Risk Factors for Repeatedly Reactive HIV-1 EIA and Indeterminate Western Blots

A Population-Based Case-Control Study

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**Objectives:** Causes of indeterminate results of Western blot testing (IWB) for human immunodeficiency virus (HIV) type 1 include seroconversion, HIV-2 cross-reactivity, and autoimmune disease, but most IWB results remain unexplained. This case-control study assessed risk factors for IWB results, including early HIV infection, other retroviral infection, autoantibodies, and other medical conditions.

**Design:** Prospective study to determine HIV seroconversion rate, with a case-control design to assess other risk factors for IWB. Cases (persons with one or more repeatedly reactive HIV-1 enzyme immunoassay with IWB), their current sexual partners, and controls (persons with negative enzyme immunoassay and Western blot results) were recruited from blood banks, health department and prenatal clinics, and private providers in Washington and Oregon.

**Results:** Of 244 cases enrolled, 206 were followed up for 6 months or longer, and six (3.0%; 95% confidence interval [CI], 0.7% to 5.3%) with recent HIV risk behaviors seroconverted. The Western blot banding patterns differed among groups; cases usually had p17 or p24 bands, while controls and cases' sexual partners usually had polymerase bands. Conditional logistic regression indicated that independent risk factors for IWB among male cases and controls were a tetanus booster in the past 2 years (odds ratio, 3.2; 95% CI, 1.2 to 8.6) and sexual contact with a prostitute (odds ratio, 3.0; 95% CI, 1.0 to 9.5). Independent risk factors for women were parity (odds ratio, 1.2; 95% CI, 1.02 to 1.4) and autoantibodies, either rheumatoid factor or antinuclear antibodies (odds ratio, 2.3; 95% CI, 1.03 to 5.6). No cross-reactivity was detected with HIV-2, human T-lymphotrophic virus type 1, feline immunodeficiency or feline leukemia, or bovine immunodeficiency viruses.

**Conclusions:** Evaluation of persons with reactive HIV-1 enzyme immunoassays and IWB should include an assessment of HIV risk and other possible risk factors, such as allotnimmunization (ie, parity or recent immunization) or autoantibodies (ie, antinuclear antibodies and rheumatoid factor). The relationship of IWB among men who reported sex with prostitutes is intriguing and warrants further study.

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**The Sensitivity and Specificity of the Human Immunodeficiency Virus (HIV) Type 1 Enzyme Immunoassay (EIA) are Greater than 99%.** Specimens that are repeatedly reactive by HIV-1 EIA are confirmed by a supplemental test, usually the Western blot, which detects antibodies to de-natured HIV-1 proteins. The HIV-1 Western blot has a reported specificity of 98%. Between 4% and 20% of serum samples that are repeatedly reactive by HIV-1 EIA are interpreted as indeterminate by Western blot, with the proportion subsequently classified as indeterminate varying according to the immunoblot used, the prevalence of HIV-1 infection in the population tested, and the interpretive criteria used. In reports of EIA-negative volunteers for HIV-1 vaccine trials, up to 33% had indeterminate banding patterns on Western blot.

Indeterminate HIV-1 Western blots (IWBs) in HIV-1-infected individuals may result from antibody production against viral core antigens early in HIV-1 infection or loss of core antibodies late in HIV-1 infection. In HIV-1–negative ind...
**SUBJECTS AND METHODS**

**STUDY POPULATION**

This case-control study was initiated at the University of Washington, Seattle, in March 1988. Cases included males and females 16 through 70 years of age with repeatedly reactive EIAs and IWBs in the past, who were referred from testing sites in Washington and Oregon. The HIV-1 Western blot interpretation for Western blots performed on subjects before study enrollment was accepted for study entry. Individuals with a previous diagnosis of HIV seropositivity or acquired immunodeficiency virus (AIDS) and recipients of experimental HIV-1 vaccine were excluded from the study. Cases were recruited by the enclosure of study information with laboratory reports sent to providers with the HIV-1 Western blot result (ie, the IWBs). The rate of response by cases was undefined, given the recruitment from providers and occasional use of anonymous testing. Cases were asked to refer current sexual partner(s) to the study for examination and HIV-1 antibody testing. Controls who had a negative HIV-1 EIA within the past 3 months were recruited from the same HIV-1 testing sites and were matched by HIV-1 testing site.

