Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10

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IL10 is a powerful TH-2 cell cytokine produced by lymphoid cells that limits HIV-1 replication *in vivo*, ostensibly by inhibiting macrophage/monocyte and T-cell lymphocyte replication and secretion of inflammatory cytokines (IL1, TNFα, IL6, IL8, and IL12). A genetic epidemiological scan of patients enrolled in AIDS cohorts for candidate gene-linked short tandem repeat polymorphisms revealed significant genotype associations for HIV-1 infection and progression to AIDS with markers adjacent to and tracking (by linkage disequilibrium) common single nucleotide polymorphic variants in the *IL10* promoter region. Individuals carrying the *IL10-5′−592A* (*IL10-5′A*) promoter allele possibly were at increased risk for HIV-1 infection, and once infected they progressed to AIDS more rapidly than homozygotes for the alternative *IL10-5′−592 C/C* (*IL10*-+/+) genotype, particularly in the later stages of HIV-1 infection. An estimated 25–30% of long-term nonprogressors (who avoid clinical AIDS for 10 or more years after HIV-1 infection) can be attributed to their *IL10*-+/+ promoter genotype. Alternative *IL10* promoter alleles are functionally distinct in relative IL10 production, in retention of an avian erythroblastosis virus transcription factor recognition sequence and in binding to specific putative nuclear transcription factors, suggesting a potential mechanism whereby *IL10-5′A* down-regulation of inhibitory IL10 facilitates HIV-1 replication *in vivo*, accelerating the onset of AIDS.

IL10 promoter variant (*IL10-5′A*)

Allelic variants in the human genome are likely to regulate susceptibility or resistance to HIV-1 infection and disease progression (1–3). A hope to resolve the etiology of HIV-1 infection and the mechanisms of disease outcome has prompted a search for genetic variants in gene candidates thought to play a role in HIV-1 disease. Genetic epidemiologic analysis of AIDS cohorts has to date implicated at least eight human loci whose alleles exert differential influence on HIV infection and/or AIDS pathogenesis among infected individuals: chemokine (c-c) receptor (*CCR*)*5-Δ32, CCR5-P1, CCR2-64I,* stromal cell-derived factor (*SDF*)*1-3′A,* mannose-binding lectin (*MBL*), and *HLA-A, -B,* and *-C* (4–13).

To detect additional genetic effects of polymorphic variants in genes whose products have been implicated in HIV-1 infection and AIDS progression, we identified 17 candidate loci with 19 closely linked short tandem repeat (STR) polymorphisms (also called microsatellites) (Table 1). Distortions in allele frequency, genotype frequency, and Hardy–Weinberg equilibrium (the tendency for genotype frequencies to occur according to a polynomial distribution) among clinical subdivisions of AIDS cohorts were measured as indicators for association with HIV-1 infection and with the rate of progression to AIDS. STRs are typically (but not always) located outside the coding region of functional genes. Because they are abundant (over 100,000 in the human genome) and nearly randomly

Table 1. Genotype association analysis for alleles of STR loci adjacent to HIV-1/AIDS candidate genes

Locus (chromosome)	Infection *P* value (df)	Progression (AIDS-1993) *P* value (allele)
IL10 (1q)*	**0.03** (10)	0.17 (157)
IL10 (1q)*	0.43 (5)	**0.003** (283)
IL1A (2q)	0.65 (10)	0.14 (327)
IL1RA (2q)	0.39 (10)	0.15 (93)
CCR2/CCR5 (3p)*	0.07 (3)	**0.05** (214)
CCR2/CCR5 (3p)*	0.06 (4)	**0.0009** (197)
STRL33 (3p)*	0.18 (6)	0.05 (137)
IL5RA (3p)	0.33 (10)	0.48 (251)
IL2 (4q)	0.44 (14)	0.11 (202)
GC (4q)	0.36 (6)	0.14 (268)
IL9 (5q)	0.97 (11)	0.21 (425)
TBP (6q)	0.07 (13)	0.17 (183)
TNFB (6p)	0.28 (1)	0.19 (162)
IFNA (9p)	0.69 (6)	0.34 (145)
CD4 (12p)	0.27 (8)	0.10 (226)
IFNG (12q)	0.82 (8)	0.19 (189)
ERBAL2 (19p)	0.38 (11)	0.13 (183)
IL2RB (22q)	0.33 (11)	0.53 (285)
PFC (Xp)	0.10 (12)	0.41 (240)

Analyses of HIV-1-infected ($n = 420$) seroconverters and antibody-negative individuals ($n = 90$) were performed for Caucasian Americans (*P* values of allele frequency differences are shown). AIDS progression tests were performed for 410 Caucasian seroconverters for the AIDS-1993 (14) outcome by Cox analyses (15, 16). Homozygotes and heterozygotes were analyzed separately for alleles with frequencies >0.25 and were combined when the frequencies were >0.03 by stepwise relative hazard regression. STR allele names reflect the molecular size in nucleotide base pairs of the allelic PCR segment. When *P* values were corrected (17) by the number of tests performed at that locus for AIDS-1993, *IL10* was $P' = 0.02$, whereas *CCR2/5* $P' = 0.20$ and $P' = 0.005$. A step-down Holm–Sidak correction (17–19) for the 19 loci as a family of tests produced significant association of *CCR2/5*-linked STRs ($P' = 0.016$) and *IL10−R(−3975)* ($P' = 0.054$). A full version of this table, contingency tables of the infection tests, and full results of Cox analyses for progression tests are listed at http://lgd.nci.nih.gov.

