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Evidence That Prior Immune Dysfunction Predisposes to Human Immunodeficiency Virus Infection in Homosexual Men

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Summary: To investigate the role of host susceptibility to HIV-1 infection, we studied subsequent seroconversion in 161 individuals, initially seronegative to HIV-1, who underwent skin testing for cutaneous anergy at an index visit within a prospective study of homosexual men. There were 23 seronversions in these men by 45 months following the skin testing, yielding a crude rate of seroconversion of 14.3%. While results of purified protein derivative (PPD), *Candida,* and *Trichophyton* skin tests were not associated with subsequent course, anergy to dinitrochlorobenzene (DNCB) was predictive of subsequent seroconversion. Kaplan–Meier estimates for the risk of seroconversion during 45 months of follow-up in those men initially anergic and reactive to DNCB were 28.9 and 11.1%, respectively, yielding a relative risk of 2.6 ($p = 0.006$). The estimated relative risk was stable with adjustment by Cox regression for annual number of male sexual partners and frequency of receptive anal intercourse, and was not sensitive to various changes in the definition of seroconversion time and of eligibility criteria. These data suggest that an impaired host immune status may be associated with an increased risk of HIV-1 infection that is independent of risk of exposure to the virus, supporting earlier speculations that HIV-1 may itself be opportunistic. The notion of varying host susceptibility to infection, at least with regard to sexual transmission in homosexual men, may help to explain the frequent observation of individuals who have been repeatedly exposed to the virus and yet have remained uninfected. **Key Words:** Human immunodeficiency virus—Host susceptibility—Dinitrochlorobenzene.

Several studies have confirmed that the predominant risk factors for infection with human immunodeficiency virus type 1 (HIV-1) in homosexual men are an elevated number of male sex partners and an increased frequency of receptive anal intercourse (1–3). These variables act presumably as exposure factors, the former by increasing the probability of contact with an infectious partner, and the latter by providing an efficient means of transmission given such contact. The role of host factors is less clear although there is recent evidence that positive herpes serology is associated with increased host susceptibility to HIV-1 in homosexual men (4).

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While the relationship between HIV-1 infection and subsequent impairment of immune function is well recognized, it is noteworthy that the converse has also been proposed, namely that independently of the other known risk factors, prior immune dysfunction might act as a host susceptibility factor to HIV-1 infection (5). The purpose of the present study is to test the hypothesis that, among uninfected homosexual men, immune dysfunction as measured by failure to respond to certain skin test antigens (namely tuberculin, *Candida, Trichophyton,* and/or dinitrochlorobenzene) might be predictive of subsequent HIV-1 infection. The degree of association between various laboratory parameters and later seroconversion is also reported.

METHODS

As described previously (3,6,7), the Vancouver Lymphadenopathy–AIDS Study (VLAS) is an ongoing prospective study of over 700 homosexual men who were recruited from six general practices located in the central area of Vancouver, Canada, during the period of November 1982 to February 1984. During each visit, a questionnaire was administered, a complete physical examination performed by the subject's physician, and a blood sample drawn for immunologic and HIV-1 antibody testing. Participants may have had additional HIV-1 antibody tests between study visits at their physician's discretion. Visits were conducted on a semiannual basis until October 1986; since that time they have occurred annually, with the eighth cycle of visits nearing completion in September 1988.