Because some cases referred with IWBs had negative Western blot tests at the time of study entry, and some persons selected for controls based on a nonreactive EIA had IWB, a subanalysis was done in which such cases and controls were excluded to compare cases with repeatedly reactive HIV-1 EIA and IWBs with controls who were negative for HIV-1 by both EIA and Western blot. We thus performed a subset analysis of cases with repeatedly reactive EIAs and IWBs and controls with nonreactive EIA and negative Western blot results at visit 1, the time at which information on independent variables was also collected.

After informed consent was obtained at the first study visit, cases and controls were interviewed about history of sexually transmitted diseases, number of sexual partners in the past 3 months and 1 year, HIV risk behaviors since 1978, and general medical history, and they were examined. Medical history included questions about parity, autoimmune illness (defined as thyroiditis, juvenile-onset diabetes, lupus erythematosus, and rheumatoid arthritis), liver disease, immunizations, recent viral-like illness, and exposure to cats and farm animals.

**STUDY DESIGN**

Cases who did not seroconvert within 6 to 9 months of follow-up were compared with EIA-negative controls to ascertain possible risk factors for IWB other than acute HIV-1 seroconversion. The Centers for Disease Control and Prevention recommends 6 months of serologic follow-up for persons with IWBs. In an analysis by Horsburgh et al., 95% of persons seroconverted within 6 months after sexual exposure to HIV-1.

**LABORATORY TESTING**

The University of Washington Virology Laboratory and the Washington State Public Health Laboratory, Seattle, performed HIV-1 EIAs and Western blots for the study. Both laboratories subscribe to the College of American Pathologists Proficiency Panel for HIV-1 antibody testing. Cases were followed up prospectively, with HIV-1 EIAs and Western blots repeated every 3 months for 6 to 9 months to detect seroconversion. Controls were tested once at the time of study enrollment for HIV-1 EIA and Western blot.

Enzyme immunoassays (Dupont, Biotech Research Laboratory Inc, Rockville, Md; and Genetic Systems, Seattle, Wash) and Western blot testing (Epitope, Beaverton, Ore) were performed on study subjects. The Centers for Disease Control and Prevention interpretive criteria were used for Epitope Western blots; a Western blot was considered positive if antibodies were present to two of the following HIV-1 viral proteins: p24, gp41, and gp120 or gp160.

Individuals, IWBs have been shown to result from cross-reactive antibody to HIV-1 or cross-reactive autoantibodies and alloantibodies. Etiologies of IWBs have not been well characterized and have been assessed primarily in case series and case reports, which have reported associations of IWBs with medical problems, including T-cell lymphoma, multiple sclerosis, and dermatologic disorders, injection drug use, alcoholic liver disease, and autoimmune diseases, such as Sjögren's disease.

Individuals with a reactive HIV-1 EIA and IWB are currently excluded from donating blood and have had difficulty obtaining life and disability insurance, US immigration status, and visas for foreign travel, regardless of their risk history. Persons notified of a reactive HIV-1 EIA and IWB are often concerned not only about their risk of HIV seroconversion but also about whether the IWB reflects any underlying medical condition. This population-based case-control study assessed risk factors for IWB; persons with repeatedly reactive EIAs and IWBs with and without risk factors for HIV infection were studied. We found that IWBs were associated with a low risk of HIV seroconversion and that the major risk factors identified for IWBs related to alloimmunization or autoantibodies.

**RESULTS**

Two hundred forty-four persons with IWBs for HIV-1 were referred and enrolled in the study as of August 1991.
Western blots without any bands were interpreted as negative, and blots with bands not meeting the criteria for a positive blot were interpreted as indeterminate. The presence of nonviral bands only was not considered an IWB for the case-control analyses. The diagnosis of HIV-1 infection was based on seroconversion to a positive Western blot with persistently reactive EIA. Positive Western blots were repeated. An HIV-1 culture, polymerase chain reaction, serum p24 antigen, and a recombinant p24, gp41 EIA (Syva Microtrak, Syva Corp, Palo Alto, Calif) were performed on study subjects with positive Western blots, as previously described.

