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Consistent Associations of HLA Class I and II and Transporter Gene Products with Progression of Human Immunodeficiency Virus Type 1 Infection in Homosexual Men

Ireneus P. M. Keet,[1,a] James Tang,[2] Michèl R. Klein,[3,b] Susan LeBlanc,[2] Cheryl Enger,[4] Charles Rivers,[2] Raymond J. Apple,[5] Dean Mann,[6] James J. Goedert,[7] Frank Miedema,[3] and Richard A. Kaslow[2]

[1]Municipal Health Service, Amsterdam, The Netherlands; [2]Program in Epidemiology of Infection and Immunity, School of Public Health, University of Alabama at Birmingham, Birmingham, Alabama; [3]Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service, Amsterdam, The Netherlands; [4]Oncology/Biostatistics, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland; [5]Roche Molecular Systems, Alameda, California; [6]Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland; [7]Viral Epidemiology Branch, National Cancer Institute–EPN, National Institutes of Health, Bethesda, Maryland

Polymorphic products of genes in the HLA region contributing to variability in the course of human immunodeficiency virus type 1 (HIV-1) infection were identified by screening 375 Caucasian seroconverters who were aggregated from 3 cohorts. AIDS-free time was related to numerous (15) class I alleles, alone or in conjunction with transporter protein variants, to homozygosity at the A or B locus, and to alleles of two class II haplotypes. A prognostic scoring algorithm derived from the 3 cohorts captured multiple HLA contributions to protection or to risk (relative hazard = 0.57–60 per unit increase in score, all $P << .001$). The impact of HLA was strong and appeared independent of the effects of chemokine receptor/ligand polymorphisms and antiretroviral treatment. The algorithm also predicted divergent rates of $CD4^+$ cell decline in 2 other groups, totaling 227 seropositive persons ($P = .06 - <.001$). Confirmation of these relationships should encourage investigation of HIV-1 antigen processing and presentation mediated by polymorphisms in the HLA region.

Control of human immunodeficiency virus type 1 (HIV-1) infection involves the processing of specific viral peptides and their presentation to both cytotoxic T lymphocytes (CTL) restricted by highly polymorphic HLA class I alleles and $CD4^+$ cells [1–4]. However, it has been difficult to establish whether specific HLA alleles are strong or consistent determinants of HIV-1 disease progression in populations. Inconsistencies have arisen from differences in ethnicity, small sample sizes in early studies, investigative designs, laboratory techniques, and statistical methods, as well as the extreme polymorphism in HLA [5–7].

Kroner et al. [8] found that pairs of infected hemophilic siblings who shared HLA haplotypes had similar rates of $CD4^+$ cell decline and progression to AIDS. Subsequently, Kaslow et al. [9] derived an algorithm using polymorphisms screened in the Multicenter AIDS Cohort Study (MACS) seroconverters, to summarize individual HLA profiles, and then demonstrated significant predictive capacity of the algorithm in the DC Gay cohort (DCG), as well as in the MACS. Selected alleles, by themselves or combined with genes-encoding transporters associated with antigen processing (TAPs) [10, 11], were associated with rapid or slow disease progression [9].

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Informed consent was obtained from all MACS and DCG participants according to the human experimentation guidelines of the US Department of Health and Human Services and from all AMCO participants according to the guidelines applied by the Municipal Health Service, Amsterdam, The Netherlands.

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[a] Present affiliation: Willem Arntsz Huis (can be reached at Municipal Health Service, P.O. Box 20244, 1000 HE, The Netherlands);

[b] Medical Research Council Laboratories, Fajara, Gambia, West Africa.

Reprints or correspondence: Dr. Richard A. Kaslow, Dept. of Epidemiology, Medicine, and Microbiology, School of Public Health, Ryals Bldg. 220A, University of Alabama at Birmingham, 1665 University Blvd., Birmingham, AL 35294-0022 (rkaslow@epi.soph.uab.edu).

Further application of that MACS algorithm has verified the general concept of a cumulative effect of multiple HLA gene products (unpublished data). However, in previous work, individual associations could have been unintentionally generated or overlooked as a result of selection and small numbers of patients, length of follow-up, and MACS screening criteria. Now, with extended follow-up and screening in seroconverters from 3 aggregated cohorts, plus molecular typing for class I, we have refined the algorithm to detect associated markers previously missed, to reject spurious relationships, to disentangle confusing effects due to linkage disequilibrium, to confirm two-locus interaction, and to incorporate homozygosity at class I loci. The applicability of the new algorithm has been tested in 2 studies separately designed and conducted in seroprevalent men.

Materials and Methods

Patients and outcome measurements. In this study, we distinguished a seropositive person whose date/duration of infection was known to within a fairly narrow interval ("seroconverter") from a seropositive person who was already infected upon entry into a cohort and whose date/duration of infection was much less certain ("seroprevalent"). In the screening phase, homosexual Caucasian seroconverters were aggregated from the 3 cohorts (MACS, DCG, and The Amsterdam Cohort Studies on AIDS [AMCO]), as described elsewhere [12–14]. MACS began enrolling homosexual men in 1984 at 4 sites (Baltimore, Pittsburgh, Chicago, and Los Angeles) [12] and contributed, to this analysis, 135 initially HIV-1–negative men who seroconverted within a 6- to 12-month interval between 1984 and 1991 (99% before 1990). DCG originated in 1982 in New York and Washington, DC, and here contributed 100 homosexual men who seroconverted within comparably narrow follow-up intervals between 1980 and 1988 (100% before 1990) [13]. AMCO drew on a previous hepatitis B vaccine trial plus additional men who seroconverted under close observation between 1984 and 1995 (140 included here; 74% before 1990) [14]. Follow-up of all 3 cohorts was censored in July 1997.

Single, combination, and more potent antiretroviral treatment regimens (in general use after January 1990, January 1993, and July 1995, respectively) were prescribed selectively in these cohorts for men with symptoms and signs of deteriorating immunity. We evaluated the HLA effects independent of treatment in seroconverters by censoring follow-up at those 3 dates.

