Effects of Exposure to Factor Concentrates Containing Donations From Identified AIDS Patients

A Matched Cohort Study

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We compared recipients of eight lots of factors VIII and IX voluntarily withdrawn from distribution because one donor was known to have subsequently developed the acquired immunodeficiency syndrome with a nonexposed cohort matched by age, sex, and factor use. The factor VIII recipient cohorts did not differ in prevalence of antibody to human immunodeficiency virus (HIV) (exposed, 75%; nonexposed, 86%), T-cell subset numbers (median: exposed, 619 T-helper cells per cubic millimeter; nonexposed, 659 T-helper cells per cubic millimeter), T-helper to T-suppressor ratios, or immunoglobulin levels. Exposed individuals had higher levels of immune complexes by C1q binding and staphylococcal binding assays and lower responses to phytohemagglutinin and concanavalin A. However, only the staphylococcal binding assay values were outside the normal range for our laboratory. Factor IX recipient cohorts did not differ in HIV antibody prevalence (exposed, 30%; nonexposed, 40%) or any immune tests. Although exposed and nonexposed individuals did not differ from each other in a clinically meaningful fashion at initial testing, both the exposed and nonexposed cohorts had high rates of HIV seroprevalence. Market withdrawals were clearly insufficient means of limiting the spread of HIV in hemophilic patients; however, the currently available methods of donor screening and viral inactivation of blood products will prevent continued exposure within this population.

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SINCE August 1983, four companies producing and/or distributing factor VIII or factor IX concentrate products have initiated seven separate voluntary distribution withdrawals of factor concentrate lots to which one of the donors was found to have later developed the acquired immunodeficiency syndrome (AIDS).[1-7] These donors were well at the time of donation, and their donations were pooled with those of thousands of other persons in the manufacturing of these lots; therefore, the companies were being highly conservative in choosing to withdraw these products.

Because of anxieties already prevalent in the hemophilic community concerning AIDS and because of additional concerns raised by these voluntary withdrawals, we decided to evaluate whether individuals exposed to these lots of factor concentrate differed in their immune function or in seroprevalence to a number of viruses, including human immunodeficiency virus (HIV), when compared with persons receiving other lots of factor concentrates. These cohorts will be followed up prospectively for up to five years; their initial evaluation is reported herein.

PARTICIPANTS AND METHODS

We contacted all hemophilia treatment centers and physicians caring for hemophilic patients in the distribution area of two withdrawn lots of factor VIII distributed by the American Red Cross and of two withdrawn lots of factor IX distributed by Hyland Corporation (withdrawal A). (These companies voluntarily supplied their distribution lists for these lots.) These lots included blood components from one individual who developed AIDS nine months following his donation. We also contacted hemophilia treatment centers and physicians in the distribution area for three withdrawn lots of factor VIII and one withdrawn lot of factor

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IX distributed by Cutter Corporation (withdrawal B). (This company voluntarily supplied its distribution lists for these lots.) These lots included donations made by a person who later developed AIDS; the donations for the four lots were made as follows: (1) three donations, nine and ten months prior to the development of AIDS; (2) four donations, eight months prior to the development of AIDS; (3) three donations, six months prior to the development of AIDS; and (4) one donation, ten months prior to the development of AIDS. The patients were first evaluated 12 to 19 months following factor distributions. For withdrawal A, this evaluation was six to 26 months following the last use of the withdrawn lots (median, 16 months); for withdrawal B, it was four to 12 months following the last use (median, five months).