The screen for autoantibodies included antinuclear antibodies (ANAs) and rheumatoid factor. Testing for ANAs was performed by means of an initial 1:40 serum dilution on rat liver and 1:100 dilution on Hep-2 cells. Latex agglutination was used to test for the presence of rheumatoid factor. A titer of 1:40 or more was considered a positive ANA or rheumatoid factor. Lymphocyte subset analyses were performed by flow cytometry. The screen for other infectious diseases included a serum VDRL, antibody to herpes simplex types 1 and 2 by Western blot, and hepatitis B surface antigen and antibody. The EIAs for human T-lymphotrophic virus (HTLV) type 1 and HIV-2 were assessed in 116 and 91 cases, respectively. To ascertain possible cross-reactivity with animal retroviruses, serum from the subset of the first 27 cases who reported raw milk ingestion or farm animal contact were tested for antibodies to bovine leukemia and bovine immunodeficiency virus by p24 agar immunodiffusion and Western blot. Serum samples from the first 26 cases who owned a pet cat were tested for reactivity on Western blots for feline leukemia virus and for feline immunodeficiency virus.

Serum samples from 78 cases were screened for the presence of antibodies to class I HLA antigens by means of a panel of T lymphocytes from 50 donors of known HLA type. Serum samples were screened for the presence of antibodies to class II HLA antigens by means of a panel of B lymphocytes from 25 donors of known HLA type. A modified microlymphocytotoxicity assay was used to detect complement-fixing antibody.

STATISTICAL METHODS

Demographic and HIV risk factors were compared by the chi-squared test and Fisher's Exact Test for categorical data and Student's t test for continuous data. The Mann-Whitney test was used for comparing continuous distributions when the assumption of a normal distribution was not appropriate. Ninety-five percent exact binomial confidence intervals (CIs) for the seroconversion risk were calculated.

To assess risk factors for IWBs other than HIV-1 infection, the nonseroconverter cases and controls were compared in terms of medical history and risk behaviors for HIV since 1978. Conditional logistic regression was used to compare the nonseroconverter cases and controls for risk factors for IWBs, stratified by testing site. Cases without matched controls (ie, cases referred from private providers, immigration, or life and disability insurance screening) were not included in the logistic regression, which was conditioned on testing site. Variables with \( P < .1 \) on univariate analysis were considered for possible entry in forward stepwise multivariate runs with the use of conditional logistic regression. Analyses were also performed for males and females combined and separately.

A second set of conditional logistic regression analyses were performed in which only the cases who were still repeatedly reactive on EIA and had IWBs at visit 1 and controls who were both EIA and Western blot negative at visit 1 were included to reduce potential misclassification bias from inclusion of EIA- or Western blot-negative cases and EIA-negative and Western blot-indeterminate controls. The female cases and controls referred from prenatal clinics were also excluded from the second analyses to avoid the potential confounder of current pregnancy in examining the relationship of parity to IWB.

A third logistic regression analysis was performed among blood donor cases and controls only, excluding cases and controls from all other testing sites.

Seven cases were referred from private physicians, life and disability insurance screening, and military screening and were excluded from the case-control comparisons because of the inability to recruit controls randomly from the same testing sites. Of the remaining 197 cases, 76 (39%) were from health department clinics, including the Sexually Transmitted Diseases Clinic and AIDS Prevention Project, 106 (54%) were referred from blood banks in Washington and Oregon, and 15 (8%) were referred from prenatal and women's clinics.

The risk of seroconversion was determined for cases with more than 6 months of follow-up, as described above. Of the 244 cases, 206 (84%) were followed up with repeat HIV-1 EIA and Western blots at least 6 months apart. The remaining 38 cases were followed up for less than 6 months because of unavailability of follow-up (n=26), incarceration (n=3), and residence out of the area (n=9). The cases with less than 6 months of follow-up were significantly more likely to be African-American, never have been married, be injection drug users, and have an income less than $20,000 than were the cases with 6 months or longer follow-up (data not shown). The risk of seroconversion was six (3.0%) of 206 (95% CI, 0.7% to 5.3%), and all six individuals had recent HIV risk behavior. We previously reported on 89 of these individuals, of whom four (4.5%) seroconverted. The risk of seroconversion may be underestimated by the unavailability for follow-up of 11 injection drug users.

One hundred thirty-one EIA-negative controls were recruited from the same HIV-1 testing sites as the cases:
77 controls (59%) were recruited from blood banks, 48 (37%) from the AIDS Prevention Project and Sexually Transmitted Diseases Clinic, and six (5%) from prenatal and women’s clinics.