We validated the results from seroconverters in 2 separate studies of MACS and AMCO subjects, who were seropositive at entry and were followed up to 13 years [15, 16]. Seroprevalent men who had relatively high initial $CD4^+$ cell counts and virtually stable or slowly declining trajectories of $CD4^+$ cell counts were compared with men who had similar initial cell counts but intermediate or rapid trajectories of decline.

Laboratory methods. HLA class I alleles were initially typed by use of standard serologic typing reagents on cryopreserved peripheral blood mononuclear cells [9, 17]. To verify results of serologic typing, to resolve ambiguous allele designations, to assess the role of C alleles, and to improve accuracy in designating homozygotes, the 275 seroconverters in MACS and AMCO were subsequently typed by use of a preformulated class I sequence-specific primer (SSP) typing kit (Imperial Cancer Research, London) or a modified set of primers yielding comparable or greater resolution. Direct sequencing of polymerase chain reaction–amplified DNA fragments resolved typing ambiguities left by SSP in ~15% of men, including all apparent homozygotes. Serologically-based designation of alleles was retained, because all the men could not be retyped and sufficient numbers were seldom available for individual analysis of the many more sequence-specific class I alleles. The impact of changes in allele assignments due to retyping by molecular techniques was examined carefully.

Alleles at DRB1 and DQB1 and major variant variants at TAP1 and TAP2 loci were identified by molecular techniques. Class II alleles were determined by sequence-specific oligonucleotide hybridization in the MACS and by single-strand conformation polymorphism (SSCP) in the DCG [9]. We used SSP (DQB1) [18] and selective automated sequencing (DRB1 [Pharmacia Biotechnology, Uppsala, Sweden]) in AMCO. TAP variants were typed by SSCP [9]. Chemokine receptor/ligand mutations (CCR5 Δ32, CCR2b-64I, and SDF-1 3′A) were identified separately for each cohort by recognized methods [19–22].

Analyses by alternative outcomes of infection and HLA groupings. In all analyses presented here, the term "marker" refers to any HLA class I allele, HLA class II haplotype, single-nucleotide TAP variant, or a combination of these. We based the assessment of individual markers in the seroconverters on clinically defined AIDS [23], because follow-up for AIDS was more complete. However, we confirmed the overall relationship of the algorithm markers to $CD4^+$ cell loss to <200 cells/mm^3 and then validated it in groups of seroprevalent persons who were originally defined by clearly contrasting rates of $CD4^+$ cell decline. To exclude further the possibility that apparent associations with progression of immunodeficiency were actually due to associations with specific AIDS-defining conditions, we examined the effects of markers contributing to the final algorithm for each of the most common AIDS diagnoses.

We explored class I relationships in a variety of ways. Class I relationships (individually and for class I–TAP combinations) were examined by use of each broad serogroup cluster (e.g., A10) and its serologically dissociated allele specificities (e.g., A25 and A26); in two instances, the degree of concordance for alleles in a cluster justified treating them as a group (see table 1 footnote). Resolving alleles to the molecular level subdivided them into more numerous subsets, but few of the sequence-specific alleles were frequent enough to support meaningful analysis, and none demonstrated an association distinguishable from that occurring with markers grouped on the basis of serologic designations. Therefore, in order to preserve analytic power and uniformity across the cohorts, we have reported results using serologic allele designations.

Certain associations with outcome, as described elsewhere [9], were shown epidemiologically to depend on interaction between specific HLA class I alleles and specific TAP variants. We analyzed 4 common but not ubiquitous TAP variants: TAP1 val 333, TAP1 gly 637, TAP2 ile 379, and TAP2 ala 665 (TAP1.2, 1.4, 2.1, and 2.3, respectively [24]), because nomenclature for TAP alleles is not settled, and some would be inferred incorrectly in double heterozygotes. Class II associations were analyzed in consideration of

Table 1. Markers (class I alleles, class I–TAP combinations, and DRB1-DQB1 haplotypes) contributing +1 or −1 to HLA profile in 3 cohorts of Caucasian homosexual human immunodeficiency type 1 seroconverters.

Marker	*n*	RH	*P*≤
RH ≤ 0.67 (*n* ≥ 15 = + 1)			
A29-33 (A19) plus TAP2 ala 665[a]	33	0.46	.006
B27	30	0.40	.003
B57	31	0.54	.02
DRB1*1300-DQB1*0603	45	0.67	.07
RH ≤ 0.55 (*n* = 10–14 = + 1)			
A25/26 (A10) plus TAP2 ala 665[b]	12	0.31	.02
RH ≥ 1.5 (*n* ≥ 15 = −1)			
A24	50	1.57	.004
B8 plus TAP2 ile 379	15	1.88	.02
Cw4[c] minus TAP2 ala 665	46	1.79	.001
DRB1*1200-DQB1*0301	15	1.83	.04
Homozygosity at A or B[d]	68	1.71	.003
RH ≥ 1.8 (*n* = 10–14 = −1)			
A23 minus TAP2 ala 665	12	2.04	.02
A28(68) plus TAP2 ala 665	12	1.88	.08
B40/60 plus TAP2 ile 379	14	2.24	.005

NOTE. RH, relative hazard of AIDS-free time in men with and without marker; TAP, genes-encoding transported associated with antigen processing.

[a] Most (A29-32) of the A19 group plus TAP2 ala 665 had similar effects; A33 (A19) (*n* = 4) did not, but it was included for consistency.

[b] Effect of A25 (A10) plus TAP2 ala 665 explained ostensible B18 effect, and closely related A26 (A10) plus TAP2 ala 665 was included because it had similar effect.

[c] Cw4 is in tight disequilibrium with B35, but neither single markers nor combination met criteria; Cw4-TAP effect shown here appeared stronger than B35-TAP or B35-Cw4-TAP effect.

[d] Homozygosity based on molecular typing (*n* = 64) or serologic identity at A-B-C or B + DRB1-DQB1) (*n* = 4).

individual DRB1 and DQB1 alleles and DRB1-DQB1 haplotypes, which could be confidently assigned in ~98% of individuals. Haplotype analyses yielded relationships as strong as or stronger than for any individual component allele. We also searched for individual and combined effects of homozygosity at class II loci.