Participants

All patients who had taken the withdrawn lots were asked to participate in the study. Hemophilia treatment centers were asked to match each participant according to the following criteria: type of factor received (VIII or IX), age (±5 years), sex, race, type of hemophilia (A or B), and, for factor VIII recipients, approximate amount of factor received per year (<15 000 units; 15 000 to 29 999 units; 30 000 to 44 999 units; 45 000 to 59 999 units; and ≥60 000 units). Participating centers attempted to match each exposed individual with someone in their own hemophilia treatment center; when this was not possible, other centers were contacted with the assistance of the National Hemophilia Foundation, and these centers attempted to find a nonexposed individual from a nearby area. Individuals who had received products involved in any AIDS-related market withdrawals were excluded from the nonexposed population; any individuals receiving more than one withdrawn lot were excluded from the exposed group. In total, 237 hemophilia treatment centers or physicians were contacted and 159 (67%) responded; 32 of these provided 119 exposed participants, and 43 provided 95 nonexposed participants.

All participants gave their informed consent, completed a questionnaire confirming that they had no risk factors for AIDS other than hemophilia, and attempted to specify (1) the quantity of factor concentrate used in the previous year (N=76 pairs) to three years and (2) the lot numbers of all concentrates used in that time (N=74 pairs).

When data on factor usage were evaluated at the Centers for Disease Control, it was clear that the factor use matching done on the basis of estimates made at the local centers was frequently inaccurate. Exposed individuals were therefore rematched to the above enrolled nonexposed persons on the basis of substantiated and recorded data, using the following criteria: age (±6 years), sex, type of hemophilia, and, for factor VIII recipients, average yearly dosage in the previous three years, or dosage in the previous year ±20 000 units. (Location criteria were not used in this matching, since data from the above nonexposed individuals and for other hemophilic patients tested by us indicate that HIV seroprevalence in hemophilic patients does not vary by location within the United States.[8]) Matching was possible for 57 exposed persons from withdrawal A and 27 exposed persons from withdrawal B, for a total of 84 matched pairs (54 pairs of factor VIII recipients and 30 pairs of factor IX recipients), leaving six exposed participants from withdrawal A and 29 exposed participants from withdrawal B unmatched.

Methods

Serum specimens were tested for antibody to HIV by Western blot analysis as previously described,[8] at a 1:100 dilution, and banding patterns were compared with those of a known positive control serum (51 factor VIII and 30 factor IX recipient pairs). Serologic reactions with any combination of the 18kd, 25kd, and 41kd proteins of HIV were scored as positive.

For mitogen assays and lymphocyte subpopulation quantification, blood was collected by venipuncture into vacuum tubes containing ethylenediaminetetraacetic acid (purple top), acid citrate dextrose (yellow top), or heparin (green top). All blood was kept at room temperature until analysis or further processing. Samples for each exposed individual were collected within approximately one month of those for the matched nonexposed individual. Cell separations were performed by density centrifugation and the separated cells were washed twice by centrifugation in phosphate buffered saline.

Mononuclear cells (1 to 2×10^7/mL) for mitogen assays were frozen in RPMI 1640 containing 50% newborn calf serum and 10% dimethyl sulfoxide. The cells were first placed in double book bags and stored at −70°C for up to 48 hours before being transferred to the vapor phase of a liquid nitrogen freezer. Cells from each exposed individual were thawed and tested for mitogen responsiveness at the same time as cells from the matched nonexposed individual. Cells were thawed by warming at 37°C until the last ice crystal melted. Ten volumes of RPMI 1640 with 10% newborn calf serum were added dropwise, and the cells were centrifuged. Viability was always greater than 80%, and normally 50% to 60% of the cells were recovered. Viability was determined by trypan blue exclusion and differential cell counts were performed on Wright's-stained preparations.