Eighty-three current sexual partners of 83 cases were enrolled in the study. Forty (48%) were partners of index cases who were blood donors, 18 (22%) had partners from the AIDS Prevention Project or the Sexually Transmitted Diseases Clinic, four (5%) were male partners of women referred from prenatal clinics, 12 (14%) were from private providers, eight (10%) were from insurance screening, and one (1%) was from immigration screening.

**HIV-1 SEROLOGIC FINDINGS AMONG CASES, CONTROLS, AND CASES’ SEXUAL PARTNERS**

Results of HIV-1 EIA and Western blots from the first study visit among the 244 cases, 131 controls, and 83 cases’ sexual partners are shown in Table 1. Of 244 cases referred to the study because of previous reactive EIA and IWB, 139 still had repeatedly reactive EIA at study visit 1, and of these, 124 also had IWBs at the first study visit and did not seroconvert to a positive Western blot. Five of the six seroconverters had a positive Western blot at visit 1, which was 1 month or less after they first tested indeterminate with a p24 band only initially. The seroconverters were confirmed to be HIV positive by supplemental tests, including HIV-1 culture, polymerase chain reaction, serum p24 antigen, and recombinant p24, gp41 EIA.

Two low-risk cases and one case with multiple heterosexual partners had false-positive Western blots during follow-up, with the presence of p24 and envelope bands, but were determined not to be HIV infected through HIV-1 culture, polymerase chain reaction, and recombinant envelope EIA. Of the 131 EIA-negative controls, 33 (27%) had IWBs and six were recruited from prenatal and women’s clinics. The remaining 90 controls with negative immunoblots were included as controls for the second set of risk factor analyses, as described above. Banding patterns included p17 and p24 (core) bands in 138 (88%) of the 156 cases who had IWBs at study visit 1 compared with 19 (54%) of the 33 controls with IWBs and three (14%) of the 21 cases’ sexual partners with IWBs (P<.001). Five partners of the cases had repeatedly reactive EIA; one was negative by Western blot and one was indeterminate with a p55 band. Three (4%) of the cases’ sexual partners were confirmed HIV-seropositive by Western blot, two of whom had HIV risk behaviors (a bisexual man and a hemophiliac). The third partner was married to a recent immigrant from West Africa who had a stable p17 band on Western blot.

**RISK FACTORS FOR IWB AMONG THE CASES**

We first analyzed potential causes of IWBs for HIV-1 among the nonseroconverter cases only. These factors included cross-reactivity with HIV-2, HTLV-1, feline and bovine retroviruses, HLA antibody cross-reactivity, and current pregnancy. We then compared cases and controls for potential risk factors for IWB on the basis of historical data (eg, autoimmune history, parity, and high-risk behavior), as well as laboratory data (eg, autoantibodies, hepatitis B and herpes simplex type 2 antibodies, and lymphocyte subsets).

**CROSS-REACTIVITY AMONG OTHER HUMAN AND ANIMAL RETROVIRUSES**

Of 116 cases tested, only one had a weakly reactive HTLV-1 EIA and an IWB for HTLV-1 with p19 and p29 bands. Ninety-one cases tested for HIV-2 by a synthetic peptide for HIV-2 gp36 were negative. Two cases with residence in West Africa or West African sexual partners were tested for HIV-2, one by HIV-2 EIA and radioimmunoprecipitation assay and one by HIV-2 polymerase chain reaction; both were negative.

Among 27 cases who reported raw milk ingestion or farm animal contact, none had antibodies to bovine immunodeficiency virus. All of the first 26 cases who reported a cat as a pet were tested by Western blot for antibodies to feline leukemia virus and to feline immunodeficiency virus. Eight (31%) of 26 had bands on Western blot corresponding to p15e of feline leukemia virus, the transmembrane protein, compared with four (29%) of
14 controls (P=.8). No cases or controls had antibodies against feline immunodeficiency virus.

**HLA ANTIBODIES**

In 78 cases HLA antibodies were tested; anti-class I HLA reactivity was found in 13 (17%), and anti-class II HLA reactivity was not identified in any of the samples. All but one of the cases who demonstrated anti-class I HLA reactivity were multiparous women. There was no correlation between HLA antibodies, a positive ANA or rheumatoid factor, and presence of p17 or p24 banding patterns on Western blot.

