyte stimulation and result in an HIV lympholytic cycle or spread of HIV infection to a lymphocyte precursor population in the bone marrow, other lymphoid organs or the central nervous system.

Although we did not detect significant changes in CD4 or CD8 cell counts with seroconversion, a marginally significant decline in the ratio of CD4 to CD8 cell counts was found, which supports the view that this combined marker may be more sensitive to subtle changes than either lymphocyte subset alone.[20]

We also detected mild decreases in both hemoglobin concentration and leukocyte count associated with seroconversion. However, since the changes were of marginal significance, they may have occurred by chance. Furthermore, the magnitude of both decreases was small. It is conceivable that these observations were due to some degree of marrow suppression associated with acute HIV infection; however, direct evidence to support such a hypothesis is lacking.

The central finding of our investigation was that the occurrence of symptoms (including fatigue, fever, night sweats, unintentional weight loss, diarrhea, joint pains, cough unrelated to smoking, shortness of breath, oral thrush and herpes zoster), individually or as complexes of two or more symptoms, did not differ with seroconversion. This is consistent with the finding of Fox and colleagues[21] that most seroconverters in their cohort were asymptomatic or had relatively trivial symptoms. Similarly, of 15 seroconverters studied by Weber and associates[22] none reported or was observed to have a mononucleosis-like illness associated with seroconversion. Berthier and coworkers[23] described 22 young patients with hemophilia who seroconverted while under study; only 6 reported any associated clinical illness, which consisted of lymphadenopathy alone in 3. The infrequency of symptoms in our study could have been a result of the insensitivity of a self-administered questionnaire or, in some cases, the time between the approximate date of seroconversion and completion of the questionnaire at the first visit after seroconversion. However, no changes in self-reported symptoms were noted for a subgroup of seroconverters seen within 3.5 months of the estimated time of seroconversion. Questioning these seroconverters and control subjects about symptoms in the past may prove more sensitive. In contrast, Tindal and collaborators[24] found that most seroconverters reported an acute illness that was of a longer duration than symptoms reported by seronegative controls. The characteristic findings were lethargy, malaise, fever, sore throat, myalgia, anorexia, sweats, arthralgia and headache. Resolution of these discrepancies can be addressed in future prospective studies by frequently determining the serologic status of an HIV-negative cohort and monitoring symptom diaries.

The increased prevalence of generalized lymphadenopathy after seroconversion found in our

study is consistent with the observations in other reports.[1,2,24] It is noteworthy that 46% of the seroconverters for whom paired observations were available had generalized lymphadenopathy before the estimated date of seroconversion. One possible explanation is that HIV infection occurred before the estimated time of seroconversion. Alternatively, since lifestyle practices that predispose to HIV infection also entail a risk of prior exposure to other agents, such as EBV, CMV and *Toxoplasma*,[25] lymphadenopathy not due to HIV may have preceded HIV infection in some subjects. Modification of these lifestyle practices and, hence, of the risks could account for the trend toward resolution of lymphadenopathy not due to HIV in the persistently seronegative men. The proportion of patients with acute HIV infection in our cohort in whom persistent generalized lymphadenopathy develops is currently under study.

We conclude that certain changes in laboratory results, including increased serum IgG and immune complex levels, are found in people with early HIV infection. We speculate that the immune complexes may consist, at least in part, of HIV–anti-HIV complexes that may be identified with newer antigen detection assays. Our failure to document a profound decrease in the absolute number of CD4 cells in early HIV infection suggests that loss of these cells may be a longer-term effect of HIV infection. The lower CD4 counts in HIV-infected populations appear to be due to the fact that the seropositive subjects under study have usually been infected for a considerable period before being tested. We have provided some evidence that most patients with HIV seroconversion are asymptomatic or have only minor symptoms, although self-administered questionnaires may be insensitive in this regard. On the other hand, the appearance of (or a change in) generalized lymphadenopathy seems to accompany a significant proportion of cases of seroconversion.

