tion analysis was performed to calculate the CTL precursor frequency (*6*).
15. Lymph node cells from B6.2.16 RAG-1–deficient mice (C57BL/6 × 129Sv, H-2^b) were labeled with CFSE and purified (*7*). The cells were analyzed by FACS (>95% B6.2.16 CD8$^+$ cells) and adoptively transferred to female RAG-1–deficient mice (*13*) and analyzed after 21 and 70 days by limiting dilution analysis (*6*).
16. A. Lalvani *et al.*, *J. Exp. Med.* **186**, 859 (1997); K. Murali-Krishna *et al.*, *Immunity* **8**, 177 (1998).
17. M. Geisberg and B. Dupont, *Int. Immunol.* **4**, 1273 (1992); D. Hamann *et al.*, *J. Exp. Med.* **186**, 1407 (1997); C. Renner *et al.*, *Immunobiology* **197**, 122 (1997).
18. Ex vivo cytolytic assays were performed on day 2.5 in vitro–activated B6.2.16 CD8$^+$ cells (activated), cells from female B6.2.16 transgenic mice (naïve), and B6.2.16 CD8$^+$ cells harvested (spleen and mixed lymph nodes) from antigen-free hosts to which activated B6.2.16 CD8$^+$ cells (4-day in vitro culture with 100 nM antigen with 10 U/ml rIL2) had been adoptively transferred (2 × 10^6) and parked for 70 days (memory). All cells were positively sorted with anti-Thy1.2 microbeads (Miltenyi Biotec), after which a sample was checked for purity by flow cytometry (>80% B6.2.16 CD8$^+$ cells). CTL assays were performed (*10*). Spontaneous lysis never exceeded 10% at maximum E:T ratio, and lysis in the absence of antigen was 1% in all E:T ratios and conditions.
19. T. Renno, H. R. Hahne, H. R. MacDonald, *J. Exp. Med.* **181**, 2283 (1995).
20. Y. Liu, R. H. Wenger, M. Zhao, P. L. Nielsen, *ibid.* **185**, 251 (1997).
21. Y.-J. Liu *et al.*, *Nature* **342**, 929 (1989); Y.-J. Liu *et al.*, *Eur. J. Immunol.* **21**, 1107 (1991); I. C. MacLennan, *Annu. Rev. Immunol.* **12**, 117 (1994); C. A. Turner, D. H. Mack, M. M. Davis, *Cell* **77**, 297 (1994); E. Stuber, M. Neurath, D. Calderhead, H. P. Fell, W. Strober, *Immunity* **2**, 507 (1995); M. F. Neurath, E. E. Max, W. Strober, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5336 (1995).
22. N. L. Letvin, *Science* **280**, 1875 (1998).
23. D. Baltimore and C. Heilman, *Sci. Am.* **279**, 98 (1998); A. McMichael, *Curr. Opin. Immunol.* **10**, 379 (1998).
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HLA and HIV-1: Heterozygote Advantage and *B*35-Cw*04* Disadvantage

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A selective advantage against infectious disease associated with increased heterozygosity at the human major histocompatibility complex [human leukocyte antigen (*HLA*) class I and class II] is believed to play a major role in maintaining the extraordinary allelic diversity of these genes. Maximum *HLA* heterozygosity of class I loci (*A*, *B*, and *C*) delayed acquired immunodeficiency syndrome (AIDS) onset among patients infected with human immunodeficiency virus–type 1 (HIV-1), whereas individuals who were homozygous for one or more loci progressed rapidly to AIDS and death. The *HLA* class I alleles *B*35* and *Cw*04* were consistently associated with rapid development of AIDS-defining conditions in Caucasians. The extended survival of 28 to 40 percent of HIV-1–infected Caucasian patients who avoided AIDS for ten or more years can be attributed to their being fully heterozygous at *HLA* class I loci, to their lacking the AIDS-associated alleles *B*35* and *Cw*04*, or to both.

HLA class I and class II loci located within the human major histocompatibility complex (MHC) comprise the most polymorphic set of genes known in humans (*1*–*3*). Products of these genes present antigenic peptide to T cells, initiating an immune response and clearance of the foreign material. Evolutionary and population studies have led to the general idea that the great diversity and even distribution of allelic frequencies observed in the class I and class II genes of the MHC (*HLA* in humans) are maintained through selective forces, such as infectious disease morbidity (*4*, *5*). The hypothesis of overdominant selection (heterozygote advantage) at the MHC proposes that individuals heterozygous at *HLA* loci are able to present a greater variety of antigenic peptides than are homozygotes, resulting in a more productive immune response to a diverse array of pathogens (*6*).

Compelling evidence for the selective maintenance of MHC diversity has come from analyses of the population distribution of *HLA* allele frequencies (*7*), the high incidence of nonsynonymous (codon altering) base substitutions among peptide-binding regions of *HLA* transcripts (*8*), persistence of numerous polymorphic MHC amino acid motifs for several million years through the emergence of multiple species (*9*), and a concordant increase in infectious disease sensitivity of species with increased MHC homozygosity (*10*). Several examples of *HLA* influence on human pathogen sensitivity have been described, particularly for interaction with malaria and hepatitis B (*11*).