Analytic strategy and statistical methods for individual markers. Using Cox proportional hazards analysis in the aggregated seroconverter cohorts, we computed the relative hazard (RH) of AIDS-free time from a marker by comparing men with and men without it, as reported elsewhere [9]. All markers (single alleles and combinations) present in ≥4 men were screened, but only a subset of these were accepted as contributory. Markers in ≥15 men with RH ≤0.67 or in 10–14 men with RH ≤0.55 were considered contributory for longer time to AIDS, and markers in ≥15 men with RH ≥1.5 or in 10–14 men with RH ≥1.8 were considered contributory for shorter time to AIDS, regardless of the significance-test result.

Further analyses of these markers were performed. (1) We systematically assessed real and "apparent" linkage disequilibrium, including such linkage between class I or class II and TAP markers, by use of a screening $P < .1$ in stratified analyses. (2) Interaction between HLA and TAP was accepted if a ≥2-fold difference between the RHs or a 0.5 difference in the βs (regression coefficient) quantifying association with an HLA class I allele was seen in the presence versus the absence of a relevant TAP variant, where both strata contained ≥4 men. These interactions (table 2) were tested, as described elsewhere [9]. (3) We excluded any marker that did not exert its effect independently of other contributing markers in ≥4 men. Markers that met all criteria for association with longer or shorter time to AIDS were assigned scores of +1 or −1, respectively, and were incorporated into a scoring algorithm similar to that described elsewhere [9].

Homozygosity at the A and B loci but less uniformly at the C locus has been associated with more rapid progression in these and other cohorts ([25], Carrington, unpublished data). We therefore compared homozygotes at the A or B locus with heterozygotes at all class I loci. Because the RH for homozygosity at A and B, singly or jointly, was comparable with RHs for single specific risk markers evaluated as described above, −1 was added to the score for homozygosity at A or B. In contrast, homozygosity for DRB1 or DQB1 or their haplotypes did not appear related to the rates of progression in previous studies [25] or in the current analysis and was not incorporated into the scoring algorithm.

Scoring in screening and validation studies. The algebraic sum of scores for specific markers and homozygosity displayed by each subject represented an HLA profile (HP). In the seroconverter-screening cohorts, the effects of HPs on AIDS-free time were summarized in Kaplan-Meier plots and tabulations, with log-rank test for significance of comparisons. In the 2 validation studies of seroprevalent persons, the strength of association between the HP and the rate of $CD4^+$ cell decline was computed by logistic regression, to estimate the relative odds (RO) of decline per unit change in HP. The χ^2 and exact tests for proportions and the Jonckheere-Terpstra test for trend were used.

Adjustment for effects of chemokine receptor/ligand polymorphisms. To determine whether the observed HLA associations were independent of the effects of reportedly protective receptor/ligand variants, we compared the overall patterns of HLA associations among the seroconverters who were homozygous for wild type for CCR5 and CCR2b at their respective polymorphic sites with the pattern of associations among those carrying either a Δ32 or a 64I variant. For the small number of men homozygous for SDF-1 3′A variant, the association with outcome was inconsistent with previous findings, and it was excluded from consideration.

Results

Characteristics of seroconverter cohorts. The mean age of the 375 seroconverters was 35 years, with similar distribution among the 3 cohorts. Of these men, 239 developed AIDS by July 1997: 97 (72%) of 135 in the MACS (median AIDS-free interval, 7.8 years), 74 (74%) of 100 in the DCG (median interval, 7.6 years), and 68 (49%) of 140 in AMCO (median interval, 8.6 years). Graphic and statistical comparison of the differences in median time to AIDS indicated that they were unimportant ($P_{\text{log-rank test}} = .76$).

Concordance between serologic and molecular methods for participants typed by both was similar to that reported elsewhere [26]. Resolving discrepancies in A and B allele designations (9% and 8%, respectively) altered the HP in ~2% of the seroconverters typed by both methods and produced negligible net change in overall findings. Frequencies of HLA alleles were broadly comparable with those in Caucasians of Eu-

Table 2. Interactions between combinations of class I alleles and TAP variants meeting criteria as determinants of AIDS-free time.

Class I allele-TAP variant combination[a]	Interaction							
	Effect with listed TAP combination[a]			Effect with reciprocal TAP combination[a]				
	RH	β[b]	n	RH	β[b]	n	χ^2[c]	P[c]
A23 minus TAP2 ala 665	2.0	0.72	12	0.30	−1.20	4	4.6	.03
A25/26 plus TAP2 ala 665	0.31	−1.17	12	1.32	0.28	21	4.5	.03
A28 (A68) plus TAP2 ala 665	1.9	0.63	12	0.78	−0.25	19	6.9	.009
A29-33 plus TAP2 ala 665	0.46	−0.76	33	1.32	0.28	58	5.9	.01
B8 plus TAP2 ile 379	1.9	0.63	15	0.86	−0.16	69	2.2	.14
B40/60 plus TAP2 ile 379	2.2	0.81	14	0.96	−0.04	31	2.3	.13
Cw4 minus TAP2 ala 665	1.8	0.58	46	0.78	−0.25	39	3.2	.07

[a] RH, relative hazard of AIDS-free time in men with and without marker; RHs are reported first with genes-encoding transported associated with antigen processing (TAP) combination listed and then with opposite TAP combination (e.g., A23 without and then A23 with TAP2 ala 665).
[b] β = coefficient of regression in RH calculation.
[c] Test of interaction based on 2–main-effects model.

ropean origin. Comparison of frequencies of the contributing markers among the seroconverter cohorts revealed only a higher frequency of A25/26 in the MACS than in the other two and, again, a negligible effect on the overall findings.