As well, at the time of the first two visits, a consecutive subsample of subjects were scheduled for skin testing for cutaneous anergy. This included epicutaneous sensitization with 2,4-dinitrochlorobenzene (DNCB). DNCB was dissolved in acetone to form a solution of 2 mg/0.1 ml. A total of 0.1 ml of this solution was then applied to a 2 cm diameter circle on the skin of the upper arm using a tuberculin syringe. Each site was covered with a porous tape (Micropore) that was removed in 24 h. The patients were instructed not to wash until the tape had been removed. The sites were examined at 48 h and at 14 days. Sensitization was defined as the presence of an acute eczematous reaction at 14 days. If no sensitization reaction was present at 14 days, the patients were patch tested with DNCB (1% wt/vol) in petrolatum using an aluminum-back patch test occluded with porous tape. This patch test was read at 48 h with reactivity defined as the presence of a localized acute eczematous reaction. Patients who were nonreactive at the first visit were resubmitted to sensitization at the second set of skin tests. Reactivity to specific recall antigens was tested as well by intradermal injection of test antigens using a 1 ml tuberculin syringe. The test antigens included tuberculin (Connaught Laboratories, Willowdale, Ontario, Canada), as well as *Candida albicans* and *Trichophyton mentagrophytes* (Hollister-Stiehr, Spokane, WA, U.S.A.). The doses of antigen used were 0.1 ml for tuberculin (5 TU/ml) and 0.025 ml for *Candida* and *Trichophyton* (1,000 PNU/ml). The tests were read at 48 h, with reactivity defined by the presence of erythema and induration at least 10 mm in diameter. All skin tests were administered and interpreted by one dermatologist (W.A.M.) who did not have access to the participants' questionnaire responses or laboratory results.

HIV-1 antibody tests were performed at the Laboratory Centre for Disease Control in Ottawa, Canada using the ELISA assay with positive and equivocal results confirmed by Western blot. A small number of samples were later thawed and tested for the presence of HIV p24 core antigen using an antigen capture ELISA method (Dupont/NEN Research Products). We also measured the following laboratory parameters as previously described (8): hemoglobin, WBC, absolute lymphocyte count, IgG, IgA, IgM, C1q binding, CD4 count, CD8 count, and CD4/CD8 ratio. During the initial phase of the study, lymphocyte subsets were performed only on a random subsample of the cohort.

For the purpose of this investigation, the second visit will be referred to as the index visit and all laboratory and skin test data will be those obtained from that visit. To be eligible for the analysis, a participant was required to have been skin tested at the index visit and to have been HIV-1 seronegative at that time. Seroconversion in this group was defined by the occurrence of a positive HIV-1 antibody test result subsequent to the index visit. The date of seroconversion was estimated as the midpoint of the time interval between the last negative and first positive HIV-1 antibody test.

Baseline comparisons of subgroups defined by initial skin test status were made using the nonparametric Wilcoxon rank sum test. Time to subsequent

seroconversion starting from the date of the index visit was examined using methods of survival analysis (9). Individuals who remained persistently seronegative were considered right-censored at the time of their last HIV-1 result. The cumulative seroconversion rate in various subgroups and the mean time to seroconversion were estimated by the product-limit method of Kaplan and Meier. Seroconversion rates in different subgroups were compared by the log rank test. For comparing seroconversion in those reacting to a skin test versus those not reacting, one-sided p values were computed because of the prior hypothesis about the direction of the association (that anergy would be associated with seroconversion). In examining the association between skin test results and later seroconversion, nominal p values were adjusted by the Bonferroni method to take account of multiple hypothesis testing. After checking the adequacy of the proportional hazards assumption, Cox regression was used to estimate the relative hazard of seroconversion in various subgroups with adjustment for potential confounding variables.

The association between several laboratory measurements performed on baseline blood specimens and later seroconversion was examined using the Wilcoxon rank sum test.

RESULTS

A total of 310 men in the study were seronegative at the index visit. A total of 306 men (139 seropositive, 167 seronegative) underwent one or more skin tests at the index visit. Of the 167 individuals who were both initially seronegative and skin tested, 6 had no follow-up information available and were eliminated from the analysis, leaving a cohort of 161 eligible men. Not all of these men underwent all four skin tests; the numbers who received *Trichophyton*, tuberculin, *Candida*, and DNCB were 146, 143, 154, and 149, respectively.