**IWB AMONG PREGNANT WOMEN**

Of the total sample of 125 female cases referred to the study, 15 (13%) were pregnant when screened for HIV antibody and when enrolled into the study. Seven of the 15 pregnant patients had risk factors for HIV, but none seroconverted. Of the seven women with postpartum serum samples available for HIV serologic testing, six had persistent indeterminate banding patterns on Western blot a mean (±SD) of 5.3 ± 3.6 months post partum. The one woman whose Western blot turned negative after delivery had a positive rheumatoid factor (titer, 1:160) that also became negative post partum.

**RISK FACTORS FOR IWB: CASE-CONTROL COMPARISONS**

Cases and controls were compared in terms of demographics and HIV risk factors, medical history (ie, autoimmune history, parity, immunizations), and laboratory tests (ie, ANAs, rheumatoid factor, hepatitis B and herpes simplex type 2 serologic tests, and lymphocyte subsets). The demographics and HIV risk factors of the 191 cases and the 131 controls, matched by testing site, were similar except that the cases had less education and were less likely to have had a previous sexually transmitted disease (Table 2).

The medical histories and results of laboratory studies of the 191 cases and 131 controls were similar except that the cases were more likely to have received a tetanus booster in the past 2 years (22% vs 9%; P=.005), have had a positive tuberculin skin test (9% vs 3%; P=.04), and have a positive rheumatoid factor (6% vs 0.8%; P=.05). There was a trend toward a higher prevalence of positive ANAs among the cases (27%) than the controls (14%; P=.14). The cases with autoantibodies in our study usually had low-titer ANAs (median titer, 40; range, 40 to 640) with a speckled or homogeneous pattern and moderate titers of rheumatoid factor (median titer, 320; range, 40 to 10 240). The absolute CD4 count did not differ between cases and controls (median, 1.020 and 1.126×109/L [1020 and 1126/μL], respectively, for cases and controls). The prevalence of hepatitis B surface antibody was similar between cases and controls (11% and 13%, respectively). The prevalence of antibody to herpes simplex virus type 1 was 26 (72%) of 36 cases tested and 13 (56%) of 23 controls tested. The prevalence of herpes simplex virus type 2 specific antibody was eight (22%) of the 36 cases and four (17%) of the 23 controls (P=.61 for differences in herpes simplex virus type 2 seropositivity).

Multivariate analyses were performed separately for females and males to examine gender-specific risk factors for IWB (Table 3). The female cases were more likely to be parous (odds ratio [OR], 1.2; 95% CI, 1.02 to 1.4) and to have autoantibodies (either ANAs or rheumatoid factor; OR, 2.3; 95% CI, 1.03 to 5.6). The male cases were more likely to have received a tetanus booster in the past.
2 years (OR, 3.2; 95% CI, 1.2 to 8.6) and to have had sexual contact with a prostitute since 1978 (OR, 3.0; 95% CI, 1.0 to 9.5).

To reduce potential bias from misclassification, we performed a second set of multivariate analyses excluding cases with a negative HIV-1 EIA or Western blot at the time of study enrollment and controls with IWBs at visit 1 (Table 4). We also excluded cases referred from prenatal clinics, because current pregnancy could serve as a confounder in looking at parity as a risk factor for IWB. This final subset included 74 cases and 90 controls. The demographics of the 74 nonseroconverter cases who were EIA reactive and had IWBs at visit 1 and the 90 EIA- and Western blot-negative controls were similar, except that the median number of years of education was lower for cases than controls, and a higher proportion of cases reported sexual contact with a prostitute since 1978 (data not shown).

Gender-specific analyses of these 74 cases and 90 controls showed results similar to those obtained in Table 3, except that CIs were wider with the smaller sample. Risk factors implicated for IWBs among females were parity (OR, 1.3; 95% CI, 1.1 to 1.7) and autoantibodies (OR, 2.4; 95% CI, 0.8 to 6.6); among males the risk factor was sex with a prostitute (OR, 3.8; 95% CI, 0.9 to 16.4).

Analyses limited to blood donor cases and controls found that significant risk factors were tetanus booster in the past 2 years (OR, 2.3; 95% CI, 0.9 to 5.8) and autoantibodies (OR, 2.0; 95% CI, 0.98 to 4.0). Among female blood donor cases and controls, cases were more likely to be parous (77% vs 47%; OR, 3.0; 95% CI, 1.1 to 8.2).