Impact of individual HLA markers. Fifteen class I alleles, alone or in combination with a TAP variant, fulfilled criteria for association with time to AIDS (table 1) in the 375 seroconverters. By our approach, 3 class I alleles (A24, B27, and B57) showed strong independent effects, whereas the effects more often appeared to depend on statistically supported interaction with TAP variants. Table 2 summarizes the class I–TAP interactive combinations that met criteria for inclusion in the algorithm.

Alternative explanations for each relationship based on linkage disequilibrium or interaction could not be found. TAP interactions were not determined primarily by the class II haplotypes in known disequilibrium with them [27]. The effects due to certain combinations contributed by TAP2 ala 665 were at least as strong as those of any of the 5 class II haplotypes in disequilibrium with the TAP variant; that is, when paired with the interacting class I alleles, the TAP variant without one of those linked class II haplotypes was more strongly associated with an altered rate of progression than was any of the haplotypes without the TAP variant. Results were similar for effects of TAP2 ile 379 plus its interacting class I alleles.

For class II, only two DRB1-DQB1 haplotypes examined met the tests for independent association with AIDS-free time (table 1). DRB1*1300-DQB1*0603 appeared protective in the absence of TAP2 ala 665, with which it is in strong disequilibrium. The risk from DRB1*1200-DQB1*0301 could not be explained by any other marker.

We analyzed potential relationships of each marker in the algorithm associated with time to AIDS to each of the more common individual AIDS-defining conditions. No such relationship was strong or unique enough to consider it a more plausible alternative explanation for the association of any marker with progression (data not shown).

Associations of homozygosity with AIDS-free time in seroconverters. Homozygotes at the A or B locus progressed more rapidly than heterozygotes at both loci ($n = 68$, RH = 1.71, $P = .003$) in the seroconverters typed by sequence-specific methods (table 1). Doubly homozygous men (at A and B) were at higher risk than were singly homozygous men (at A or B), but not so clearly higher as to warrant a greater contribution to the scoring algorithm (RH = 2.08, $P = .13$). Excess risk from class I homozygosity depended largely on more prevalent alleles not contained in the algorithm (e.g., A1, A2, B7, and B44).

Associations of HLA HP with AIDS-free time and CD4$^+$ cell loss in seroconverters. Sums of integers for markers that met sample size and RH criteria (+1 for specific markers of protection and −1 for each specific marker of risk or homozygosity at the A or B locus) produced individual HPs ranging from −4 to +4. Because only 7 and 2 men, respectively, had HPs of ≤ -3 or $\geq +3$, we formed 5 categories of HP: ≤ -2, −1, 0, +1, and $\geq +2$. In the aggregated seroconverters, the RH of progression to AIDS for each successive HP category was 0.59 (95% confidence interval [CI], 0.51–0.67, $P << .001$); the cumulative RH was 0.12 for the 23 men whose HPs were $\geq +2$, compared with the 41 men whose HPs were ≤ -2. The composite survival analysis (figure 1*A*) reflects the strong incremental effect, with a difference of ≥ 7 years in median time to AIDS, between individuals in the two extreme HP categories. Corresponding findings for each cohort individually (RH = 0.57–0.60, all $P < .001$) are consistent (figure 1*B*–1*D*). About 70% of the subjects were homozygous at the A or B locus or carried at least one specific marker contributing to the scoring algorithm, but only 10.9% and 6.1% showed scores reflecting the more extreme risk (≤ -2) or protection ($\geq +2$), respectively. Discrimination across HP categories, by using disappearance of CD4$^+$ cells as the measure of disease progression, was comparable with that seen when AIDS-free time was used (RH = 0.62, $P < .001$; data not shown).

Censoring data in seroconverters on sequentially later dates, when single, dual, and triple therapies were in general use, had successively decreasing impact on the results. In the most strictly censored Kaplan-Meier analyses (i.e., after January 1990), results were the most altered, but paradoxically so, with prognosis more favorable in the absence than in the presence