A total of 23 seroconversions were observed in these 161 men during the 45 months subsequent to the index visit for a crude seroconversion rate of 14.3%. For the HIV-1 seroconverters, time to seroconversion ranged from 2 to 27 months, with an estimated mean time of 11.0 months. Among the 138 eligible men who did not seroconvert, follow-up time ranged from 4 to 48 months, with an estimated mean time of 30.5 months. The comparison of seroconversion rates among men reacting and men not reacting (anergic) to a skin test is presented for all four tests in Table 1. There was no association between anergy to *Trichophyton* or to tuberculin and later seroconversion. While there is a trend toward greater seroconversion in those anergic to *Candida* relative to those reactive, this association did not approach statistical significance.

There was, however, a strong observed association between DNCB anergy and later HIV-1 seroconversion. Among the 149 men who underwent this test, 116 responded to DNCB sensitization, while 33 (22.1%) did not and were classified as DNCB anergic. As seen in Table 1, among the 33 DNCB-anergic subjects, 9 (27.3%) subsequently seroconverted as compared to only 12 (10.3%) of 116 DNCB-reactive men. The Kaplan–Meier estimates for the 45 month seroconversion rates in the DNCB-anergic and reactive groups were 28.9 and 11.1%, respectively, with a nominal one-tailed p value of 0.006.

Figure 1 presents a comparison of the cumulative seroconversion curves for the DNCB-anergic and DNCB-reactive groups. The 9 seroconversions in

TABLE 1. *Comparison of subsequent seroconversion rates by response to skin testing at index visit in a cohort of homosexual men*

Skin test	Response	Proportion seroconverting	Kaplan–Meier estimate[a]	p Value[b]
Trichophyton	Anergic	14/106	14.2%	0.48
	Reactive	6/46	14.4%	
Tuberculin	Anergic	17/121	15.1%	0.77
	Reactive	4/22	20.0%	
Candida	Anergic	8/41	22.0%	0.13
	Reactive	14/113	13.1%	
DNCB	Anergic	9/33	28.9%	0.006
	Reactive	12/116	11.1%	

[a] Kaplan–Meier estimate for cumulative risk of seroconversion within 45 months following index visit.
[b] Based on log rank test, one-sided p value.