With increasing pressure to screen more of the population for antibodies to HIV-1,38 more indeterminate HIV-1 serologic results will be generated. Previous studies that have examined causes of IWBs other than acute HIV-1 seroconversion or HIV-2 infection consisted of case series primarily of low-risk persons, such as blood donors or persons with known autoimmune illnesses.22,23,28,37 This is the first case-control study to examine risk factors for IWBs for HIV-1 in persons at either high or low risk for HIV infection. We compared both high- and low-risk cases with controls for medical conditions and exposures that might result in autoantibodies or alloimmunization, and prospectively followed up the cases for 6 months or longer to determine whether they seroconverted to a positive Western blot. The risk of seroconversion was 3.0%, comparable with that of earlier reports in blood donor cohorts,23,25,37 and seroconversion was observed only among persons with recent high-risk behavior.

Immunoblot banding patterns of cases differed from those of the controls and the current sexual partners of the cases. Cases more often had anti-core (p17 or p24) antibodies. In previous studies, up to 33% of EIA-nonreactive low-risk individuals had IWBs, often with p24 or polymerase reactivity.11,12 Thus, it appears that EIA screening followed by Western blot testing of EIA-reactive serum samples tends to select IWB patterns with antibody to core epitopes. Although core epitopes tend to be retrovirus-group specific, we found no association of HIV-1 IWBs with serologic reactivity to other known human and animal retroviruses (ie, HTLV-1, HIV-2, or bovine or feline leukemia or immunodeficiency viruses). Similarly, a recent study of 99 Minnesota blood donors with IWBs for HIV-1 also found no evidence of HIV-1 or HIV-2 infection.37 One case series from the New York Blood Center in Syracuse suggested an association between indeterminate HIV-1 immunobLOTS and bovine immunodeficiency virus, but this was not subsequently confirmed.24

Table 3. Analyses of Risk Factors for Indeterminate HIV-1 Western Blots*

<table>
<thead>
<tr>
<th>Case Characteristics</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity, No. (%)</td>
<td>104</td>
<td>83</td>
<td>1.2 (1.0-1.4)</td>
</tr>
<tr>
<td>Autoantibodies (ANA or rheumatoid factor), No. (%)</td>
<td>53 (32)</td>
<td>11 (17)</td>
<td>2.5 (1.1-6.0)</td>
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</tbody>
</table>

*Comparisons of 191 nonseroconverter cases and 131 controls. Cases in this table are restricted to the nonseroconverter cases for whom controls were available for matching by testing site (eg, blood centers, high-risk testing sites, ie, Seattle-King County AIDS Prevention Project and Sexually Transmitted Diseases Clinic), and prenatal clinics. The six cases who seroconverted and the 47 cases referred from private providers, life and disability insurance screening, and military screening, for whom site-matched controls were not available, were excluded from the table. HIV-1 indicates human immunodeficiency virus type 1; OR, odds ratio; CI, confidence interval; and ANA, antinuclear antibodies.
Risk factors for IWB among the nonseroconverter female cases in the present study were parity and autoantibodies, suggesting cross-reactive antibody. The association with parity persisted even after cases and controls from prenatal clinics were excluded. Parity could reflect alloimmunization during pregnancy, producing antibody to cellular proteins that comigrate with HIV proteins on the Western blot. False-positive HIV EIA tests in multiparous women have been associated with the presence of HLA-DR4 antibodies. Although 12 of 13 cases in our study with anti-class I HLA reactivity were multiparous women, we found no significant relationship of HLA antibodies to IWBs, and anti-class II reactivity was not observed. Golding and colleagues demonstrated DNA homology between HIV-1 gp41 and human class B1 domains; however, most of our cases had cross-reactivity to core proteins, either p17 or p24. We found no independent association between pregnancy at the time of HIV testing and IWBs, but our power to detect an association was only 37% given the sample size of female cases and controls. Since HIV testing of pregnant women is increasing, and if pregnancy is a risk factor for IWB, rapid evaluation protocols and counseling will be necessary to avoid unnecessary anxiety related to an indeterminate test result and to determine the HIV status of the mother and fetus.