The AIDS epidemic is characterized by extreme heterogeneity in the clinical course as well as in the incidence of HIV-1 infection among exposed individuals (*12*, *13*), which is probably a result of genetic variants among HIV-1 strains and of host genetic differences such as variants in chemokine and chemokine receptor structural genes (*14*, *15*). More than 50 reports examining a role for *HLA* variation in AIDS outcomes have appeared, however the reported associations have been difficult to generalize or to affirm in multiple cohorts (*12*, *16*). Potential explanations for this difficulty involve a paucity of patients, limitations in patient clinical descriptions, failure to correct for multiple comparisons, and a reliance on serological typings that can miss allele differences found by molecular typing. Nonetheless, concordant AIDS outcomes in sib pair analyses (*17*), the recurrent implication of two *HLA* haplotypes (*A1-Cw7-B8-DR3-DQ2* and *Cw4-B35-DR1-DQ1*) (*12*, *16*, *18*), plus the quasi-species pattern of HIV-1 change in infected patients (*19*) are strong indicators of *HLA* involvement in HIV pathogenesis.

We performed survival and genetic association analyses to address two hypotheses: (i) that overall or specific locus heterozygosity at the *HLA* class I loci confers relative resistance to AIDS progression and (ii) that individual alleles at the class I loci vary in their influence on progression to AIDS. *HLA* class I loci were molecularly typed with DNA from individuals enrolled in five AIDS cohorts: Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), Hemophilia Growth and Development Study (HGDS), San Francisco City Clinic Cohort (SFCC), and AIDS Linked to Intravenous Experience (ALIVE) Study (*20*, *21*). Survival analyses incorporated data derived from HIV-1–positive individuals with known dates of infection and

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tested *HLA* association with progression to three endpoints (*15*, *22*): (i) AIDS-1993, which includes HIV-1 infection plus an AIDS-defining illness, a decline of $CD4^+$ T lymphocytes to <200 cells/mm^3, or death; (ii) AIDS-1987, which includes HIV-1 infection plus an AIDS-defining illness or death; and (iii) death.

An individual who is homozygous at *HLA-A*, *HLA-B*, and *HLA-C* displays a limited variety of class I molecules available for antigen presentation to cytotoxic T lymphocytes (CTLs) relative to an individual heterozygous for each class I locus. If maximum diversity in the repertoire of antigen-presenting molecules is advantageous in prolonging time to AIDS after HIV-1 infection, then homozygosity at class I genes should associate with more rapid progression to AIDS. This idea was tested by a genotype survival analysis of 498 seroconverters (patients who had enrolled in at-risk AIDS cohort studies when their HIV-1 antibody status was negative and subsequently became HIV-1 antibody positive). The analysis used a Cox proportional hazards model (*23*) to examine the rate of progression to three AIDS endpoints in Caucasian, African American, and combined ethnic groups. The results demonstrate a highly significant association of *HLA* class I homozygosity with rapid progression to AIDS in Caucasians, African Americans, and the combined ethnic group analyses for all three AIDS endpoints (Fig. 1A and Table 1). The observation is apparent when a single *HLA* class I locus is homozygous while the other two are heterozygous (RH = 1.5 to 4.4, Table 1; Fig. 1, A to D) and becomes more pronounced when two or three *HLA* class I loci are homozygous (RH = 2.9 to 4.8, Table 1; Fig. 1, A to D).

Because *HLA* class I loci are tightly linked and occur in linkage disequilibrium in certain populations (particularly *HLA-B* and *-C*, which are separated by 100 kb on chromosome 6) (*24*), the effect of homozygosity may be due to a single class I locus, rather than to all three. However, homozygosity for *HLA-A*, *HLA-B*, or *HLA-C* each predisposed their carriers to more rapid progression to AIDS compared with heterozygous individuals at each respective locus (Fig. 1, B to D, and Table 1). Furthermore, the relatively rapid progression of individuals with any single locus homozygosity (*HLA-A*, *-B*, or *-C*) was enhanced when a second or third *HLA* class I locus was also homozygous (Fig. 1, B to D), as suggested in Fig. 1A. The data suggest that each locus contributes separately to the protective effect associated with class I heterozygosity. Three of four patients in our cohorts who progressed to AIDS-1987 in less than 12 months of HIV-1 infection were homozygous at one or more *HLA* class I loci.

To rule out the possibility that observed *HLA* class I homozygosity might be marking overall homozygosity at other AIDS-influencing loci within or outside the *HLA* region, nine short tandem repeat (STR or microsatellite) loci within a 10-Mb region encompassing *HLA* (*25*) were genotyped in these patients and tested in a Cox analysis. In addition, a group of 16 STR loci located on other human chromosomes were similarly tested in the combined cohorts. No statistically significant association [with corrections for multiple tests (*26*)] was found for any AIDS outcomes ($P \geq 0.2$) nor was any significant correlation among *HLA* homozygosity and STR homozygosity detected in these cohorts (*27*), indicating that the observed effects of *HLA* class I homozygosity on AIDS progression are due to the *HLA* class I genes or other very closely linked genes.

Although *HLA* class I heterozygosity is strongly associated with protection against AIDS, about 75% of the patients that progressed to AIDS within 4 years of HIV-1 infection were heterozygous at all three class I loci. It was of interest to determine whether individual class I alleles had an effect on progression, some of which may override the protective effect of heterozygosity at class I loci. Analysis of 330 Caucasian and 144 African American seroconverters [analyzed separately for allele and genotype distribution because of the differences in allele frequencies between the two ethnic groups (*2*, *24*)] revealed six alleles (*A29*, *B27*, *B35*, *B41*, *Cw*04*, and *Cw*12*) of 63 detected that showed significant association with disease progression (*28*). Two of these, *B*35* and *Cw*04*, were highly significant in Caucasians (*28*) (RH = 2.34, $P = 2 \times 10^{-6}$; and RH = 2.41, $P = 2 \times 10^{-7}$, respectively); this was also true when corrections for multiple tests were used ($P = 1 \times 10^{-4}$ and 1×10^{-5}, respectively).