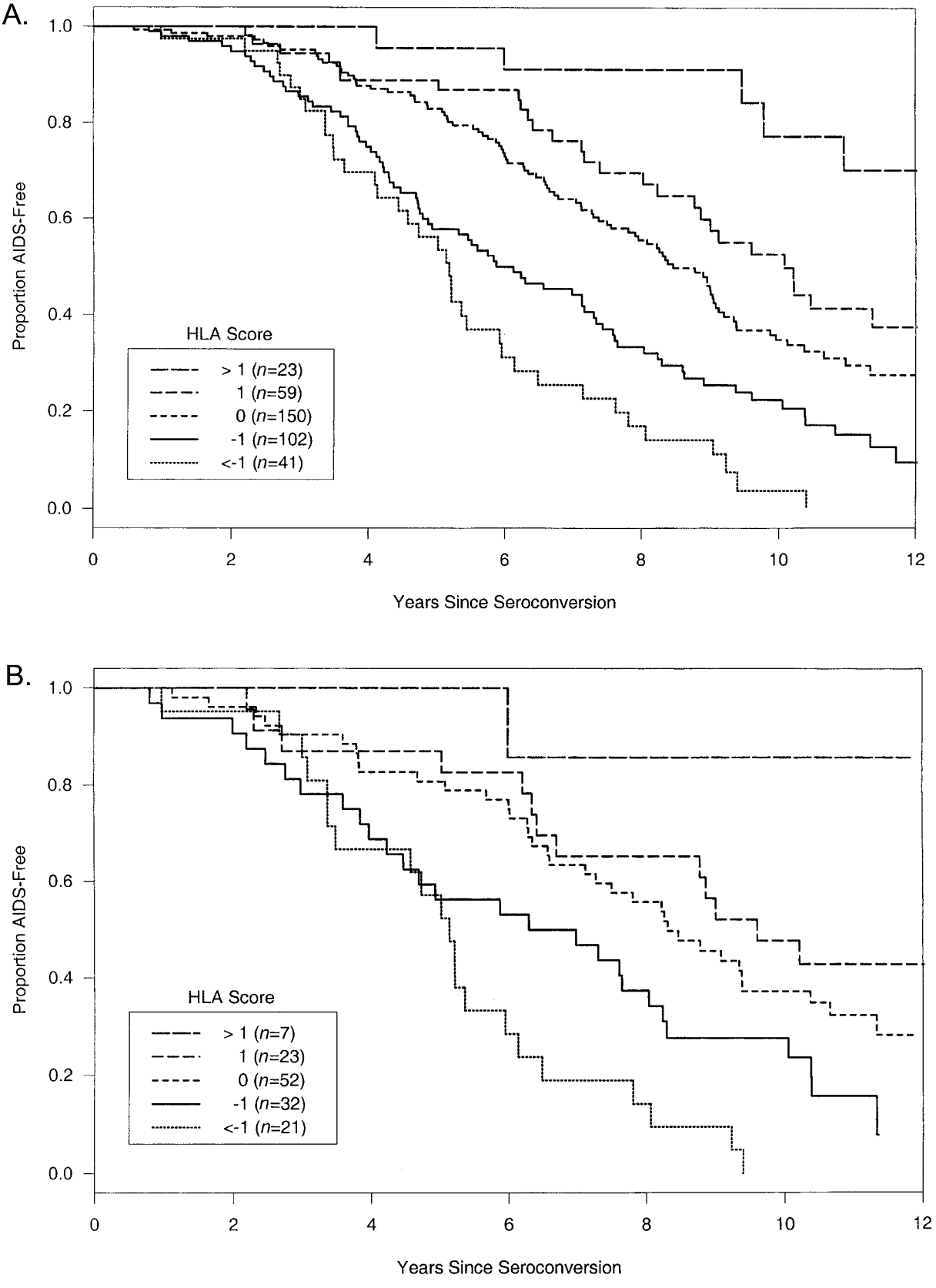

Figure 1. *A,* AIDS-free time in 375 seroconverters aggregated from Multicenter AIDS Cohort Study (MACS), DC Gay cohort (DCG), and Amsterdam Cohort Studies on AIDS (AMCO) and assigned to 5 categories of HLA profile (HP). HP is based on markers listed in table 1. No. of persons in each category is shown for each plot. Wilcoxon and log-rank tests for comparison of 5 categories ($P < .001$). *B,* AIDS-free time in 135 MACS seroconverters assigned to 5 categories of HP. Wilcoxon and log-rank tests for comparison of 5 categories ($P < .001$).

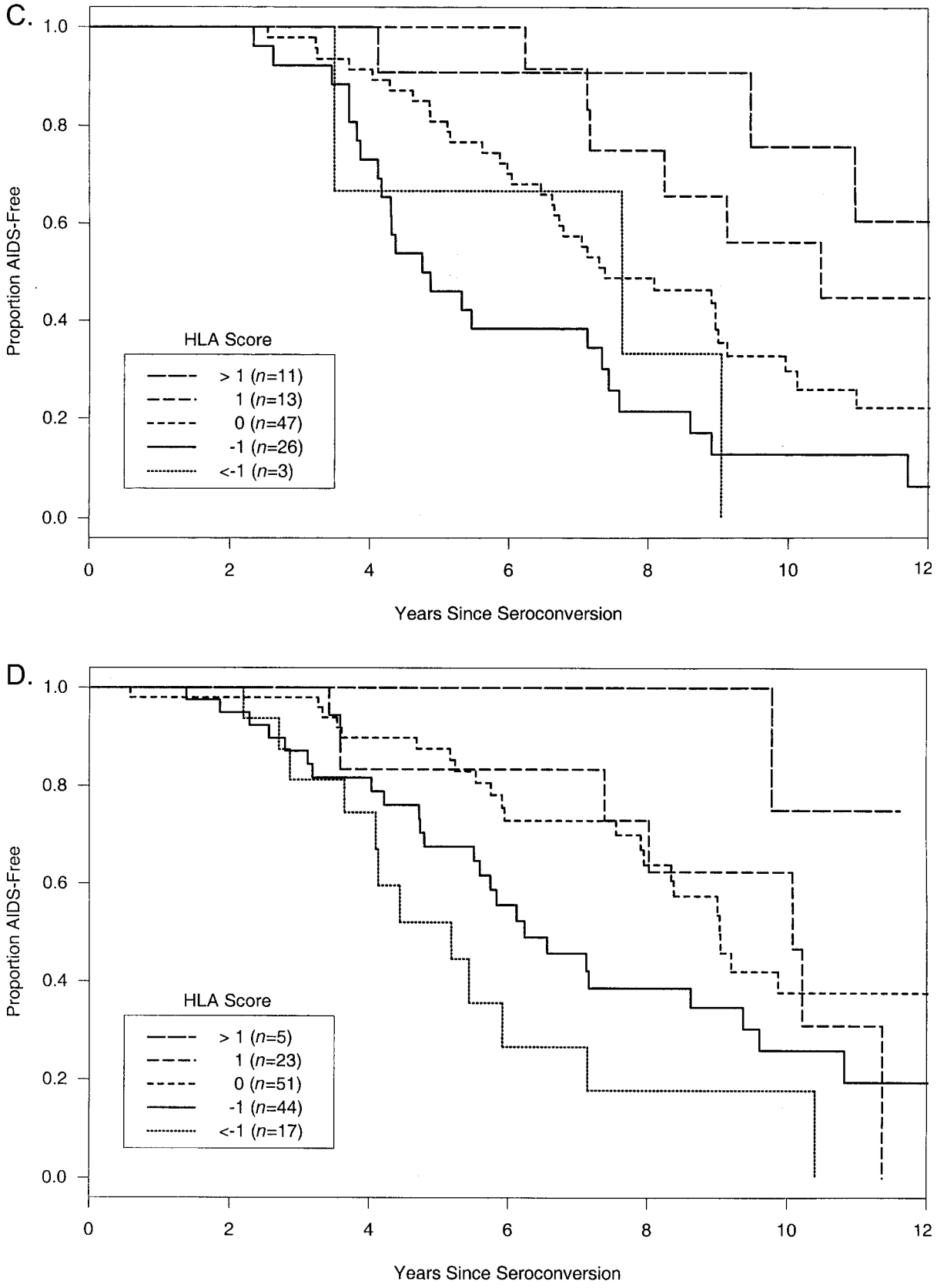

Figure 1. *(Continued). C,* AIDS-free time in 100 DCG seroconverters assigned to 5 categories of HP. Wilcoxon and log-rank tests for comparison of 5 categories ($P < .001$). *D,* AIDS-free time in 140 AMCO seroconverters assigned to 5 categories of HP. Wilcoxon and log-rank tests for comparison of 5 categories ($P < .001$).

