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Western Blot Banding Patterns of HIV Rapid Progressors in the U.S. Navy Seropositive Cohort: Implications for Vaccine Development

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ABSTRACT

Although human immunodeficiency virus (HIV) infection is progressive, the rate of decline in CD4+ lymphocyte counts varies. The role of immune system components in limiting HIV infection has yet to be defined, but a previous report on the U.S. Navy HIV Seropositive Cohort reported that strong reactivity in the anti-p55 (core precursor), p24 (core) and p53 (reverse transcriptase) Western blot bands was associated with higher CD4+ lymphocyte counts at the first clinical evaluation for HIV. The previous report examined the cross-sectional association between Western blot banding patterns and initial CD4+ lymphocyte counts. This report examines the association between these banding patterns in individuals who progressed rapidly as compared with patterns of patients who did not, based on their trends in repeated CD4+ lymphocyte counts as a marker of progression. Rapid and slower progressors were identified from a cohort of 3414 Navy and Marine Corps personnel who had a first positive HIV Western blot during 1986–1991. For purposes of this study, rapid progressors were defined as individuals whose CD4+ lymphocyte counts declined to < 500 cells/mm³ within 1 year of seroconversion. A total of 325 individuals met these criteria. A comparison group of 63 slower progressors also was identified; this group consisted of those whose CD4+ lymphocyte counts remained at ≥ 500 cells/mm³ for a minimum of 5 years of follow-up after their first positive Western blot. Rapid progressors were slightly younger than slower progressors and were more likely to be never married but did not differ significantly from slower progressors in race or sex. Rapid progressors had weaker reactivity in the anti-p55 core precursor ($P < 0.0001$), p15 core ($P < 0.01$), gp41 transmembrane ($P < 0.01$) and p31 endonuclease ($P < 0.05$) bands on the Western blot. The odds ratio for rapid progressor status associated with weak or absent reactivity was 7.8 in the anti-p55 band and ranged from 2.0 to 3.2 in the anti-p31, p15, and gp41 bands. These associations remained significant after adjustment for age, race, and sex. The p55 association persisted in repeated Western blots during routine clinical evaluation during a period of 5 years after the first positive Western blot. It was concluded that several possible explanations may account for the weaker reactivity of rapid progressors: (i) weak anti-p55 reactivity might have been a marker of early immune system damage; (ii) high concentrations of p55 or related proteins in the serum may have bound the available anti-p55 antibodies in rapid progressors, making them difficult to identify on the Western blot; or (iii) lack of anti-p55, p15, gp41, or p31 reactivity might have allowed more rapid progression. *Ann Epidemiol* 1996;6:341–347.

KEY WORDS: Human immunodeficiency virus, Western blotting, CD4+ lymphocyte count, HIV antibodies, AIDS vaccines, cohort studies.

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INTRODUCTION

Although the course of human immunodeficiency virus (HIV) infection is progressive, the rate of decline in CD4+ lymphocyte counts (1–4) and immunological competence (5–8) varies. The principal roles of various components of the immune system in HIV infection have not been fully characterized, but almost all individuals exposed to HIV from transfused blood will develop antibodies, usually within 3 months of exposure (9). The presence of specific antibodies to viral proteins and glycoproteins is detectable in specific bands on the Western blot (10, 11), which is the main confirmatory test for the diagnosis of HIV infection (6, 8, 12, 13). Although the Western blot has been used to characterize the natural history of the antibody response to

HIV (14, 15), relatively few previous studies have examined comprehensively the extent to which the pattern of immune response to individual viral antigens is associated with progression (14, 16-21).

The Western blot includes nine principal bands that reflect the presence of antibodies to viral proteins and glycoproteins (10, 11). There are three antibodies to core proteins encoded by the *gag* gene: anti-p55 (core precursor), p15 (core), and p24 (core); three to reverse transcriptase or endonuclease components encoded by the *pol* gene, anti-p31, p53, and p64; one to a transmembrane glycoprotein (gp41) encoded by the *env* gene; and two to envelope glycoproteins (gp120 and gp160) also encoded by the *env* gene (10, 11, 15, 18). One study found that declining titers of anti-p55 and anti-p51/65 antibodies were significantly related to progression to AIDS, while declining titers of other antibodies were not (19). A study that followed 76 HIV-infected heroin users for a mean of 9 months found that progressors had substantially lower anti-p24 antibody titers at first diagnosis than did nonprogressors, but that progressors did not differ from nonprogressors in anti-gp41, or gp120 titers (18). Another study of intravenous drug users reported that progressors had weaker anti-p24, p15, p31, p53, p64, and gp41 reactivity at first examination than nonprogressors but did not differ significantly in the proportion of subjects reactive in the p55 or gp120/160 bands at first examination (20). In other studies declines in titers to specific core antigens, such as p24 (17) and p15 (22) were found to be associated with loss of antibody-dependent cellular cytotoxicity against HIV-infected cells.