The association of *HLA-B*35* and *HLA-*

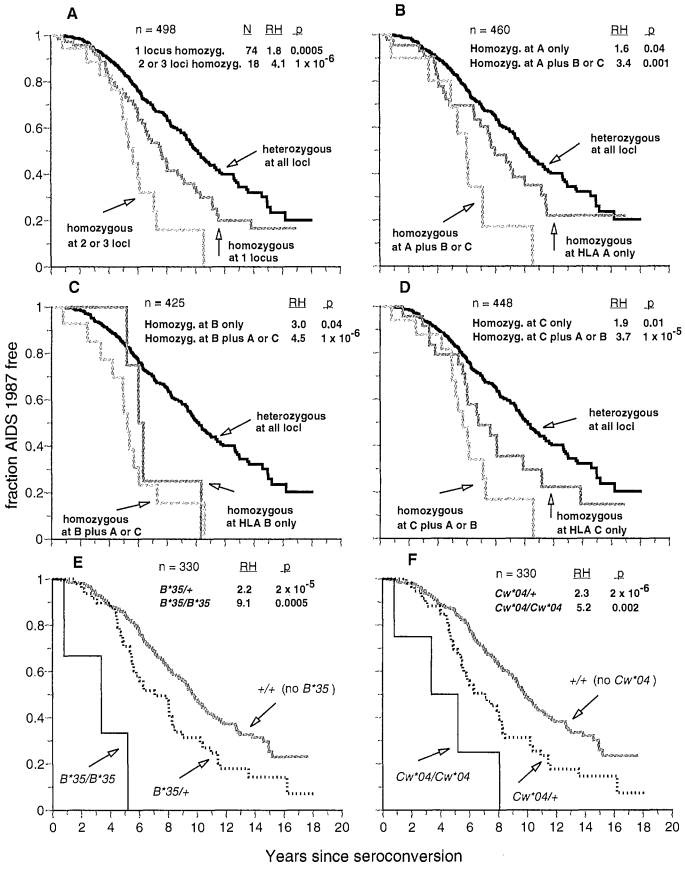

Fig. 1. Kaplan Meier survival curves for seroconverters from combined cohorts that included all ethnic groups [panels (A) to (D)] for *HLA* class I genotype association with progression to AIDS-1987. The seroconverters were study participants with a known HIV-1 antibody negative test 36 or fewer months before the first HIV-1 positive test. (**A**) Association between homozygosity at one or more class I loci and progression to AIDS-1987. Relative hazards, RH, and the corresponding *P* values calculated by the Cox proportional hazards model (*23*) are given for the singly and multiply homozygous groups compared with the completely heterozygous group (*41*). The analyses were stratified for age groups (<30, 30 to 40, and >40 years) and for race in analyses where ethnic groups were combined. Mixed racial groups were analyzed because there is no a priori reason to expect that a heterozygosity effect would differ among ethnic groups. Because the Kaplan Meier curves suggest that the relative hazards vary over time, we also applied nonparametric numerical tests (log-rank and Wilcoxon) to the Kaplan Meier analysis. The *P* values from this analysis were comparable to those obtained from the Cox model analysis. (**B** to **D**) Association between homozygosity for individual class I loci [*HLA-A* (B), *HLA-B* (C), and *HLA-C* (D)] and progression to AIDS-1987. Study participants were divided into three groups: individuals who were heterozygous at all three loci; individuals homozygous at the specified locus, but heterozygous at both of the other two loci; and individuals homozygous at the specified locus and also at one or both of the other two loci. RH and *P* values for the two groups are given as a comparison of homozygotes with heterozygotes at all three loci. Influence of *HLA-B*35* (**E**) and *HLA-Cw*04* (**F**) on progression to AIDS-1987 among combined Caucasian cohorts. Survival analysis of individuals with one or two copies of the *B*35* allele compared with individuals lacking *B*35* are shown. RH and corresponding *P* values are given for the two *B*35*-bearing genotypes (*+/B*35* and *B*35/B*35*) compared with the *B*35*-negative genotypes. Analyses for (F) were the same as those for (E) with *Cw*04* substituted for *B*35*.

*Cw*04* was analyzed for both homozygous and heterozygous influence on three AIDS endpoints among combined and separate Caucasian cohorts (Fig. 1, E and F, and Table 2). The results affirm the strong association of *HLA-B*35/+* heterozygotes (+ indicates any *HLA-B* allele except *B*35*) with rapid progression (Fig. 1E: RH = 2.2, $P = 2 \times 10^{-5}$), whereas even more rapid AIDS progression was observed among *HLA-B*35/B*35* homozygotes (Fig. 1E: RH = 9.1, $P = 5 \times 10^{-4}$). Similarly *HLA-Cw*04/+* heterozygosity promotes rapid progression (RH = 2.3, $P = 2 \times 10^{-6}$) as does *HLA-Cw*04/Cw*04* homozygosity (RH = 5.2, $P = 0.002$). Associations determined with a dominant genetic model (which combines *+/B*35* and *B*35/B*35* individuals; Table 2) were highly significant for MACS and com-