of treatment for men in each HP category, reconfirming that in our study, selective intervention with monotherapy in early seroconverters showing relatively poor immunologic and clinical condition was associated with somewhat worse group prognosis. More potent combinations of drugs were taken by so few men so late in their courses, that censoring for introduction of these agents produced virtually no change in the plots. In short, our analysis demonstrated similar highly significant discrimination of disease course by the HP in data censored and uncensored for a treatment effect ($P_{\text{log-rank test}} << .001$).

Associations of HP with CD4⁺ cell decline in seroprevalent populations. The algorithm based on AIDS in seroconverters (table 1) was applied to 2 independent studies designed to compare seroprevalent men with distinct rates of decline in $CD4^+$ cells. For the 166 MACS subjects matched for baseline $CD4^+$ cell count ± 100 cells, there was a strong association between $CD4^+$ cell decline and HP score over the −1, 0, and +1 categories (RO = 0.32 for each +1 change in HP; $P < .001$; table 3). For 61 subjects of the AMCO seroprevalent study, matched for baseline $CD4^+$ cell count ± 250 cells, the association was of similar magnitude but predictably less statistically significant (RO = 0.50, $P = .06$; table 4). The consistency of the overall relationship in the 227 seroprevalent study subjects compared with the 375 seroconverters was borne out by broad consistency of individual marker relationships: all contributing markers carried by ⩾10 individuals showed similar trends in both sets of analyses.

Finally, the capacity of the HP for prognostic discrimination was quite similar among the men with any of the chemokine receptor/ligand variants and the men with only wild type forms (figure 2*A*, 2*B*). The presence of one or more of these variants in the aggregated seroconverters prolonged median AIDS-free time by 0.7–1.4 years for the 2 middle categories with the largest numbers of men (HP = −1 and 0). Analysis, including the HP and the 2 chemokine receptor variants, detected their separate effects ($RH_{HLA} = .53$, 95% CI, .35–.78; $RH_{CCR5/2} = .85$, 95% CI, .63–1.16) but offered no evidence for interaction ($RH_{Interact} = 1.09$, 95% CI, 0.82–1.45).

Table 3. Relationship between HLA profile (HP) and human immunodeficiency virus type 1 disease progression measured by rate of decline in $CD4^+$ cell count in MACS seroprevalent study.

	Progression				
HP category[a]	None ($n = 44$)	Slow ($n = 11$)	Intermediate ($n = 56$)	Rapid ($n = 55$)	Total ($n = 166$)
−1	7	5	32	30	74
0	19	4	16	18	57
+1	18	2	8	7	35

NOTE. MACS, Multicenter AIDS Cohort Study.

[a] Relative odds of increasingly rapid $CD4^+$ cell decline with each +1 change in HP score = 0.32; 95% confidence interval, 0.20–0.52; $P < .001$ (by logistic regression and Jonckheere-Terpstra test for trend).

Table 4. Relationship between HLP profile (HP) and human immunodeficiency virus type 1 disease progression measured by rate of decline in $CD4^+$ cell count in AMCO seroprevalent study.

	Progression		
HP category[a]	None and slow ($n = 21$)	Intermediate and rapid ($n = 40$)	Total ($n = 61$)
−1	6	20	26
0	9	15	24
+1	6	5	11

NOTE. AMCO, Amsterdam Cohort Studies on AIDS.

[a] Relative odds of more rapid $CD4^+$ cell decline with each +1 change in HP score = 0.50; 95% confidence interval, 0.24–1.04; $P = .06$ (by logistic regression and Jonckheere-Terpstra test for trend).

Discussion

Polymorphic products of genes in the HLA region collectively exert substantial influence on AIDS-free survival [9]. Previous demonstration of that influence has been extended to the AMCO and other HIV-1–infected individuals as further "proof of principle" (unpublished data). Here in an expanded screening population of 375 men from 3 seroconverter cohorts, the previously published algorithm [9] was recast in order to incorporate the effects of individual markers that met more rigorous sample size and RH criteria and of A or B locus homozygosity, as described elsewhere ([25], Carrington et al., unpublished data), to confer risk of relatively rapid disease progression.

Concordance between serologic and molecular assignments of class I alleles was high, nearly 98% in subjects with alleles contributing to the algorithm. Molecular typing made only minor differences in conclusions about individual markers, but permitted incorporation of the risk due to homozygosity. In larger numbers of HIV-1 clade B–infected Caucasians, molecular typing will probably further refine the observed relationships and may reveal additional ones (e.g., with C alleles), but it is unlikely to alter the overall pattern of these relationships in any fundamental way.

The sample size and RH statistical criteria were necessarily arbitrary, balancing the risk of introducing noncontributory variables against that of omitting relevant ones. Typical screening of individual HLA markers by significance testing, with corrections for multiplicity, would have been counterproductive. Anticipating multiple small effects of individual markers from earlier work, we avoided prematurely omitting them during screening. We could be more inclusive, because larger numbers than previously studied [9] improved discrimination, and studies in seroprevalents were available to confirm the findings. However, requiring consistency with individual markers across 3 cohorts would have been too stringent: even with 375 men, associations with less frequent markers varied somewhat among the 3 cohorts. Relatively few combinations fulfilled the restrictive interaction criteria, and requiring a larger effect would have eliminated several that were subsequently found to be consistent in the validation groups. Despite the range of RHs and *P* values,