Lymphocyte subpopulations were quantitated by indirect immunofluorescence on a fluorescence-activated cell sorter with commercial monoclonal antibodies OKT3 for T cells, OKT4 for T-helper/inducer cells (T_H) (45 factor VIII and 28 factor IX recipient pairs), and OKT8 for T-suppressor/cytotoxic cells (T_S) (45 factor VIII and 28 factor IX recipient pairs), and fluorescein-conjugated anti-mouse immunoglobulin (Centers for Disease Control).[9,10] (Ratio of T_H to T_S [T_H/T_S] was obtained for 49 factor VIII and 30 factor IX recipient pairs.) Immunoglobulins G, A, and M were quantitated by nephelometry (12 factor VIII and seven factor IX recipient pairs). The staphylococcal binding assay and the iodine 125-labeled C1q binding assay were performed as previously described (23 factor VIII and 15 factor IX recipient pairs).[11,12]

Lymphocyte transformation responses were quantitated by a micromethod, with the mitogens phytohemagglutinin (PHA) (46 factor VIII and 26 factor IX recipient pairs), concanavalin A (Con A) (46 factor VIII and 26 factor IX recipient pairs), and pokeweed mitogen (14 factor VIII and eight factor IX recipient pairs).[13] Mononuclear cells were placed in RPMI 1640 medium supplemented with glutamine, penicillin, streptomycin, and 10% heat-inactivated AB+ serum. At a concentration of either 5×10^4 or 2.5×10^4 cells per well, they were incubated with optimal concentrations of the mitogens for three days (PHA and Con A) or six days (pokeweed mitogen) in a total volume of 0.125 mL. Sixteen hours before the end of this incubation, the cultures were pulsed with tritiated thymidine. Cultures were harvested on glass-fiber filter paper using a multiple, automated cell harvester (Titertek) and filter paper disks were placed in scintillation fluid and counted in a liquid scintillation counter. Cultures were done in triplicate. Mitogen responses were "normalized" by comparison with a single normal control donor

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whose mononuclear cells had been collected and frozen down in bulk and were used as a control each time that patients' cells were studied. This formula was used to determine the normalized mitogen response with stimulated and unstimulated values expressed in counts per minute:

$$\frac{\text{stimulated} - \text{unstimulated (patient)}}{\text{stimulated} - \text{unstimulated (control)}} \times 100.$$

Serum specimens on some participants were tested for antibodies to cytomegalovirus by indirect hemagglutination (26 pairs)[14]; herpes simplex virus types 1 and 2 by indirect hemagglutination (26 and 25 pairs)[15]; and the Epstein-Barr viral capsid antigen (48 pairs), nuclear antigen (47 pairs), and early antigen (48 pairs).[16] Tests for hepatitis B virus surface antigen and antibody to hepatitis B virus surface antigen (38 pairs) were done by radioimmunoassay.

Statistical Analysis

For factor VIII and IX recipients separately, the exposed and nonexposed groups were compared using the following matched-pair analyses.[17] The immunologic tests were compared between the two groups with the Wilcoxon signed rank test.[17] The comparison of the HIV results was analyzed using the binomial test, if the expected frequency was less than 5, or the McNemar test.[18] Serologic results were also analyzed using the binomial test or the McNemar test. Significance level was .05 for all testing.

RESULTS

The exposed and nonexposed groups did not differ significantly in age or factor use, confirming that our matching was adequate (Table 1). They also had similar regional distributions (data not shown) and did not differ significantly in number of different lots used (Table 1).

For withdrawals A and B combined, HIV seroprevalence did not differ significantly for exposed and nonexposed factor VIII recipients (74.5% and 86.3%, respectively) or for exposed and nonexposed factor IX recipients (30.0% and 40.0%, respectively). Exposed and nonexposed factor VIII recipients did not differ significantly in total lymphocyte counts, T_H numbers, T_S numbers, ratio of T_H to T_S, or serum levels of IgG, IgA, and IgM. This was also true for exposed and nonexposed factor IX recipients (Table 2). Exposed factor VIII recipients had significantly higher levels of immune complexes than did nonexposed recipients, by C1q binding assay and by staphylococcal binding assay; median values of all groups by C1q binding assay were within our laboratory's normal range (Table 3). Exposed and nonexposed factor IX recipients did not differ in either C1q binding assay or staphylococcal binding assay. Exposed and nonexposed factor VIII recipients did not differ significantly in seroprevalence to cytomegalovirus, herpes simplex virus types 1 and 2, Epstein-Barr virus, or hepatitis B virus, nor did exposed and nonexposed factor IX recipients.