Table 1. Survival analysis of the effect of homozygosity at the three *HLA* class I loci *-A*, *-B*, and *-C* on susceptibility to progression to three AIDS endpoints (AIDS-1993, AIDS-1987, and death) for seroconverters of MACS, MHCS, SFCC, and ALIVE (*20–22*). HLA class I typing is described in (*40*). Homozygosity at one locus only and homozygosity at two or three loci were considered as covariables in a Cox model analysis (only five individuals were homozygous at *HLA-A*, *-B*, and *-C* loci, precluding a robust statistical treatment of triple homozygotes). Relative hazards (RH) and their *P* values are given for AIDS-1993, AIDS-1987, and death. There were not a sufficient number of African Americans homozygous for two or three *HLA* class I loci to provide a robust analysis. Homozygosity at each of the loci (*HLA-A*, *-B*, and *-C*) was considered as a Cox model explanatory variable. Each analysis was performed on seroconverters in combined cohorts, separately for Caucasians and African Americans, and for all ethnicities. Analyses were performed without considering specific allele protection and also as adjusted for the AIDS accelerating effects of *B*35* and *Cw*04* (*41*). RH values were similar, and adjusted values are presented here. A survival analysis was also performed after adjusting the RH values for the protective effects of *CCR5-Δ32*, *CCR2-64I*, and *SDF1-3'A* (*22*). The results (*27*) revealed no epistatic interaction of these loci with *HLA* homozygosity because the adjusted RH and *P* values were largely consistent with those reported here. Dashes indicate no data available.

AIDS outcome	N/events	Homozygous at one HLA-I locus		Homozygous at two or three HLA-I loci		Homozygous for locus					
						HLA-A		HLA-B		HLA-C	
		RH	P	RH	P	RH	P	RH	P	RH	P
					All ethnicities						
AIDS-1993	497/338	1.68	4×10^{-4}	2.91	1×10^{-4}	1.62	0.005	2.81	4×10^{-5}	1.92	3×10^{-4}
AIDS-1987	498/257	1.78	5×10^{-4}	4.07	1×10^{-6}	1.69	0.006	3.83	5×10^{-7}	2.23	5×10^{-5}
Death	498/200	1.47	0.05	3.84	2×10^{-5}	1.58	0.04	3.09	2×10^{-4}	1.96	0.002
					Caucasians						
AIDS-1993	329/252	1.56	0.01	3.38	2×10^{-5}	1.52	0.03	3.32	1×10^{-5}	2.07	4×10^{-4}
AIDS-1987	330/208	1.55	0.02	4.80	1×10^{-7}	1.71	0.009	4.48	7×10^{-8}	2.06	0.001
Death	330/167	1.39	0.13	4.33	4×10^{-6}	1.56	0.06	3.54	4×10^{-5}	2.00	0.004
					African Americans						
AIDS-1993	144/67	2.37	0.007	—	—	2.98	0.01	2.57	0.20	1.80	0.15
AIDS-1987	144/36	4.40	2×10^{-4}	—	—	2.68	0.13	1.32	0.79	3.64	0.005
Death	145/25	2.70	0.06	—	—	2.62	0.20	0.00	0.99	2.31	0.19

Table 2. Caucasian seroconverters were analyzed for MACS, MHCS, and SFCC cohorts and the combination of all cohorts. The alleles *B*35* and *Cw*04* are each considered as codominant allele variables in a Cox proportional hazards model, that is, the allele variable has a value of 0, 1, or 2 corresponding to the number of copies of the allele carried by the individual. The analysis for *B*35/+* and *B*35/B*35* considers the effect of *B*35* without taking into account the presence or absence of *Cw*04;* likewise, the analysis of *Cw*04* ignores the effect of *B*35*. Because these two alleles are in strong, positive linkage disequilibrium in Caucasians (*24*) (that is, on most chromosomes where one of these alleles is present the other is present also), an additional analysis was made for individuals carrying one of these alleles but not both. The three pairs of columns on the right give the results of analyses in which heterozygosity for *B*35* but not *Cw*04*, for *Cw*04* but not *B*35*, and for *B*35* and *Cw*04* together were considered as explanatory variables in a Cox model. Homozygotes for either or both of these alleles were excluded from this analysis (*39*). The results of a survival analysis in which the RH was adjusted by considering the protective genotypes of *CCR5*, *CCR2*, and *SDF1* (*15*) as additional covariants were virtually identical to the unadjusted RH and *P* values presented here (*27*). Failure to observe significant association for either *HLA-B*35* or *HLA-Cw*04* for MHCS and SFCC may reflect small sample size or a biased depletion of very rapid progressors to AIDS in both of these cohorts (*20, 32*), diminishing ability to observe *HLA B*35* and *Cw*04* influence on rapid progression. Dashes indicate no data available.