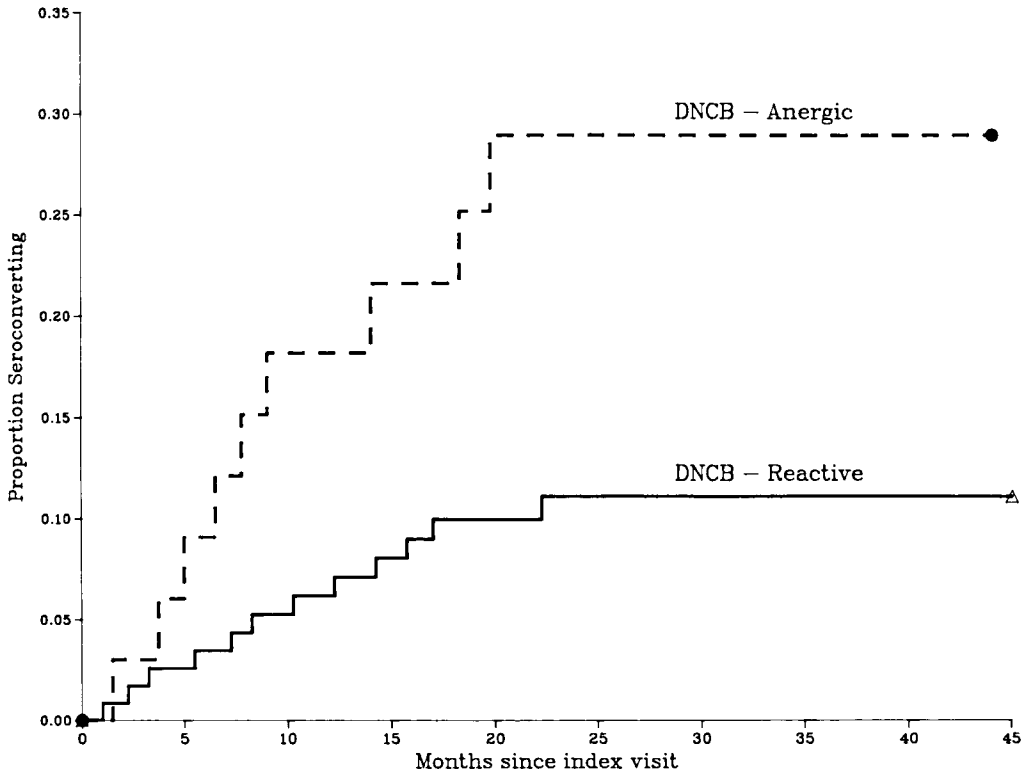

FIG. 1. Comparison of cumulative seroconversion curves for DNCB-anergic and DNCB-reactive groups.

the DNCB-anergic group occurred a mean of 10.7 months following the index visit, while the 12 seroconversions in the reactive group occurred at a mean of 11.0 months following the index visit (p = 0.95; log rank test). Among those who did not seroconvert, the follow-up times for the DNCB-anergic and -reactive subjects were similar, with mean censoring times of 29.8 and 31.1 months, respectively (p = 0.39; log rank test).

We conducted a further analysis restricted to those 140 individuals still seronegative at least 3 months after the index visit and found that the estimated relative risk of seroconversion in those DNCB anergic relative to those DNCB reactive was 2.7 (p = 0.03).

Of the nine DNCB-anergic seroconverters, frozen sera from the index visit were located in sufficient amount for p24 antigen testing for four subjects (with no serum located for one case, and insufficient serum for testing in four others). All four samples tested were negative for p24 antigen.

The men who responded to DNCB were compared to those who did not on a number of baseline characteristics measured at the time of the index visit. The results of these comparisons may be seen in Table 2. These two groups were similar with respect to age, behavioral characteristics including number of sex partners and frequency of receptive anal intercourse, and with respect to a number of laboratory parameters including lymphocyte subsets.

The Cox proportional hazards model was used to check the possibility that the observed association between DNCB anergy and later seroconversion might be due to confounding by other known risk factors for HIV-1 infection. The unadjusted relative risk from the Cox model for seroconversion in DNCB-anergic individuals relative to DNCB-reactive individuals was 2.84 (95% CI: 1.20–6.08). When adjusted for number of episodes of receptive anal intercourse with distinct partners in the preceding year, the relative risk was 2.71 (1.13–7.33). The relative risk was also stable under adjustment for all of the baseline factors listed in Table 2 (taken singly), and for the three other skin test results.

None of the laboratory parameters obtained at the index visit was associated with subsequent seroconversion in our cohort. The results of compar-

TABLE 2. *Baseline characteristics in a cohort of 149 homosexual men classified by DNCB result*

Variable	DNCB reactive			DNCB anergic			p Value[a]
	N	Mean	SD	N	Mean	SD	
Age	116	33.7	7.45	33	32.6	6.48	0.68
Partners[b]	115	6[d]	—	33	6[d]	—	0.82
Receptive anal intercourse[c]	115	3.6[d]	—	33	3.6[d]	—	0.80
Hemoglobin (g/L)	109	15.5	1.07	32	15.4	.65	0.71
WBC (per mm^3)	111	6563	1842	33	6052	1583	0.16
Lymphocytes (per mm^3)	110	2211	732	32	1999	794	0.07
IgG (mg/dl)	95	997	248	29	1095	358	0.53
IgA (mg/dl)	95	200	94	29	226	135	0.25
IgM (mg/dl)	95	132	52	29	137	51	0.77
C1q binding (%)	84	7.08	3.1	26	7.35	4.4	0.57
CD4 count (per mm^3)	75	926	343	25	848	365	0.26
CD8 count (per mm^3)	75	562	197	25	525	187	0.43
CD4/CD8 ratio	75	1.71	.55	25	1.65	.49	0.65

[a] Wilcoxon rank sum test.
[b] Number of different partners in preceding year.
[c] Number of episodes involving different partners in preceding year.
[d] Median.

isons of these parameters in seroconverters and nonconverters are presented in Table 3. Similarly, among the 157 men who were seronegative at the index visit but who were *not* skin tested, there was no association between laboratory parameters at the index visit and later seroconversion (data not shown).