Exposed factor VIII recipients had significantly lower stimulation indexes to the T-cell mitogens PHA and Con A than did the nonexposed factor VIII recipients, but median values for both exposed and nonexposed were within our laboratory's normal ranges (Table 3). They did not differ in their response to the T-cell-dependent B-cell mitogen, pokeweed mitogen. Exposed and nonexposed factor IX recipients did not differ significantly in their response to any mitogen (Table 3).

We were able to match 22 pairs of factor IX recipients, using the factor-use matching scheme used with factor VIII recipients. The results of analyses were consistent with those presented above, ie, there were no significant

Table 1.—Median Age and Factor Use of Exposed and Nonexposed Participants, by Type of Factor Used

Age and Factor Usage	Factor VIII		Factor IX	
	Exposed	Nonexposed	Exposed	Nonexposed
Age, y	23	24	21	22
Range	3-66	7-66	10-65	9-59
Factor units 1983	67 440	61 265	33 324	23 800
Range	7210-265 956	1000-255 250	0-808 420	0-407 510
Yearly average*	66 373	64 627	36 959	18 165
Range	7265-314 017	1000-185 042	3240-478 817	755-329 265
Different lots used	19	20	12	10
Range	6-47	1-67	3-85	1-16

*Average use per year; up to three years of usage information obtained.

Table 2.—Median T-Cell Subset Counts, Ratios, and Immunoglobulin Levels, by Exposure Status and Type of Factor Received*

Immune Test	Factor VIII		Factor IX	
	Exposed	Nonexposed	Exposed	Nonexposed
T-helper cells/mm^3	619	659	897	869
Range	87-1682	234-1263	303-2277	135-1764
T-suppressor cells/mm^3	623	686	591	659
Range	160-3548	218-1872	163-1659	196-1298
T-helper/T-suppressor cells ratio	1.0	1.0	1.4	1.6
Range	0.3-2.5	0.1-2.1	0.5-2.8	0.4-3.1
IgG, mg/dL (g/L)	1845 (18.45)	1790 (17.90)	1460 (14.60)	1660 (16.60)
Range	1100-3350 (11.00-33.50)	1040-5550 (10.40-55.50)	1270-1890 (12.70-18.90)	1430-2230 (14.30-22.30)
IgA, mg/dL (g/L)	229 (2.29)	296 (2.96)	280 (2.80)	235 (2.35)
Range	103-905 (1.03-9.05)	50-549 (0.50-5.49)	160-359 (1.60-3.59)	145-428 (1.45-4.28)
IgM, mg/dL (g/L)	156 (1.56)	189 (1.89)	169 (1.69)	143 (1.43)
Range	74-476 (0.74-4.76)	86-457 (0.86-4.57)	78-240 (0.78-2.40)	54-200 (0.54-2.00)

*Laboratory normal ranges are as follows: T-helper cells, 408 to 1583/mm^3; T-suppressor cells, 190 to 820/mm^3; T-helper/T-suppressor ratio, 1.0 to 3.9; IgG, 786 to 1647 mg/dL (7.86 to 16.47 g/L); IgA, 94 to 420 mg/dL (0.94 to 4.20 g/L); and IgM, 54 to 290 mg/dL (0.54 to 2.90 g/L).

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differences between exposed and nonexposed factor IX recipients.

When persons from withdrawal A and withdrawal B were analyzed separately from one another, findings for both withdrawals were in a direction consistent with those above, although significance was not reached in some of these analyses (ie, factor VIII recipients: for withdrawal A, $P=.2$ for difference in C1q binding assay; for withdrawal B, $P=.2$ for difference in staphylococcal-binding assay, $P=.2$ for PHA, and $P=.5$ for Con A).