AIDS outcome and cohort	N/event	*B*35/+* and *B*35/B*35*		*Cw*04/+* and *Cw*04/Cw*04*		*B*35*, no *Cw*04* genotypes		*Cw*04*, no *B*35* genotypes		*Cw*04* and *B*35* genotypes	
		RH	P	RH	P	RH	P	RH	P	RH	P
AIDS-1993:											
Combined	329/252	1.83	9×10^{-5}	1.82	2×10^{-5}	1.89	0.28	1.84	0.04	1.79	0.001
MACS	195/173	1.69	0.003	1.57	0.005	16.0	3×10^{-4}	1.77	0.07	1.82	0.008
MHCS	58/34	1.28	0.60	1.67	0.26	0.0	0.99	2.29	0.43	1.54	0.37
SFCC	68/43	1.36	0.52	0.89	0.84	4.51	0.17	0.00	0.99	1.01	0.99
AIDS-1987:											
Combined	330/208	2.35	3×10^{-7}	2.29	3×10^{-8}	2.73	0.09	2.41	0.004	2.28	2×10^{-5}
MACS	196/151	2.57	1×10^{-6}	2.18	3×10^{-6}	14.5	4×10^{-4}	2.41	0.01	2.93	1×10^{-5}
MHCS	58/30	1.60	0.33	2.25	0.08	0.00	0.99	7.25	0.08	1.95	0.17
SFCC	68/26	2.06	0.22	1.35	0.65	16.3	0.03	0.00	0.99	1.58	0.49
Death:											
Combined	330/167	2.20	2×10^{-5}	2.10	5×10^{-6}	1.64	0.50	1.93	0.06	2.01	0.001
MACS	196/124	2.76	1×10^{-6}	2.12	2×10^{-5}	12.3	0.001	2.28	0.02	2.93	6×10^{-5}
MHCS	58/24	0.92	0.87	0.98	0.97	0.00	0.99	0.00	1.00	0.99	0.99
SFCC	68/18	0.81	0.78	0.95	0.95	0.00	0.99	—	—	0.92	0.91

bined analyses ($P \leq 0.003$ for all endpoints) which included considerably more individuals than the other cohorts. Caucasians having *HLA-Cw*04* also progressed more rapidly to both AIDS-1993 and AIDS-1987 ($P \leq 2 \times 10^{-5}$; Table 2).

Among Caucasians, progression to AIDS-1987 and to death was accelerated to about the same extent among individuals who were heterozygous for *HLA-B*35* alone (that is, without *HLA-Cw*04*), for *HLA-Cw*04* alone (without *HLA-B*35*), or for both alleles (Table 2). The Cox analyses revealed highly significant P values for *HLA +/B*35;+/Cw*04* double heterozygotes ($P = 2 \times 10^{-5}$) and for single *HLA +/Cw*04* (without *B*35*; $P = 0.004$) individuals to AIDS-1987, but there were too few *HLA +/B*35* (without *Cw*04*) to achieve statistical significance ($N = 4$ individuals; $P > 0.05$).

To quantify the effect of locus homozygosity and *HLA-B*35* plus *HLA-Cw*04* on accelerating disease relative to alternative genotypes, we computed the relative risk (which examines the strength of the effect) and the attributable fraction (*29*, *30*) (which estimates the fraction of individuals who progress rapidly or survive without AIDS progression because of their *HLA* genotype) for various AIDS endpoints (Table 3). The relative risk for early progression (≤6 years) to AIDS ranged from 1.3 to 2.5 for *HLA* homozygosity, for *B*35* or *Cw*04* genotype susceptibility, or for both. Conversely, full *HLA* heterozygosity for all genotypes other than those with *B*35* or *Cw*04* alleles increases the likelihood of longer survival (that is, greater than 10 years before AIDS) by a factor of 1.4 to 2.3 (Table 3).

Because of the high frequency of *HLA* class I locus homozygosity and of *B*35-* or *Cw*04-* bearing individuals (26 and 24% respectively; Table 3), there is an appreciable fraction of rapid progressors and long-term survivors that can be attributed to *HLA* genotype. More precisely, the attributable fraction for rapid progression (within 6 years of infection) to AIDS is 8 to 11% for class I homozygosity, 7 to 26% for bearers of *B*35* or *Cw*04* or both genotypes, and 19 to 32% for both. Alternatively between 28 to 40% of the study participants who avoid AIDS for 10 or more years do so because they are fully heterozygous for alleles other than *B*35* or *Cw*04* (*31*).

Our data reveal a strong association of the alleles *B*35* and *Cw*04* with accelerated AIDS progression, but it is not certain whether one or both of these alleles, or undiscovered loci in the *HLA* acting on AIDS progression, are responsible. A direct effect of *HLA* allele products on progression to AIDS is plausible, given their role in antigen presentation to T cells and the evidence indicating that CTLs play an important role in protection against HIV-1 (*12*, *32*). However, the failure of *B*35/Cw*04* to protect against AIDS progression may not reflect a simple failure to present HIV-1 epitopes because significant HIV epitope presentation has been reported for both of these alleles (*18*, *33*).

One mechanism to explain rapid progression to AIDS in individuals with certain *HLA* genotypes may involve the regulation of natural killer (NK) cell activity. A number of studies have indicated that rapid disease progression after HIV-1 infection is correlated with decreased NK cell activity (*34*). Like CTLs, NK cells are involved in surveillance and killing of foreign or infected cells through a mechanism involving *HLA* molecules (*35*). Furthermore, both *HLA* homozygosity and the *B*35-Cw*04* haplotype have been shown to be associated with reduction in NK cell number and activity (*36*). Recently, the HIV-1–encoded *nef* gene product has been shown to down-regulate *HLA* class I molecules (*37*) and perhaps protect infected cells from CTL-mediated killing (*38*). The relations among *HLA* genetic effects described here, the immunological consequences of HIV-1 *nef*, and NK cell activity are areas that merit further investigation.