The 149 eligible men who were DNCB skin tested were compared to the group of men who were seronegative at the index visit and who had subsequent follow-up but who were not skin tested (i.e., the group that was not eligible solely on the basis of not having been skin tested). The results of this comparison are presented in Table 4. The distributions of these baseline characteristics are seen to be quite similar for the two groups.

DISCUSSION

There are several possible explanations for the observed association between DNCB anergy and later seroconversion that are worthy of discussion. These include chance, bias, confounding, reverse causation, and, finally, causation.

With regard to the possibility that this association with DNCB is a chance finding, the probability that one would find an association by chance alone at a nominal significance level of 0.006 is 0.024, under the conservative assumption that the four skin tests are independent. The true significance level may be less considering that the various skin-test results are in fact correlated.

Several possible sources of bias were considered.

TABLE 3. *Comparison of laboratory measurements at the index visit in subsequent seroconverters and nonconverters in a cohort of homosexual men*

Variable	Seroconverters			Nonconverters			p Value[a]
	N	Mean	SD	N	Mean	SD	
Hemoglobin (g/L)	21	15.1	1.26	120	15.5	0.93	0.30
WBC (per mm^3)	21	6386	1703	123	6456	1815	0.88
Lymphocytes (per mm^3)	20	2140	915	122	2167	723	0.71
IgG (mg/dl)	20	1074	323	104	1010	270	0.60
IgA (mg/dl)	20	184	73	104	210	110	0.49
IgM (mg/dl)	20	127	46	104	135	52	0.78
C1q binding (%)	16	7.6	3.2	94	7.1	3.5	0.43
CD4 count (per mm^3)	15	898	384	85	908	344	0.96
CD8 count (per mm^3)	15	541	183	85	555	197	0.88
CD4/CD8 ratio	15	1.69	0.54	85	1.70	0.54	0.97

[a] Wilcoxon rank sum test.

TABLE 4. *Baseline characteristics in a cohort of 302 homosexual men classified by whether DNCB skin testing was done or not*

Variable	DNCB tested			Not DNCB tested			p Value[a]
	N	Mean	SD	N	Mean	SD	
Age	149	33.5	7.24	152	32.6	7.35	0.23
Partners[b]	148	6[d]	—	152	6[d]	—	0.14
Receptive anal intercourse[c]	148	3.6[d]	—	152	3.6[d]	—	0.28
Hemoglobin (g/L)	141	15.5	.99	147	15.4	.95	0.58
WBC (per mm^3)	144	6446	1793	146	6492	2749	0.44
Lymphocytes (per mm^3)	142	2164	749	149	2159	713	0.79
IgG (mg/dl)	124	1020	279	131	1017	273	0.59
IgA (mg/dl)	124	206	105	131	192	94	0.22
IgM (mg/dl)	124	133	51	131	145	60	0.26
C1q binding (%)	110	7.15	3.5	127	7.65	4.7	0.69
CD4 count (per mm^3)	100	907	349	80	934	355	0.57
CD8 count (per mm^3)	100	553	194	80	599	245	0.27
CD4/CD8 ratio	100	1.70	.53	80	1.67	.63	0.40

[a] Wilcoxon rank sum test.
[b] Number of different partners in preceding year.
[c] Number of episodes involving different partners in preceding year.
[d] Median.

There was no evidence of any difference in the distributions of censoring times for the nonconverters in the anergic and reactive groups, suggesting that more intensive follow-up of the anergic group did not take place. Nor was there a difference in the frequency of visits or in the time interval between visits for the two groups. The method of calculating the seroconversion time as the mid-time point between the last negative test and the first positive test might be questioned. The analysis was therefore repeated assuming seroconversion immediately after the last negative HIV-1 serology date, with the results essentially unchanged.