COMMENT

The hemophilic community has been appropriately anxious concerning their risk of AIDS. Voluntary withdrawals of factor lots known to contain donations from persons subsequently developing AIDS are laudably conservative on the part of pharmaceutical companies, but these withdrawals have the unfortunate effect of increasing the anxiety level of recipients of the withdrawn lots. Because of this emotional impact, we evaluated recipients of some of these withdrawn lots in a prospective, controlled fashion.

The results of our first evaluation of these recipients are not reassuring, in that they support previous data indicating a high prevalence of HIV antibody in persons with hemophilia A in the United States.[8,19-21] These results are reassuring only in regard to the central question being asked in the study, ie, we found that persons exposed to withdrawn lots did not differ from those not exposed to these lots, in their HIV seroprevalence or in the majority of their immune tests, including T_H numbers. The two groups did differ in their levels of immune complexes and their responses to T-cell mitogens, and in the direction that might have been hypothesized if the withdrawn lots had been differentially contaminated with HIV. The results, however, were all well within normal range for our laboratory, suggesting no clinically relevant defect in the exposed cohort. Furthermore, given the lack of difference in HIV seroprevalence, it might be suggested that these immune test differences could, if real, be due to contaminants other than HIV in the withdrawn lots.

The negative results of this study are unlikely to be due to a lack of statistical power, at least in regard to factor VIII recipients, for at least three reasons. First, any differences that are present are in favor of the exposed group having less exposure to HIV than the nonexposed. Second, we did have sufficient power to detect a difference in responses to the mitogens PHA and Con A and in immune complex levels. Third, for factor VIII recipients, our numbers provided at least a 95% probability of detecting an HIV seroprevalence rate difference of 30% (100% for exposed and 70% for nonexposed recipients), at the 5% significance level.

There are, however, a number of other potential reasons for the negative results of this study. First, the donor may not have been infectious at the time of donation. Second, even if he had been infectious, the viral burden would have been diluted by donations of over 1000 other donors and thus may not have been adequate for infectivity. Third, other donors to these and other lots (ie, "exposure" and "nonexposure" lots) may have been infectious with the AIDS virus at the time of their donation, although these donors never themselves developed AIDS. Thus, the withdrawn lots may not have differed from other lots in their content of HIV. Both the cohort "exposed" to the withdrawn lots and the cohort "not exposed" to these lots may have been exposed to HIV, to the extent that any effect due specifically to the withdrawn lots would not be detectable. This possibility is a plausible one, given that one study found 0.25% of US blood units collected in spring of 1985 were repeatedly reactive for HIV.[22] Because this testing was done using enzyme-linked immunosorbent assay test kits, presumably some portion of these reactions represent false-positives. However, the plasma of 1000 to 20 000 donors is pooled to produce a given lot of factor concentrate; therefore, if antibody-positive individuals are frequently viremic,[23,24] with this range of seroprevalence, before donor screening, contamination of lots might not have been unusual. A fourth reason for the negative results of this study is that this evaluation may have been done too soon after exposure for us to detect immune differences between the exposed and nonexposed cohorts. This possibility is also plausible, given that the incubation period for AIDS is now estimated to be approximately five years.[25,26] However, it is less likely given that immune test changes have already reached measurable levels in overlapping and other infected cohorts.[8,27]

Table 3.—Median Values for Immune Complex Levels* and Lymphocyte Transformation to Mitogens,† by Exposure Status and Type of Factor Used

	Factor VIII		Factor IX	
Immune Test	Exposed	Nonexposed	Exposed	Nonexposed
C1qBA, %	8	7	7	7
Range	5-30	5-15	4-12	6-11
P‡	.01		NS§	
SBA, %	94	75	41	38
Range	0-383	11-214	10-164	5-94
P	<.01		NS	
PHA, %	132	168	157	162
Range	27-258	58-370	54-387	64-326
P	<.01		NS	
Con A, %	171	219	252	245
Range	24-435	14-691	47-1308	124-526
P	.01		NS	
PWM, %	78	67	106	104
Range	17-190	21-209	78-188	60-194
P	NS		NS	

*Laboratory normal ranges are as follows: C1q binding assay (C1qBA), less than 9%; and staphylococcal binding assay (SBA), less than 31%.