The average time to AIDS after infection is about 10 years (*13*), and an effective CTL response is thought to keep the virus in check for many years in most individuals. However, HIV-1 undergoes a high rate of mutation within a single host and escape mutant isolates that evade the host immune system evolve in most individuals, resulting in AIDS (*19*). The data presented here show that maximal heterozygosity at the *HLA* class I loci slows progression to AIDS and AIDS-related death. A parsimonious explanation for this finding is that, overall, heterozygotes present a broader range of HIV-1 peptides than do homozygotes and, therefore, it takes longer for escape mutants to arise in heterozygotes than in homozygous individuals. In any case, that heterozygotes have a significantly delayed time to death provides strong support for the hypothesis of overdominant selection at the MHC class I loci and affirms the previous indications that infectious diseases play a role in selection for heterozygosity (*6*, *39*).

Table 3. Combined Caucasian cohorts were partitioned into two arbitrarily defined disease categories: (i) seroconverter patients who progress to AIDS outcome in ≤6 years after HIV-1 infection and (ii) seroconverter patients who avoid AIDS outcome for ≥10 years. Relative risk (RR) and attributable fraction (AF) were computed on the basis of established methods (*29*, *30*). Freq. susc. genotype, frequency of susceptible genotype.

Risk factor and AIDS outcome	N	Freq. susc. genotype	Rapid progression to AIDS*			Long-term survival*		
			RR	P	AF (%)	RR	P	AF (%)
HLA class I homozygosity								
AIDS-1993	320	26.3%	1.46	0.007	10.8	2.23	0.005	47.6
AIDS-1987	316	26.3%	1.37	0.15	8.9	1.61	0.02	30.8
Death	313	26.3%	1.33	0.40	8.0	1.41	0.03	23.2
*B*35*, *Cw*04* homo/heterozygotes								
AIDS-1993	320	24.0%	1.31	0.07	6.8	1.82	0.02	38.3
AIDS-1987	316	24.0%	2.18	8×10^{5}	22.0	1.70	0.009	34.7
Death	313	24.0%	2.45	0.004	25.8	1.80	0.0002	37.9
HLA-homozygotes or *B*35*, *Cw*04* genotypes								
AIDS-1993	320	46.7%	1.52	0.0008	19.4	2.27	6×10^{-5}	40.4
AIDS-1987	316	46.7%	2.02	0.0002	32.3	1.78	0.0002	29.3
Death	313	46.7%	1.93	0.03	30.2	1.73	8×10^{-6}	27.9

*For rapid progressors, the risk factors include homozygosity at one or more class I loci, the presence of at least one *B*35* or *Cw*04* allele, or both. For slow- and long-term survivors, alternative protective factors (full heterozygosity, absence of *B*35* or *Cw*04* allele, or both) were considered in computing values.

References and Notes

1. B. Dupont, Ed., *Immunobiology of HLA* (Springer-Verlag, New York, 1995); J. Klein, Ed., *Natural History of the Major Histocompatibility Complex* (Wiley, New York, 1986).
2. M. P. Baur *et al.*, in *Histocompatibility Testing*, E. Albert, J. J. Baur, W. Mayr, Eds. (Springer-Verlag, Berlin, 1984), pp. 333–341.
3. W. F. Bodmer and J. G. Bodmer, in *Mathematical Evolutionary Theory*, M. W. Feldman, Ed. (Princeton Univ. Press, Princeton, NJ, 1989), pp. 315–334.
4. P. Parham and T. Ohta, *Science* **272**, 67 (1996).
5. A. Hughes and M. Yeager, *Annu. Rev. Genet.* **32**, 415 (1998).
6. P. C. Doherty and R. M. Zinkernagel, *Nature* **256**, 50 (1975); R. M. Zinkernagel, *Science* **272**, 634 (1996).
7. P. Hedrick and G. Thomson, *Genetics* **104**, 446 (1983); P. Hedrick *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5897 (1991).
8. A. L. Hughes and M. Nei, *Nature* **335**, 167 (1988); *Proc. Natl. Acad. Sci. U.S.A.* **86**, 958 (1989).
9. N. Takahata and M. Nei, *Genetics* **124**, 967 (1990); J. Klein, *Hum. Immunol.* **19**, 155 (1987); J. Klein *et al.*, *Annu. Rev. Ecol. Syst.* **29**, 1 (1998).
10. D. Watkins, F. S. Hodi, N. L. Letvin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 771 (1988); D. T. Evans *et al.*, *J. Immunol.* **159**, 1374 (1997); S. J. O'Brien *et al.*, *Science* **227**, 1428 (1985); F. L. Black, *ibid.* **258**, 1739 (1992); _____ and P. W. Hedrick, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 12452 (1997).
11. S. C. Gilbert *et al.*, *Science* **279**, 1173 (1998); M. R.