In a previous cross-sectional study from the present cohort, weak or absent anti-p55, p24, and p53 bands each were associated with lower CD4+ lymphocyte counts at first diagnosis of HIV infection, but data on progression were not then available (23). This study reports on reactivity in specific bands on the Western blot in rapid as compared with slower HIV progressors; the trend over time in CD4+ lymphocyte counts was used as a biomarker for progression (5).

METHODS

The Navy HIV Central Registry maintained at the Naval Health Research Center in San Diego, CA, was used to assemble a cohort of men and women in the Navy and Marine Corps who had a first positive HIV Western blot between January 1, 1986, and December 31, 1991. This registry contains results from the Navy HIV Screening Program and from routine follow-up clinical evaluations of HIV-seropositive individuals. Testing was done as part of the service-wide HIV screening program for active-duty Navy and Marine Corps personnel, testing of individuals in preparation for deployment overseas, screening of health care providers, and routine physical examinations or clinical evaluations.

Seropositive individuals were defined as those who had two positive paired enzyme-linked immunosorbent assays (EIAs) followed by a positive Western blot that met criteria of the Centers for Disease Control and Prevention (Atlanta, GA) for HIV positivity (24), with at least two of three bands present at p24, gp41, and gp120/160. Testing procedures have been described in detail elsewhere (25, 26).

Seroconverters were defined as those having a documented history of a negative EIA followed by two positive paired EIAs and confirmed by a positive Western blot, as above. The midpoint of the time interval between the individual's most recent negative EIA and first positive Western blot was used as the estimated seroconversion date, as in previous studies (27-30).

Western Blot Testing

The possible values for density of the Western blot bands (a marker of the intensity of the antibody response) were: 0, nonreactive; 0.5+, very weakly reactive; 1+, weakly reactive; 2+, moderately reactive; and 3+, strongly reactive. Scoring of Western blot bands was performed from January 1, 1986 to June 30, 1989 by Biotech Laboratories (Rockville, MD) using the Biotech Research Laboratories kit, and thereafter by North American Biologicals, Inc. (Miami, FL) using the Dupont Biotech kit. Results in the gp120 and gp160 bands were not reported separately during 1986-1987; results specific for these bands were therefore limited to the period beginning January 1, 1988. All blots were analyzed by technicians who were not aware of whether the individual was a rapid or slower progressor.

CD4+ Lymphocyte Counts

CD4+ lymphocyte counts on initial clinical evaluation were obtained from the Navy HIV Central Registry (25, 26), a central resource containing the results of all Navy clinical evaluations. These were performed at Naval Medical Centers in Bethesda, MD, San Diego, CA, Portsmouth, VA, and Oakland, CA. CD4+ counts were performed by use of laser-based flow cytometry: a Coulter Profile 1 (Coulter Corporation, Hialeah, FL) was used in Bethesda; a Coulter Epics Model C in Portsmouth; and a Becton-Dickinson (Immunocytometry Systems Division, San Jose, CA) FAC-Scan in San Diego and Oakland. Hematology was performed in all laboratories using a Coulter counter (Coulter Corporation, Hialeah, FL). Analyses were performed within each hospital, except Oakland, where they were performed at the Clinical Laboratories of the University of California, San Francisco, Medical Center, and at Naval Medical Center, Bethesda, during 1988 through mid-1990, when they were performed at Smith-Kline Beecham Clinical Laboratories, Rockville, MD.

The flow cytometry and quality assurance procedures

used in this study have been described previously (28, 29, 31). All laboratories (except the University of California, San Francisco, Medical Center) were participants in an ongoing comprehensive quality assurance program that provided monthly aliquots of human blood, standardized quality-control reagents, and monthly proficiency testing (31, 32). The laboratories were required to provide monthly CD4+ counts on these specimens, which were used to determine coefficients of variation. Hematology procedures for lymphocyte counts also were standardized and reported monthly for multiple identical aliquots of human blood. Coefficients of variation among laboratories for CD4+ counts were available for 1988 and subsequent years; they were 19% in 1988 and < 10% in subsequent years (W. Rickman, personal communication, 1991).