†Given as normalized stimulation indexes, expressed as a percent of the response of a single normal donor who is included in each test run. Laboratory normal ranges are as follows: phytohemagglutinin (PHA), greater than 86%; concanavalin A (Con A), greater than 61%; and pokeweed mitogen (PWM), greater than 41%.

‡Significance tested using the Wilcoxon signed rank test.

§NS indicates not significant.

CONCLUSION

We found that individuals exposed to the evaluated withdrawn lots of factor concentrate show no clinically impor-

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tant immunologic or HIV serologic differences from their comparison group. Yearly reevaluations are essential, however, because of the results concerning immune complexes and mitogens and, more importantly, because the incubation period for AIDS may be many years in length.[25,26] Given the high rate of HIV seroprevalence in both the cohort exposed to the withdrawn lots and the cohort not exposed to these lots, we suggest that market withdrawals were insufficient means of limiting the spread of the AIDS virus in the hemophilic population. Since either wet or dry heat treatment appears to be effective in inactivating HIV and related viruses,[28-30] we suggest that only viral-inactivated factor products be used in therapy for hemophilia.

Addendum

The 175 participants that returned for further evaluation between March and August 1985 included 68 paired factor VIII recipients and 36 paired factor IX recipients. Analyses of the data from the second evaluation were recently completed. For factor VIII recipients, 89.5% of the exposed and 92.1% of the nonexposed had HIV antibody (not significant). For factor IX recipients, 56.5% of the exposed and 47.8% of the nonexposed had HIV antibody (not significant). Exposed and nonexposed groups did not differ significantly in any immune test, including their serum levels of immune complexes, as measured by C1q binding assay and staphylococcal binding assay, and their responses to PHA, Con A, and pokeweed mitogen. Thus, at a one-year reevaluation, those exposed to withdrawn lots did not differ from those not exposed to these lots.