Thursz *et al.*, *N. Engl. J. Med.* **332**, 1065 (1995); A. V. Hill *et al.*, *Nature* **352**, 595 (1991).
12. B. F. Haynes, G. Pantaleo, A. S. Fauci, *Science* **271**, 324 (1996); S. Rowland-Jones, R. Tan, A. McMichael, *Adv. Immunol.* **65**, 277 (1997).
13. R. Detels *et al.*, *J. Acquir. Immune Defic. Syndr.* **12**, 1263 (1994).
14. J. M. Coffin, *Science* **267**, 483 (1995); A. J. Leigh Brown and E. C. Holmes, *Annu. Rev. Ecol. Syst.* **25**, 127 (1994); J. M. McNicholl, D. K. Smith, S. H. Qari, T. Hodge, *Emerg. Infect. Dis.* **3**, 261 (1997).
15. M. Dean *et al.*, *Science* **273**, 1856 (1996); M. W. Smith *et al.*, *ibid.* **277**, 959 (1997); C. Winkler *et al.*, *ibid.* **279**, 389 (1998); M. Martin *et al.*, *ibid.* **282**, 1907 (1998).
16. J. J. Just, *Hum. Immunol.* **44**, 156, (1995); T. Sahmoud, *AIDS* **7**, 497 (1993); H. Shiga *et al.*, *ibid.* **10**, 1075 (1996); S. Itescu *et al.*, *J. Acquir. Immune Defic. Syndr.* **5**, 37 (1991); M. R. Klein *et al.*, *J. Infect. Dis.* **169**, 1244 (1994).
17. B. L. Kroner *et al.*, *AIDS* **9**, 275 (1995).
18. H. Tomiyama *et al.*, *J. Immunol.* **158**, 5026 (1997); S. Rowland-Jones *et al.*, *Nature Med.* **1**, 59 (1995); Erratum *ibid.* **1**, 598.
19. M. A. Nowak *et al.*, *Science* **254**, 963 (1991); L. G. Phillips *et al.*, *Nature* **354**, 453 (1991).
20. J. Phair *et al.*, *J. Acquir. Immune Defic. Syndr.* **5**, 490 (1992); J. J. Goedert *et al.*, *N. Engl. J. Med.* **321**, 1141 (1989); S. P. Buchbinder *et al.*, *AIDS* **8**, 1123 (1994); M. W. Hilgartner *et al.*, *Am. J. Pediatr. Hematol. Oncol.* **15**, 208 (1993).
21. D. Vlahov *et al.*, *NIDA Res. Monogr. Ser. 103* (Public Health Service, Alcohol and Drug Abuse Administration, Washington, DC, 1991).
22. U.S. Centers for Disease Control and Prevention. *Morb. Mortal. Wkly. Rep.* **41**, 1 (1992); *ibid.* **36** (suppl. 1), 1 (1987).
23. D. R. Cox, *J. R. Stat. Soc. B* **34**, 187 (1972); Proportional Hazard Regression, SAS Release 6,10, SAS Institute, Cary, NC.
24. D. Charron, Ed., *Genetic Diversity of HLA: Functional and Medical Implication* (EDK Medical and Scientific International, Paris, France, 1997).
25. M. P. Martin *et al.*, *Immunogenetics* **47**, 131 (1998).
26. B. S. Weir, Ed., *Genetic Data Analysis* (Sinauer, Sunderland, MA, 1990); T. Schweder and E. Spjotvoll, *Biometrika* **69**, 493 (1982).
27. Tables of supplementary data are available at www.sciencemag.org/feature/data/986310.shl and at http://rex.nci.nih.gov/RESEARCH/basic/lgd/front_page.htm.
28. The following alleles were detected in seroconverters: *HLA-A*01, 02, 03, 11, 23, 24, 25, 26, 29, 30, 31, 32, 33, 34, 36, 66, 68, 69, 74, 80; HLA-B*07, 08, 13, 14, 15, 18, 27, 35, 37, 38, 39, 40, 41, 42, 44, 45, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 67, 78, 81*; and *HLA-Cw*01, 02, 03, 04, 05, 06, 07, 08, 12, 14, 15, 16, 17*. Associations with AIDS-1987 progression were not significant ($P > 0.05$, uncorrected) except for in Caucasians *B*35* (RH = 2.34, $P = 2 \times 10^{-6}$), *Cw*04* (2.41, 2×10^{-7}), and *Cw*12* (0.61, 0.03); and in African Americans *A*29* (3.96, 0.01), *B*27* (6.86, 0.01), and *B*41* (3.89, 0.03). All associations except *B*35* and *Cw*04* were not significant ($P > 0.3$) after correction for multiple comparisons. Neither *B*35* nor *Cw*04* displayed significant acceleration to AIDS endpoints among 144 African Americans. Although this failure may involve smaller sample size or the fact that the principal African American cohort, ALIVE, is younger and may not include sufficient AIDS cases ($N = 37$) (*21*), it is possible that the differential effect represents either different *B*35* or *Cw*04* alleles represented by African compared with Caucasian alleles or ethnic group differences in linkage disequilibrium for the B and C alleles. For example, *Cw*04* and *B*53* are associated by strong linkage disequilibrium in African Americans, whereas *Cw*04* and *B*35* are associated in Caucasians. Resolution of this discrepancy in examined African or African American AIDS cohorts may help resolve this question.
29. M. L. Levin, *Acta Unio Int. Contra Cancrum* **9**, 531 (1953).
30. M. J. Khoury, T. H. Beaty, B. H. Cohen, *Fundamental of Genetic Epidemiology. Monographs in Epidemiology and Biostatistics* (Oxford Univ. Press, New York, 1993).
31. Because two Caucasian cohorts, MHCS and SFCC, are nonrandomly depleted of rapid progressors [S. M. Donfield, H. S. Lynn, M. W. Hilgartner, *Science* **280**, 1819 (1998); M. W. Smith, M. Dean, M. Carrington, C. Winkler, S. J. O'Brien, *ibid.*, p. 1819; M. W. Smith *et al.*, *Nature Med.* **3**, 1052 (1997)] and because ALIVE is 94% African American (*15*, *21*), we also computed the attributable fraction (AF) for surviving 10 or more years AIDS free from the 197 Caucasian MACS seroconverters that have no such bias in ethnic or survival representation. This analysis indicated a higher attributable fraction for *HLA* class I homozygosity (AF = 70%, 47%, and 37% for AIDS-1993, AIDS-1987, and death, respectively), for the sum of *B*35/Cw*04* sensitive genotypes (AF = 61%, 62%, and 66%, respectively) and for HLA class I homozygosity plus *B*35/Cw*04* sensitivity combined (AF = 62%, 50%, and 50%, respectively).