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References

1. Cooper DA, MacLean P, Finlayson R et al: Acute AIDS retrovirus infection — definition of clinical illness associated with seroconversion. *Lancet* 1985; 1: 537–540
2. Ho DD, Sarngadharan MG, Resnick L et al: Primary human T-lymphotropic virus type III infection. *Ann Intern Med* 1985; 103: 880–883
3. Schechter MT, Boyko WJ, Jeffries E et al: The Vancouver Lymphadenopathy–AIDS Study: 1. Persistent generalized lymphadenopathy. *Can Med Assoc J* 1985; 132: 1273–1279
4. Boyko WJ, Schechter MT, Jeffries E et al: The Vancouver Lymphadenopathy–AIDS Study: 3. Relation of HTLV-III seropositivity, immune status and lymphadenopathy. *Can Med Assoc J* 1985; 133: 28–32
5. Snedecor GW, Cochran WG: *Statistical Methods*, 6th ed, Iowa St U Pr, Ames, 1976: 128–129
6. Armitage P: *Statistical Methods in Medical Research*, Blackwell Sci, Oxford, 1971: 131–135
7. Nie NH, Hull CH, Jenkins JG et al: *SPSS: Statistical Package for the Social Sciences*, 3rd ed, McGraw, New York, 1983
8. Euler HH, Kern P, Loffler H et al: Precipitable immune complexes in healthy homosexual men, acquired immune deficiency syndrome and the related lymphadenopathy syndrome. *Clin Exp Immunol* 1985; 59: 267–275
9. McDougal JS, Hubbard M, Nicholson JK et al: Immune complexes in the acquired immunodeficiency syndrome (AIDS): relationship to disease manifestation, risk group, and immunologic defect. *J Clin Immunol* 1985; 5: 130–138
10. Lightfoote MM, Folks TM, Redfield R et al: Circulating IgA immune complexes in AIDS. *Immunol Invest* 1985; 14: 341–345
11. Morrow WJW, Wharton M, Strickler RB et al: Circulating immune complexes in patients with the acquired immune deficiency syndrome contain the AIDS-associated retrovirus. *Clin Immunol Immunopathol* 1986; 40: 515–524
12. Williams RC, Masur H, Spira TJ: Lymphocyte-reactive antibodies in acquired immune deficiency syndrome. *J Clin Immunol* 1984; 4: 118–123
13. Mavligit GM, Talpaz M, Hsia FT et al: Chronic immune stimulation by sperm alloantigens: support for the hypothesis that spermatozoa induce immune dysregulation in homosexual males. *JAMA* 1984; 251: 237–241
14. Boyko WJ, Schechter MT, Craib KJP et al: The Vancouver Lymphadenopathy–AIDS Study: 5. Antecedent behavioural, clinical and laboratory findings in patients with AIDS and HIV-seropositive controls. *Can Med Assoc J* 1986; 135: 881–887
15. Pahwa S, Pahwa R, Saxinger C et al: Influence of the human T-lymphotropic virus/lymphadenopathy-associated virus on functions of human lymphocytes: evidence for immunosuppressive effects and polyclonal B-cell activation by banded viral preparations. *Proc Natl Acad Sci USA* 1985; 82: 8198–8202
16. Rosen A, Gergely P, Jondal M et al: Polyclonal Ig production after Epstein–Barr virus infection in human lymphocytes in vitro. *Nature* 1977; 267: 52–54
17. Hutt-Fletcher L, Balchandarn N, Elkins MH: B-cell activation by cytomegalovirus. *J Exp Med* 1983; 158: 2171–2176
18. Purtilo DT, Kipscomb H, Krueger G et al: Role of Epstein–Barr virus in acquired immune deficiency syndrome. *Adv Exp Med Biol* 1985; 187: 53–65
19. Birx DL, Redfield RR, Tosato G: Defective regulation of Epstein–Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. *N Engl J Med* 1986; 314: 874–879
20. Melbye M, Biggar RJ, Ebbesen P et al: Long-term seropositivity for human T-lymphotropic virus type III in homosexual men without the acquired immunodeficiency syndrome: development of immunologic and clinical abnormalities. *Ann Intern Med* 1986; 104: 496–499
21. Fox R, Polk BF, Eldred L et al: Clinical correlates of HTLV-III/LAV seroconversion in a cohort of gay men. Presented at the International Conference on AIDS, Paris, June 23–25, 1986
22. Weber JN, Wadsworth J, Rogers LA et al: Three-year prospective study of HTLV-III/LAV infection in homosexual men. *Lancet* 1986; 1: 1179–1182
23. Berthier A, Chamaret S, Fauchet R et al: Transmissibility of human immunodeficiency virus in haemophilic and non-haemophilic children living in a private school in France. *Lancet* 1986; 2: 598–601
24. Tindal B, Barker S, Donovan B et al: Characterisation of the acute illness associated with AIDS retrovirus infection. Presented at the International Conference on AIDS, Paris, June 23–25, 1986
25. Drew WL, Mintz L, Miner RC et al: Prevalence of cytomegalovirus infection in homosexual men. *J Infect Dis* 1981; 143: 188–192