Study Design

Rapid and slower progressors from the Navy HIV cohort were studied. This cohort includes 3414 Navy and Marine Corps personnel of both sexes who had a first positive HIV Western blot during 1986-1991. Since relatively few members of this cohort progress to AIDS while they remain under observation, progression was defined in terms of stability or decline in CD4+ lymphocyte counts that were monitored as part of routine follow-up. Rapid progressors were defined for the purpose of this study as individuals whose CD4+ lymphocyte count declined to < 500 cells/mm³ within 1 year of the estimated date of seroconversion. There were 325 individuals who met these criteria. For the purpose of this study, the comparison group of slower progressors was defined as individuals whose CD4+ lymphocyte counts remained at ≥ 500 cells/mm³ for a minimum of 5 years after the first positive Western blot and who developed no AIDS-associated illness (33). There were 63 such individuals. Patients with a history of receiving zidovudine (AZT) or other antiviral pharmacotherapy prior to meeting the above criteria were not encountered in this study.

Statistical Analysis

Odds ratios (OR) were used to analyze the univariate effects of demographic characteristics and immune reactivity on the Western blot (34). Age differences were tested with the χ^2 test (34). Differences according to race, sex, and marital status were tested with Fisher's exact test (35). Subjects who were separated, divorced, or of unknown marital status were grouped for statistical testing to provide adequately large expected cell frequencies (34). The occasional weakly reactive (0.5+) specimen was grouped with the nonreactive (0+) specimens. Multiple logistic regression was used to analyze the independent contributions of age, race, sex, and immune reactivity in each band (34), using the SAS LOGISTIC procedure (SAS Institute, Cary NC). A separate regression analysis was performed for each band.

TABLE 1. Age distributions of rapid and slower progressors: Navy HIV cohort, 1986-1991^a

Age group (years)	Rapid progressors		Slower progressors	
	No.	%	No.	%
17-21	82	25.2	6	9.5
22-24	85	26.2	13	20.6
25-27	66	20.3	27	42.9
≥ 28	92	28.3	17	27.0
Unknown	0	0.0	0	0.0
Total	325	100.0	63	100.0

P < 0.001

^aThe median ages of the rapid and slower progressor groups were 25 and 26 years, respectively.

A longitudinal analysis also was performed to compare anti-p55 reactivity in rapid and slower progressors for annual intervals of up to 5 years after the first positive Western blot for all subjects remaining in the cohort. Statistical significance was assessed in each interval using Fisher's exact test (35). Nominal *P* values were calculated, unadjusted for the number of tests (36).

RESULTS

Rapid progressors tended to be slightly younger than the comparison group and were more likely to be never married (Table 1). Fifty-one percent of rapid progressors and an identical percentage of slow progressors were white. There were eight women: five rapid progressors and three slower. Seventy percent of rapid progressors and 54 percent of slower progressors were never married. Slower progressors had seroconverted in 1986-1987, allowing a minimum of five years to elapse between seroconversion and the latest CD4+ count of ≥ 500 cells/mm³, while rapid progressors had seroconverted throughout 1986-1991 (data not shown).

Rapid progressors were more likely than than the comparison group to have weak or absent reactivity in the anti-p55, p15, gp41, and p31 Western blot bands (Table 2). The OR for rapid progressor status associated with weak or absent reactivity was 7.8 in the anti-p55 band, and ranged from 2 to 3.2 in the anti-p31, p15, and gp41 bands. Rapid progressors also tended to have weak or absent reactivity in the anti-p24, p64, and p53 bands, but these effects were not statistically significant.

Rapid progressors were more likely to have weak reactivity in the same (anti-p55, p15, gp41, and p31) bands after adjustment by multiple regression for age, race, and sex (Table 3). Rapid progressors were approximately half as likely as slower progressors to have strong reactivity in the anti-p55 band on the first repeated Western blot (54.1% vs. 90.4%); although the difference diminished somewhat, it remained significant at 48 months or longer (Table 4).