32. E. S. Rosenberg *et al.*, *Science* **278**, 1447 (1997); O. O. Yang *et al.*, *J. Virol.* **71**, 3120 (1997); F. Gotch *et al.*, *Immunol. Lett.* **51**, 125 (1996); T. Harrer *et al.*, *AIDS Res. Hum. Retrovir.* **12**, 585 (1996); J. E. Schmitz *et al.*, *Science* **283**, 857 (1999).
33. H. Shiga *et al.*, *AIDS* **10**, 1075 (1996); R. Paul-Johnson, A. Trocha, T. M. Buchanan, B. D. Walker, *J. Virol.* **67**, 438 (1993).
34. H. Bruunsgaard, C. Pedersen, P. Skinhøj, B. K. Pedersen, *Scand. J. Immunol.* **46**, 91 (1997).
35. G. Trinchieri, *Adv. Immunol.* **47**, 187 (1989); H. G. Ljunggren and K. Karre, *Immunol. Today* **11**, 237 (1990).
36. D. P. Dubey *et al.*, *J. Exp. Med.* **179**, 1193 (1994); *Eur. J. Immunol.* **17**, 61 (1987)
37. O. Schwartz *et al.*, *Nature Med.* **2**, 338 (1996).
38. K. L. Collins *et al.*, *Nature* **391**, 397 (1998).
39. M. R. Thursz, H. C. Thomas, B. M. Greenwood, A. V. S. Hill, *Nature Genet.* **17**, 11 (1997).
40. For *HLA* class I typing, genomic DNA was isolated from patients' lymphoblastoid B cell lines or from peripheral blood lymphocytes and amplified with a panel of 96 sequence specific primers (SSP-PCR) for *HLA-A*, *-B*, and *-C* [M. Bunce *et al.*, *Tissue Antigens* **46**, 355 (1995)]. Each reaction included positive control primers that amplify a 796–base pair fragment from the third intron of *HLA-DRB1*. *HLA* class I polymerase chain reaction (PCR) products were electrophoresed in 1.5% agarose gels containing ethidium bromide, and predicted size products were visualized under ultraviolet light. To resolve cryptic (to SSP technology) heterozygosity, all homozygotes were sequenced with the ABI Big Dye terminator cycle sequencing ready reaction kit. (Applied Biosystems Division/Perkin-Elmer, Foster City, CA). Primers in the first and third introns of *HLA-A*, *-B*, and *-C* [N. Cereb *et al.*, *ibid.* **45**, 1 (1995)] were used for locus-specific amplification of exons 2 and 3. The amplified product was purified in a Microcon-100 microconcentrator column (Amicon, Beverly, MA), subjected to cycle sequencing in both orientations, according to the manufacturer's protocol, followed by isopropanol precipitation. The samples were then run on an ABI 377 sequencer (Applied Biosystems Division/Perkin-Elmer), and the sequences were analyzed with the Match Tools and MT navigator allele identification software (Applied Biosystems Division/Perkin-Elmer). Sequence analysis of 125 homozygotes for class I loci revealed 17 individuals that were heterozygous for recognized nucleotide polymorphism subtypes within the type indicated by SSP typing. Sixteen of these were heterozygous at adjacent class I loci. Only homozygotes verified by sequence analysis were considered homozygous for all analyses in this report.
41. The class I alleles *B*35* and *Cw*04* were used as a covariable in the association analysis of homozygosity (Fig. 1 and Table 1), and homozygosity was used as a covariable in the analysis of allele association with progression to AIDS (Fig. 1 and Tables 1 and 3).
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Motor Cortical Encoding of Serial Order in a Context-Recall Task

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The neural encoding of serial order was studied in the motor cortex of monkeys performing a context-recall memory scanning task. Up to five visual stimuli were presented successively on a circle (list presentation phase), and then one of them (test stimulus) changed color; the monkeys had to make a single motor response toward the stimulus that immediately followed the test stimulus in the list. Correct performance in this task depends on memorization of the serial order of the stimuli during their presentation. It was found that changes in neural activity during the list presentation phase reflected the serial order of the stimuli; the effect on cell activity of the serial order of stimuli during their presentation was at least as strong as the effect of motor direction on cell activity during the execution of the motor response. This establishes the serial order of stimuli in a motor task as an important determinant of motor cortical activity during stimulus presentation and in the absence of changes in peripheral motor events, in contrast to the commonly held view of the motor cortex as just an "upper motor neuron."

Ever since Lashley's famous paper in 1951 (*1*), the imagination of psychologists and neuroscientists alike has been captured by the problem of serial order in behavior. Accurate representation of temporal order is crucial for both perceptual and motor functions (for example, comprehending a sentence, playing a musical instrument). Moreover, serial order information must often be transiently kept in working memory before being translated to motor output, as, for exam-