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References

1. Hemophilia Information Exchange: *AIDS Update*, advisory 1. New York, National Hemophilia Foundation, Nov 2, 1983.
2. Hemophilia Information Exchange: *AIDS Update*, medical bulletin 7, chapter advisory 8. New York, National Hemophilia Foundation, May 11, 1983.
3. Hemophilia Information Exchange: *AIDS Update*, chapter advisory 9. New York, National Hemophilia Foundation, revised Sept 7, 1983.
4. Hemophilia Information Exchange: *AIDS Update*, medical bulletin 10, chapter advisory 13. New York, National Hemophilia Foundation, Jan 24, 1984.
5. Hemophilia Information Exchange: *AIDS Update*, medical bulletin 13, chapter 18. New York, National Hemophilia Foundation, Sept 6, 1984.
6. Hemophilia Information Exchange: *AIDS Update*, medical bulletin 14, chapter 14, chapter advisory 19. New York, National Hemophilia Foundation, Oct 8, 1984.
7. Hemophilia Information Exchange: *AIDS Update*, medical bulletin 20, chapter advisory 24. New York, National Hemophilia Foundation, Dec 13, 1984.
8. Jason J, McDougal JS, Holman RC, et al: Human T-lymphotropic retrovirus type III/lymphadenopathy-associated virus antibody: Association with hemophiliacs' immune status and blood component usage. *JAMA* 1985;253:3409-3415.
9. Nicholson JKA, McDougal JS, Spira TJ, et al: Immunoregulatory subsets of the T helper and T suppressor cell populations in homosexual men with chronic unexplained lymphadenopathy. *J Clin Invest* 1984;73:191-201.
10. Hoffman RA, Kung PC, Hansen WP, et al: Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. *Proc Natl Acad Sci* 1980;77:4914-4917.
11. McDougal JS, Redecha PB, Inman RD, et al: Binding of immunoglobulin aggregates and immune complexes in human sera to staphylococci containing protein A. *J Clin Invest* 1979;63:627-636.
12. Zubler RH, Lange G, Lambert PH, et al: Detection of immune complexes in unheated sera by a modified ^{125}I-C1q binding test. *J Immunol* 1976;116:232-235.
13. Pitchenik AE, Fischl MA, Dickinson GM, et al: Opportunistic infections and Kaposi's sarcoma among Haitians: Evidence of a new acquired immunodeficiency state. *Ann Intern Med* 1983; 98:227-283.
14. Warner JL, Weller TH, Stewart JA: Cytomegalovirus, in Rose NR, Friedman H (eds): *Manual of Clinical Immunology*. Washington, DC, American Society of Microbiology, 1980, pp 622-627.
15. Stewart JA, Herrman KL: Herpes simplex virus, in Rose NR, Friedman H (eds): *Manual of Clinical Immunology*. Washington, DC, American Society of Microbiology, 1980; pp 614-619.
16. Henle W, Henle G, Horwitz CA: Infectious mononucleosis and Epstein-Barr virus-associated malignancies, in Lennette EH, Schmidt NJ (eds): *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*. Washington, DC, American Public Health Association, 1979, pp 441-470.
17. Lehmann EL: *Nonparametrics: Statistical Methods Based on Ranks*. San Francisco, Holden-Day Inc, 1975.
18. Siegel S: *Non-Parametric Statistics for the Behavioral Sciences*. New York, McGraw-Hill International Book Co, 1956.
19. Kitchen LW, Barin F, Sullivan J, et al: Aetiology of AIDS: Antibodies to human T-cell leukaemia virus (type III) in haemophiliacs. *Nature* 1984;312:367-369.
20. Ramsey RB, Palmer EL, McDougal JS, et al: Antibody to lymphadenopathy-associated virus in haemophiliacs with and without AIDS. *Lancet* 1984;2:397-398.
21. Evatt BL, Gomperts ED, McDougal JS, et al: Coincidental appearance of LAV/HTLV-III antibodies in hemophiliacs and the onset of the AIDS epidemic. *N Engl J Med* 1985;312:483-486.
22. Results of human T-lymphotropic virus type III test kits reported from blood collection centers—United States, April 22-May 19, 1985. *MMWR* 1985;34:375-376.
23. Jaffe HW, Darrow WW, Echenberg DF, et al: The acquired immunodeficiency syndrome in a cohort of homosexual men: A six-year follow-up study. *Ann Intern Med* 1985;103:210-214.
24. Gomperts ED, Feorino P, Evatt BL, et al: LAV/HTLV-III presence in peripheral blood lymphocytes of seropositive young hemophiliacs. *Blood* 1985;65:1549-1552.
25. Lui K-J, Lawrence DN, Morgan WM, et al: A model-based approach for estimating the mean incubation period of transfusion-associated acquired immunodeficiency syndrome. *Proc Natl Acad Sci USA* 1986;83:3051-3055.
26. Curran JW, Lawrence DN, Jaffe H, et al: Acquired immunodeficiency syndrome (AIDS) associated with transfusions. *N Engl J Med* 1984;310:69-75.
27. 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.
28. Update: Acquired immunodeficiency syndrome (AIDS) in persons with hemophilia. *MMWR* 1984;33:589-591.
29. Levy JA, Mitra G, Mozen MM: Recovery and inactivation of infectious retroviruses from factor VIII concentration. *Lancet* 1984;2:722-723.
30. Spire B, Dormont D, Barre-Sinoussi F, et al: Inactivation of lymphadenopathy-associated virus by heat, gamma rays, and ultraviolet light. *Lancet* 1985;1:188-189.

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