Autoantibodies have been detected in approximately 20% of the population and were found in 37% of our cases and 21% of our controls. Autoantibodies might cross-react with normal cellular determinants of the H-9 or CEM cells in which HIV-1 is cultured before Western blot production. Of 22 cases with a reported autoimmune history, 13 (59%) reported a history of autoimmune thyroid disease. However, we did not specifically measure antimicromasal antibodies or antithyroglobulins. There was no correlation between a reported history of autoimmune disease and the presence of autoantibodies (ANA or rheumatoid factor). Although a link between human retroviruses and certain autoimmune illnesses, such as Sjögren’s disease and systemic lupus erythematosus, has been suggested, only limited data support this hypothesis.

Among men, a recent tetanus booster and sex with prostitutes were implicated as risk factors for IWBs. A recent tetanus booster could result in polyclonal stimulation of B cells and production of antibody with cross-reactivity to epitopes of HIV-1 or cellular proteins. An association of trivalent influenza vaccine with false-positive EIAAs and IWBs has been reported among blood donors, we sought but did not observe an association with recent influenza vaccination in our study (data not shown).

Because sexual contact with a prostitute was a risk factor for IWBs among men, we evaluated hepatitis B surface antibody, herpes simplex virus type 2 seropositivity, and history of sexually transmitted diseases as surrogate markers for sexual activity and sexually transmitted disease risk. All of these were similar among cases and controls. Furthermore, the percentage of IWBs among sexual partners of cases was not higher than that among controls, also arguing against a sexually transmissible infection. Further studies would be needed to investigate whether a sexually transmitted infection accounts for the association between prostitute contact and IWBs among the male cases. Sex with a prostitute was not a significant risk factor for IWBs among blood donor cases. This result corroborates the finding by Dock and colleagues in which blood donors with IWBs and HIV-1-seropositive and HIV-1-seronegative blood donor controls had similar rates of serologic evidence of past cytomegalovirus, Epstein-Barr virus, hepatitis A and B virus, or herpes simplex virus 1 infections.

There are several limitations to our study. We recruited a population-based sample of high- and low-risk individuals with IWBs referred from the community, and we recruited EIA-negative controls from the same testing sites to match for the heterogeneous mix of high- and
low-risk cases. Since we were not able to recruit EIA-negative controls from private providers and insurance companies, we did not include those cases in the case-control comparisons of risk factors for IWB. Case-control studies involve examination of exposures and risk factors for a relatively rare disease outcome. The use of a case-control method to examine risk factors for a reactive laboratory test that is not always reproducible over time or with different manufacturers' tests presented methodologic issues. To reduce potential misclassification from inclusion of cases who no longer had IWBs at the first study visit and controls who had IWBs, we performed subset analyses including only those cases who still had repeatedly reactive EIAs and IWBs at visit 1 and the controls who were both EIA nonreactive and Western blot negative. The ORs for risk factors identified in the initial and subset analyses were similar, but power was sacrificed in the subset analysis.

Our data have four principal implications for facilitating the management of IWBs for HIV in the clinical setting. First, approximately one third of persons who present with IWBs will not be repeatedly reactive by follow-up EIA, and retesting within 1 month to identify this group is warranted. There is no need for further testing or follow-up of this group. Second, 3% of persons with IWBs in this study were infected with HIV-1, and the most important factor for seroconversion was high-risk behavior, which could be determined from the risk history. As previously reported, the Western blot banding pattern and selective supplemental testing for HIV can be used to counsel patients and identify those at greatest risk of seroconversion. Supplemental testing and repeat Western blot testing were also useful in identifying persons with no history of high-risk behaviors who proved to have false-positive Western blots in this study. Third, no cross-reactivity with known human retroviruses or animal retroviruses was demonstrated in our study population. However, as additional retroviruses are identified, serologic and virologic studies will be of interest among persons with reactive HIV-1 EIAs and indeterminate HIV-1 serologic findings, particularly since the antibody pattern observed most often in cases with IWBs suggested antibody to core proteins.

Fourth, the significant risk factors for IWBs identified in our study included parity and autoantibodies among the female cases, and tetanus booster in the past 2 years and sexual contact with a prostitute since 1978 among the male cases. Medical history will identify subjects with such risk factors. Study of persons with IWBs should include an assessment of HIV risk behavior as well as these potential risk factors by history and testing for autoantibodies.

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