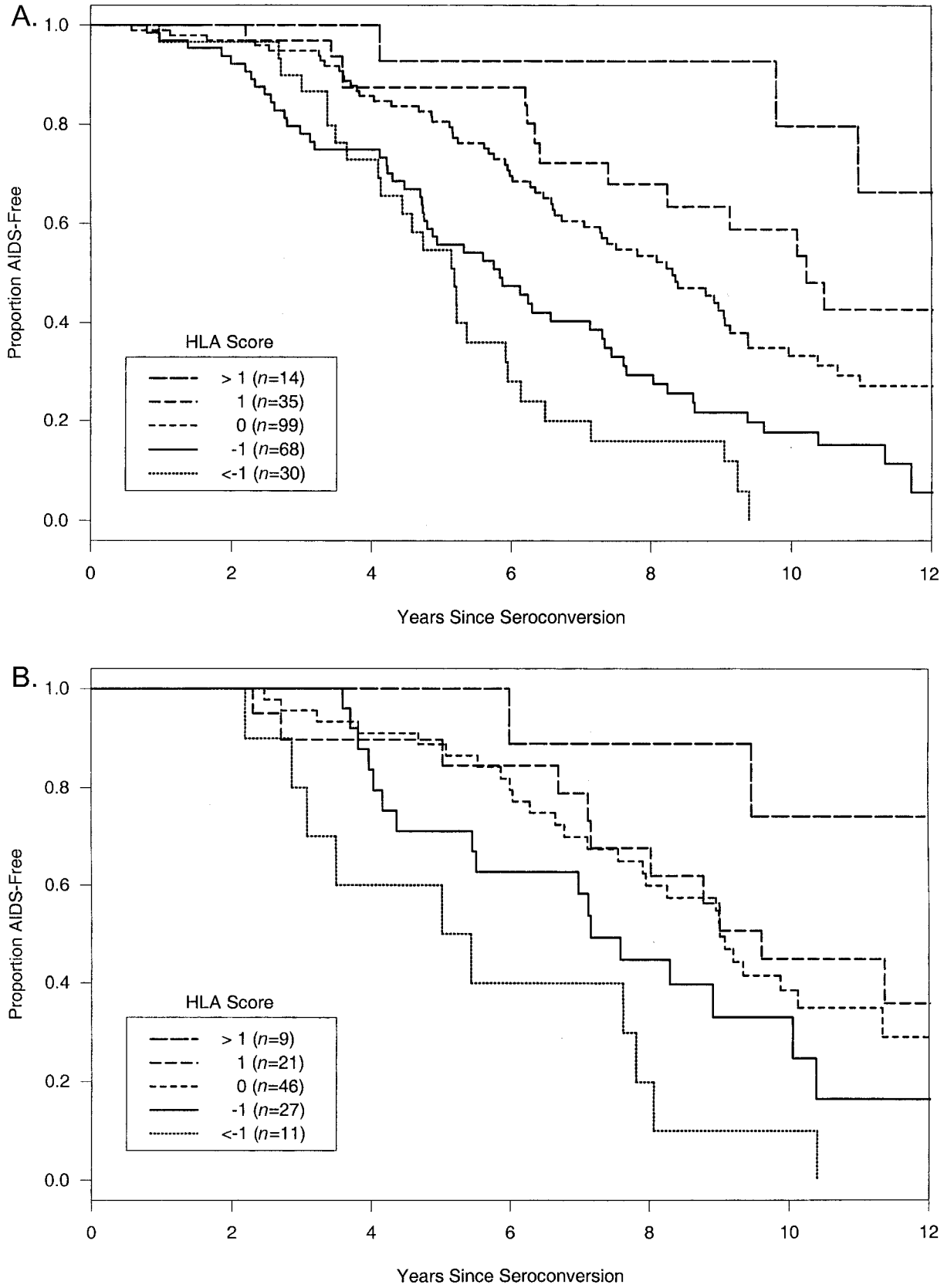

Figure 2. *A,* AIDS-free time according to HLA profile (HP) in 246 seroconverters manifesting only wild type (CCR5+/+ and CCR2b) at polymorphic sites of those genes. *B,* AIDS-free time according HP in 114 seroconverters manifesting ⩾1 of protective genotypes (CCR5Δ32, CCR2b-64I, or CCR2b 64I/64I) at polymorphic sites of those genes.

we treated all markers as equivalent in the HP, because no alternative approach (e.g., combining RH values) better captured the discriminatory power.

The clear, strong HLA relationships were evident in men who seroconverted early enough in the epidemic to diverge widely in their AIDS-free time over 13 years of follow-up. With incident clinical AIDS as the outcome measure for screening and decline in $CD4^+$ cells for independent validation of the algorithm in both seroconverter and seroprevalent groups, we have considerable confidence in the generalizability of the analytic strategy. During much of the early follow-up time, antiretroviral therapy was unavailable or only modestly effective, and selective administration to the seriously ill clouded the analysis of joint genetic and antiviral effects. By the time the more potent agents were available, most of the divergence due to differences in HP observed here had already occurred. Furthermore, whereas analysis of the HLA effects was based on events that can take years to evolve, clinical divergence according to HP was apparent relatively early—within 2–3 years of seroconversion in our cohorts (figure 1), and preliminary analysis in MACS has demonstrated a correlation of HP with plasma viral RNA concentration soon after seroconversion ([28], Kaslow, unpublished data). Together these data imply relatively early influence of HLA polymorphic variation on immunopathogenesis of HIV-1 infection.

Consensus is slowly emerging from various efforts to identify individual HLA determinants of disease progression. A number of the class I alleles (some in the context of specific TAP variants [table 1]) that showed consistent trends in our study populations have shown similar effects in another population or in an experimental (i.e., CTL) system (A24 [29], A31/A32 [30, 31], B8 [32–34], B27 [2, 35], B57 [3, 36, 37], and Cw4 ± B35 [38–41]). Reports of other associations have been less consistent (DRB1*1200-DQB1*0301 or related alleles [42] and DRB1*1301 [8, 41]). The remaining markers (table 1) were too sparse to permit assessment in the separate cohorts. Data on certain additional markers, including some proposed elsewhere [9], fell short of inclusion criteria but remained suggestive for risk (e.g., B38 without TAP1 val 333, B39, B49, B55/56, and DRB1*1401-DQB1*0503) or for protection (e.g., B14 ± Cw8 and B51 with TAP1 val 333) in the seroconverter and seroprevalent groups (data not shown).