TABLE 2. Odds ratios comparing rapid with slower progressors by reactivity on first Western blot, according to band: Navy HIV cohort, 1986-1991

Band	Description	Western blot reactivity ^a				Odds ratio	P
		Rapid progressors (n = 325)		Slower progressors (n = 63)			
		Weak	Strong	Weak	Strong		
Core precursor and core proteins							
p55	Core precursor	172	153	8	55	7.8	< 0.0001
p15	Core	133	192	14	49	2.4	0.01
p24	Core	27	298	3	60	1.9	NS
Polymerase components							
p31	Endonuclease	117	208	14	49	2.0	0.05
p53	Reverse transcriptase	85	240	13	50	1.4	NS
p64	Reverse transcriptase	50	275	7	56	1.5	NS
Transmembrane and envelope glycoprotein components							
gp41	Transmembrane	92	233	7	56	3.2	0.01
gp120	Envelope	29	268	0	8	^b	NS
gp160	Envelope	7	290	0	8	^b	NS

^aWeak refers to absent or weak (1+) reactions; strong refers to moderate (2+) or strong (3+) reactions.
^bBecause of the small number of slower progressors with data available on these bands, odds ratios were not calculated.

TABLE 3. Multiple logistic regression analysis comparing rapid with slower progressors, by reactivity on first Western blot, according to band, race, and sex: Navy HIV cohort, 1986-1991

Measurement	Regression coefficient	Odds ratio ^a	P
Western blot bands			
Core precursor and core proteins			
p55 Core precursor	+2.0256	7.6	< 0.0001
p15 Core	+0.8514	2.3	0.01
p24 Core	+0.5501	1.7	0.39
Polymerase components			
p31 Endonuclease	+0.6744	2.0	0.05
53 Reverse transcriptase	+0.2711	1.3	0.44
p64 Reverse transcriptase	+0.3738	1.5	0.40
Transmembrane glycoprotein			
gp41	+1.0619	2.9	0.01
Covariates ^b			
Age in years			
17-21	...	1.0	...
22-24	-0.2520	0.8	0.59
25-27	+1.2027	3.3	0.003
≥ 28	+0.2248	1.2	0.57
Race			
White	...	1.0	...
Black	+0.0015	1.0	0.99
Other	-0.9886	0.4	0.35
Sex			
Men	...	1.0	...
Women	+1.4304	4.2	0.11

^aOdds ratios were based on 325 rapid and 63 slower progressors and compare individuals having Western blot reactivity scores of 2+ (moderately to strongly reactive) with those having scores of 0-1 (nonreactive to weakly reactive), P values were calculated using the Wald χ^2 test. The regression model did not converge for the gp120 and gp160 bands due to smaller sample size.

^bRegressions including all covariates were performed for each band; values are shown for the regression including p55. Results for other bands were similar.

DISCUSSION

In the present study, rapid progressors had significantly weaker anti-p55, p15, gp41, and p31 bands on the Western blot, with the strongest association present in the p55 band. All of these associations persisted after adjustment by multiple regression for age, race, and sex; moreover, the weaker anti-p55 reactivity in rapid progressors persisted on repeated Western blots.

TABLE 4. Number and percentage of rapid and slower progressors with strong (2+ or 3+) reactivity in the Western blot p55 band, according to interval in months between first Western blot and subsequent blots: Navy HIV cohort, 1986-1991

Group and interval	No. tested	No. (%) with strong reactivity
Rapid progressors		
First Western blot	325	155 (47.6)
Subsequent Western blot		
0-12 months	314	170 (54.1) ^a
12-23 months	163	89 (54.6) ^b
24-35 months	108	61 (56.5) ^c
36-47 months	57	36 (63.2)
≥ 48 months	29	23 (79.3) ^c
Slower progressors		
First Western blot	63	55 (87.3)
Subsequent Western blot		
0-12 months	52	47 (90.4)
12-23 months	60	50 (83.3)
24-35 months	57	46 (80.7)
36-47 months	50	39 (78.0)
≥ 48 months	41	41 (100.0)

^aP < 0.0001.

^bP < 0.01.

^cP < 0.01 for rapid vs. slower progressors in this interval.

The results of the present study are, in general, consistent with a previous cross-sectional study of this cohort that observed a strong association of anti-p55 reactivity on the initial Western blot with low CD4+ lymphocyte counts from blood samples taken at approximately the same time (23). The cross-sectional study detected significant positive associations of anti-p55, p24, and p53 antibodies with CD4+ lymphocyte counts, but a negative association between anti-gp41 antibodies and CD4+ counts.