Another possible explanation for the observed association is confounding by other risk factors for HIV-1 exposure. If the same risk factors that give rise to HIV-1 exposure also entail a risk of anergy to DNCB, then one might anticipate the observed association. For example, DNCB anergy due to multiple exposures to other viruses and antigens might be more frequent in those men with an elevated number of sex partners and/or an increased frequency of anal receptive intercourse. Since the latter are also the predominant risk factors for HIV-1 infection, an association between DNCB anergy and subsequent seroconversion would be expected. However, since the strength of the association between DNCB status and later seroconversion (as measured by relative risk in the Cox model) is essentially unchanged with adjustment for other variables in the data set, including the known predictors of HIV-1 infection, it is unlikely that confounding by these variables could account for the observed association. Misclassification in the measurement of confounding variables may seriously impair the ability to adjust for the effect of a confounding variable (10). However, even with misclassification, one would expect to see an attenuation of the association if confounding is present and this was not seen in our data. Confounding by a factor outside of the currently established causal pathway, however, cannot be excluded.

We must also consider the possibility that some of the participants, although HIV-1 antibody negative, might have already been infected with HIV-1 at the time of the index visit. If HIV-1 infection gave rise to anergy to DNCB even before seroconversion had occurred, then the observed association could be due to the fact that anergy is an effect of HIV-1 infection rather than a cause, i.e., reverse causation. There are several considerations against this explanation, however. First, when the analysis is restricted to those individuals still seronegative 3 months after the skin test was performed (N = 140), the estimated relative risk is 2.7, with the strength of the association showing no trend towards weakening, although the statistical significance is less because of the smaller sample size. Second, baseline laboratory parameters including IgG and C1q binding are similar for the DNCB-reactive and the DNCB-anergic groups whereas one would expect increases in the latter group if early HIV infection were present. Third, mean time to seroconversion was similar in DNCB-anergic and DNCB-reactive seroconverters. Thus, there is good evidence that the relative risk is stable over time,

not initially higher as one would expect if the DNCB-anergic group were already infected at baseline. An additional minor piece of evidence is that p24 antigen testing was negative at the index visit in all four DNCB-anergic seroconverters in whom testing was carried out.

Finally, there remains the hypothesis that given equal exposure to HIV, homosexual men who are DNCB-anergic are more susceptible to infection than are DNCB-reactive men. This explanation implies that while HIV-1 exposure is certainly necessary for infection to develop, factors not directly related to exposure are important in the causal chain, at least in some cases of infection. Well before the original discovery and characterization of HIV-1 and the clarification of its role in AIDS, numerous mechanisms were proposed as possible causes of the immunodeficiency seen in AIDS (5). Hypotheses considered at that time included antigen overload resulting from repeated exposures to microbial pathogens (11), the effect of infection with immunosuppressive viruses such as Epstein–Barr virus (EBV) (12) and cytomegalovirus (CMV) (13), and immunosuppression due to exposure to alloantigens expressed on sperm (14) or transfused lymphocytes (15). With the discovery of HIV-1, however, the contribution of such factors in the pathology of AIDS was discounted, although their role as potential cofactors in disease outcome among HIV-1-infected individuals is presently under investigation in prospective studies. While such investigations may reveal a role for these as cofactors once HIV-1 infection has been established, there have been few recent reports that implicate predisposing immune factors in host susceptibility to HIV-1 infection. Previous studies in homosexual men and in hemophiliacs have shown evidence of immune dysfunction that was apparently not accounted for by HIV-1 infection (5,16–21). Tsoukas et al. (18) found that 6 of 14 hemophiliacs showed evidence of functional cellular immunodeficiency despite being HIV-1 antibody negative. Another group of seronegative hemophiliacs was shown by Ludlam et al. (19) to have altered lymphocyte subsets. Madhok et al. (20) found that 9 of 19 clinically severe hemophiliacs had an abnormal response to DNCB testing. This was not related to HIV-1 seropositivity, but an inverse relation between DNCB response and previous clotting factor exposure was detected, suggesting that an agent or agents in clotting factor other than HIV-1 may have been responsible for the immune defect.