Class I polymorphisms dominated the HLA associations. Only 3 class I alleles independently fulfilled our criteria, whereas 12 depended on the presence or absence of a specific TAP variant (tables 1, 2), as described elsewhere [9]. TAP variants that modified time-to-AIDS in the presence of certain class I alleles were unrelated to AIDS-free time in the presence of others. The unpredictable pattern of interaction with each TAP variant may seem contrived as long as the molecular mechanism of interaction remains uncertain, but reproducible applicability of our model provides a strong empirical argument for the relevance of the observed interactions, either with TAP variants or something closely related to them. Without taking these interactions into account, our investigation would have overlooked the contribution of a number of class I alleles in >100 subjects contributing a substantial proportion of the variation.

The numerous but small interactive effects seen here imply a limit to the degree of consistency attainable, within even reasonable-sized, ethnically homogeneous, and predominantly HIV-1 clade B–infected Caucasian study populations. To reconcile ethnic, clade, and other differences will require careful investigation of hundreds of well-defined patients [43]. Efforts to elucidate underlying molecular pathogenetic mechanisms should help clarify the relationships, but a comprehensive resolution may come slowly. As examples, preliminary analysis, according to the four broad supermotif categories of alleles with shared binding groove characteristics [44], demonstrated no obvious relationship between supermotif and outcome of HIV-1 infection, and the intriguing correlation between the degree of protection by a class I allele and the frequency with which its preferred binding motifs occur in HIV-1 proteins [45] cannot fully account for the findings.

Close examination of the joint and independent effects of both the DRB1-DQB1 haplotypes and the TAP variants linked with them indicated that the TAP component, not the class II haplotypes, accounted for the interaction. Early molecular analyses have not disclosed obvious clues in DNA sequence adjacent or more centromeric to the TAP variants or in DPB1 (MACS) or in DMA, DMB, or LMP (AMCO) (Tang, unpublished data). Preliminary sequencing of TAP exons near known variant sites have produced only weak suggestions of additional, potentially contributory, variation. The relationships with TAP observed epidemiologically await explanation on the immunobiologic level.

Two class II haplotypes appeared to be independently associated with the progression of infection in our populations. Both occur in disequilibrium with contributing TAP variants, but its link with TAP2 ala 665 did not seem to account for the protective effect of DRB1*1300-DQB1*0603, as suggested elsewhere [9]. Certain DRB1*1300 alleles have been suggested as favorable prognostic factors in other settings [40], but separate effects of the DRB1 and the DQB1 alleles could not be clearly distinguished here. Too few men with DRB1*1200 were available to examine further. No other class II marker alone appeared involved, and association of class II haplotypes interacting with contributory class I markers was not readily apparent in a screening evaluation. The effects of DPB1 and other specific contributory genes in the class II region have yet to be fully examined, but, so far, promotion of HLA-restricted CTL response [4] by $CD4^+$ lymphocytes does not appear to be allele-specific (i.e., highly restricted by particular class II polymorphisms). Moreover, the absence of an apparent effect of class II homozygosity, in contrast with the strong, consistent effect of class I homozygosity in these and other cohorts [25], might be taken to imply that neither diversity nor specificity

in the class II pathway is as critical as in class I for controlling HIV-1 infection.

Differential association of individual class I HLA markers with disease progression most likely signifies that different HLA molecules more or less effectively capture, bind, or display the HIV-1 peptides recognized by CTL. These peptides may occur in higher frequency in HIV-1, bind to the HLA groove with higher affinity, or interact more effectively with the available CDR3 repertoire on $CD8^+$ T cells [45, 46]. Differential down-regulation of HLA-peptide complex production by HIV-1–infected antigen-presenting cells [47] or differential expression on their surface may be HLA allele-specific. Furthermore, differential transport of peptides across the endoplasmic reticulum membrane or transfer of peptides to HLA molecules by different TAP alleles may selectively modulate formation, release, or migration of HLA-peptide complexes from the endoplasmic reticulum. As for class I homozygosity, the narrower spectrum of available class I HLA alleles presumably reduces options for CTL restriction in response to antigens generated by an evolving virus. In addition to the induction of cytotoxicity by polymorphic protein products of class I alleles, natural killer cell signaling must also be considered as a possible part of the explanation for involvement of class I molecules in HIV-1 pathogenesis [48].

Genes encoding polymorphisms in CCR5, CCR2b, and SDF-1, residing on different chromosomes from the one carrying the HLA region and mediating different immune mechanisms [16, 19–22], did not account for the HLA effects observed here. In our seroconverter cohorts, the HP and the receptor variants appeared to exert independent but not joint influence on the course of disease; however, further analysis of the strength, timing, and other aspects of their separate influence and their potential interaction is required.

In summary, relatively consistent and independent associations with both time to clinical AIDS and $CD4^+$ cell loss in 3 prospective seroconverter cohorts and with different rates of decline in $CD4^+$ cells in 2 studies of seroprevalents corroborate the previously reported strong influence of specificity and diversity in alleles of genes in the HLA region on disease progression. Replication of several associations in enough different settings in table 1 now suggests that they are at least surrogate predictors if not causal determinants of the highly variable course of HIV-1 infection. In analysis of other determinants of outcome, especially outside rigorously structured randomized clinical trials, adjustment for strong HLA effects is warranted. Beyond extending and refining the approaches taken here, work should now converge on the genetics and biology of the involved genes, on their mechanisms for mediating immune response to HIV-1, and on strategies for driving that response toward protection.

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Note added in proof. Since the acceptance of this manuscript, another group of investigators [Carrington M, Nelson G, Martin MP, et al. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* **1999**;283:748–52] has reported that, in addition to the influence of specific class I markers, homozygosity at the A, B, and C loci is epidemiologically associated with significantly less favorable long-term outcome of HIV-1 infection.