The tendency of rapid progressors to have weaker anti-p55 reactivity is consistent with results of a previous small cohort study that observed an association between declining anti-p55 and p51/65 antibody titers and rapid progression to AIDS, despite the absence of significant change in titers of antibodies to other viral antigens (19), although that study reported no initial difference in anti-p55 titers in rapid as compared with slower progressors. Another study based on data from 36 months of follow-up of seropositive individuals reported that weak anti-p24 titers were associated with progression to symptomatic disease, but that anti-gp41 and anti-gp120 titers were not (18). The study did not analyze anti-p55 titers.

Comparisons of the findings of the present study with those of the previous cross-sectional study in this cohort suggest that the effects of anti-core (particularly anti-p55) antibodies may differ from those of anti-gp41 (transmembrane) antibodies. Anti-core antibodies were associated with higher CD4+ counts at first diagnosis of HIV, and, consistent with this earlier finding, rapid progressors in the present study persistently had weaker reactivity in the anti-p55 band than slower progressors.

One possible interpretation of these results is that weak anti-p55 antibody reactivity in rapid progressors was solely a marker of the natural history of the infection. Anti-p24, p55, and p15 antibodies appear within two months of infection and are the earliest markers (15). However while these antibodies appear early in the course of the infection, they also tend to persist during at least the first 6 months and generally longer (15).

A second interpretation is that weak anti-p55 antibody reactivity could be an indirect marker of the presence of large concentrations of p55 antigens in the serum that have bound the available anti-p55 antibodies, leaving a low concentration to react with the Western blot. Reduction in antibody titers due to excess viral antigen has been described for various antibodies, particularly late in the natural history of HIV infection or in symptomatic HIV disease (14, 37-40). Such an explanation seems relatively unlikely in the present investigation, which studied individuals detected in a screened population, generally earlier in the course of infection than is possible in unscreened populations, and during a phase of the infection when antigenemia is presumably relatively low. A previous study in which serum was treated to dissociate antigen-antibody complexes found that

the association between levels of p24 antigen and its antibody in adults were largely independent of whether the serum had been pretreated (21). While acid-dissociation increased the concentration of p24 antigen in the serum, it did not increase low titers of anti-p24 antibody. Since that study did not examine p55 antibody titers after dissociation, further studies of dissociated anti-p55 antibody are needed.

A third possible explanation for the findings is that antibodies to *gag*-encoded proteins, and possibly others, may play some role in limiting the rate of progression of infection, since it is known that declines in antibodies to *gag*-encoded proteins are associated with loss of antibody-dependent cellular cytotoxicity against HIV-infected cells (17, 22). It is also thought that the *gag* gene is more strongly conserved than the *env* gene, and that *gag*-encoded gene products tend to vary less than *env*-encoded antigens (41). This pattern could work to the advantage of antibodies to *gag*-encoded proteins, since potentially they would face less antigenic diversity. It also has been hypothesized that anti-p24 reactivity may be a marker reflecting function of a cell-mediated immune cytotoxicity mechanism based on detection of a *gag*-encoded protein expressed on the surface of infected cells, possibly p15 or p55 (18). The findings of the present study are consistent with such an interpretation.

This study used CD4+ lymphocyte counts, rather than incidence of AIDS, as the biomarker of progression of HIV infection. The natural history of CD4+ counts has been well-characterized in this cohort (28, 29). Using CD4+ counts to define progression for this study made it possible to examine a larger sample of rapid progressors. This strategy was worthwhile in this cohort, whose members frequently depart before the onset of clinical HIV disease. The use of the CD4+ count as a defining biomarker of AIDS has been widely accepted, but at a lower level (200 cells/mm³) than in this study (42); however, CD4+ counts even in the range above 200 cells/mm³ are widely accepted as a marker of progression (5-7).

The prevalence of weak initial anti-p55 reactivity was 48.5% in this cohort (23). Based on the OR of 7.8 for progression that was observed in the present study, the population attributable risk for weak anti-p55 reactivity was 77% (43). This suggests that approximately three-quarters of the risk for rapid progression in this population was accounted for by weak initial anti-p55 reactivity.

These findings may have implications for vaccine development. The ultimate goal of vaccination against HIV is the reliable induction of persistent sterilizing immunity, mediated perhaps by neutralizing antibodies and a strong cell-mediated response. This and a previous study (19) suggest that strong antibody responses to products of the *gag*-gene are correlated with slower progression of HIV infection. If this association is causal rather than artifactual, then preserving the antigenic features of *gag*-encoded proteins might be important for candidate vaccines intended to re-

duce progression (44). Further studies would help to verify these findings and to further delineate the possible roles of specific antibodies at various stages of HIV infection.

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