Of the four skin tests that were performed in our study, only abnormal reactivity to DNCB sensitization was found to be predictive of subsequent infection, whereas anergy to recall antigens was not. This would suggest that it is something particular to the type of response being tested by DNCB that is associated with the increased risk. One evident difference between DNCB and the other three skin tests is that the latter measure the recall of a previous exposure and hence serve as a measure both of exposure and of immune memory to these antigens. DNCB, on the other hand, is not an environmental antigen and most individuals would not be expected to have been sensitized. The inability of some of the seronegative individuals in our study to respond to a secondary challenge with DNCB must therefore reflect either a genetic predisposition to DNCB anergy, or a more fundamental difficulty in mounting primary immune responses. One would predict on this basis that DNCB-anergic subjects would also have problems mounting immune responses to other antigens against which they had not previously been sensitized. HIV-1 may represent such a new antigen, fulfilling to a degree the role of "opportunistic infection" as originally proposed by Levy and Ziegler (5).

Questions naturally arise as to the extent of impairment of cell-mediated immunity in HIV-1-negative at-risk individuals and to factors predisposing to such impairment. External causes such as exposure to immunosuppressive viruses such as EBV or CMV could be responsible (12,13). Exposure to sperm alloantigens or other immunosuppressive factors may be another avenue by which such a defect may arise (21,22). We did not find an association in our data between anergy to DNCB and sexual risk factors that would be likely to increase the risk of these latter exposures. The number of individuals, however, may not have been sufficient to reveal a subtle association. On the other hand, exposure to clotting factor has been associated with DNCB anergy in hemophiliacs (19). Shearer and Hurtenbach (22) reported that a number of cellular immune functions were disrupted in mice by prior injections of whole sperm. Cytotoxic T-cell (CTL) responses against hapten-modified self- and alloantigens as well as mixed lymphocyte reactivity were reduced. These effects seemed to be antigen nonspecific but the onset of impairment of CTL function was detected earlier with hapten-modified cell antigens than with alloantigens. Findings of reduced responses to influenza virus-infected self-cells

among HIV-1 seronegative homosexual men were also reported by Shearer et al. (23).

The nature of the population studied deserves comment. To be eligible for this analysis, an individual had to have remained uninfected through the second cycle of our study so that men at highest risk who were infected early in the epidemic and hence already seropositive at the time of study enrollment were not eligible. The present results are thus based on a relatively low-risk group of homosexual men, beyond which the results should not be generalized. Indeed, it is tempting to speculate that if host susceptibility is truly a factor in HIV-1 infection, it is likely to have its greatest effect in low-risk homosexual men such as we have studied, where exposure may be infrequent and where host factors may be more likely to tip the balance in favor of the establishment of infection (21).

The established list of determinants of HIV-1 transmission includes the presence of the virus in a body fluid or medium, the concentration and viability of the virus in that fluid or medium, and access of the fluid or medium to a portal of entry. Our data suggest that host susceptibility should also be included as a determinant of infection following exposure to the virus, at least with regard to sexual transmission among homosexual men. As noted by Klatzmann and Gluckman (21), the notion of varying host susceptibility to infection may help to explain the frequent observation of individuals in various settings who are known to have been repeatedly exposed to the virus and yet have remained uninfected (3,24–27). However, we must caution that although we may have identified a role for host susceptibility as a determinant of HIV-1 infection, there are no data at present to support the concept of *absolute* resistance to infection in any population. There is nothing in the present data, for example, to suggest that a healthy individual with an intact immune system cannot become infected after even a single sexual encounter. Thus, the implications of these data for current public health and educational initiatives are limited. Moreover, if it is the case that the risk factors that promote host susceptibility among seronegative individuals are also risk factors for exposure to HIV-1, then the potential for further reduction of HIV-1 transmission beyond that offered by modification of exposure factors alone is also limited. Further study of the causes and extent of immune impairment in seronegative at-risk individuals is needed to determine if modifiable factors exist that might offer the potential for further reductions in the transmission of HIV-1 by lowering host susceptibility to infection.

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