*IL10, CCR2/CCR5, and STRL33 were analyzed with IL10−3975-R, IL10−1140-G, AFMB362WB9, GAAT12D11, and D3S2354, respectively.

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Abbreviations: CCR, chemokine (c-c) receptor; STR, short tandem repeat; SNP, single nucleotide polymorphism; RH, relative hazard; ETS, avian erythroblastosis virus.

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distributed, they are frequently informative in linkage mapping and population association studies (20–22). The STR association approach is based on the presumption that genetic associations of AIDS outcomes with STR alleles/genotypes mark mutational variants in adjacent functional genes that are carried on nonrandom haplotypes defined by linkage disequilibrium with STR alleles (23–26). STR genotype frequencies of HIV-1-infected patients were compared to those with documented high HIV-1 exposure who had not become infected. In addition, all prospective recessive and dominant STR genotypes were tested for association with differential rates of progression to AIDS by using a Cox proportional hazards model (15, 16).

Materials and Methods

Study Population and Outcomes. The study group includes 848 seroconverters, 1,863 seroprevalents and 627 seronegatives, for a total of 3,337 (Caucasian, 2,208; African American, 937; Hispanic, 153; and Asian, 47, including 31 Asians not in AIDS cohorts) from 5 AIDS cohorts, AIDS Link to the Intravenous Experience, Hemophilia Growth and Development Study, Multicenter Hemophiliac Cohort Study, Multicenter AIDS Cohort Study, and San Francisco City Clinic Study (27–31). Seroconversion date was estimated as the midpoint between the first positive HIV-1 antibody test date and the last negative antibody test. HIV-1-infected San Francisco City Clinic Study patients ($n = 90$) were considered seroprevalent because they had no previous HIV-1-antibody negative visit, and also because this cohort has a disproportionate number of slow/longer-term progressors to AIDS (32–34). Four separate end points reflecting advancing AIDS morbidity were considered: (*i*) CD4 $<$ 200 cells/mm^3, (*ii*) AIDS-1993, the Centers for Disease Control and Prevention 1993 definition of AIDS (14) (HIV-1 infected plus AIDS-defining illness, decline of CD4 T lymphocytes to $<$200), or death, (*iii*) the more stringent AIDS-1987 definition (35) (HIV-1-infected plus AIDS-defining illness) or death, and (*iv*) death during followup for an HIV-1-infected patient. Time to end points was calculated from seroconversion date. The possibility that the AIDS acceleration effects associated

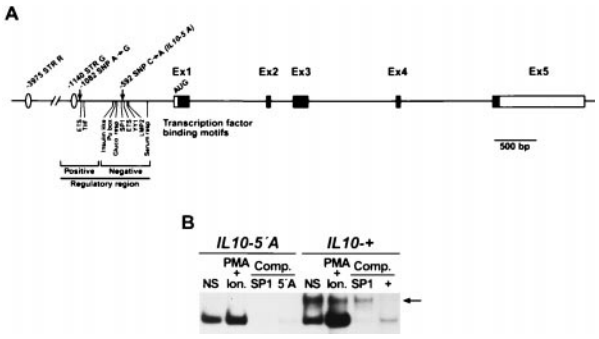

Fig. 1. Map of the human *IL10* gene on chromosome 1q31-32 (38–40). Exons, AUG start, untranslated regions, and SNP and STR polymorphisms are shown along with putative transcriptional factor-binding sites identified by sequence homology (41) in relation to negative and positive regulatory elements. (*B*) Differential DNA-protein binding between synthesized oligonucleotide probes specific for *IL10-5′A* vs. *IL10-+*. Synthetic oligonucleotides 25 base pairs around position *IL10-592* were incubated with nuclear extracts of peripheral blood mononuclear cells from random human donors and tested in an electrophoretic mobility shift assay for specific DNA-binding recognition factors (see text). Both allele nucleotides, *IL10-+* and *IL10-5′A*, resolve the faster-migrating SP-1 complex, but only *IL10-+* specifically binds the slower-migrating complex (arrow). NS, nonstimulated; Comp, cold competition with double-stranded oligonucleotides; PMA, phorbolmyristate acetate; Ion, ionomycin. Comparable results were obtained in duplicate analyses with three human donors of nuclear extracts.

Table 2. Analysis for *IL10* polymorphic sites (STRs and SNPs) and haplotype association* with HIV-1 infection and/or AIDS progression†

			HIV-1 infection‡		Progression to AIDS outcomes							
						CD4$<$200		AIDS-1993		AIDS-1987		Death	
IL10 locus	Allele (model)	Distance from AUG, kb	No. of individuals	*P* value	No. of individuals	RH	*P*	RH	*P*	RH	*P*	RH	*P*
STR−3975-R	283 (rec)	4.0	507	0.43	379	1.47	**0.007**	1.46	**0.003**	1.45	**0.009**	1.46	**0.02**
STR−1140-G	169 (dom)	1.1	506	**0.03**	379	1.25	0.26	1.18	0.36	1.44	0.06	1.72	**0.009**
SNP−1082	G (rec)§	1.1	879	0.50	419	0.89	0.18	0.85	**0.03**	0.78	**0.008**	0.77	0.39
SNP−592-5′A	5′A (dom)¶	0.6	1008	0.48‡	514	1.27	**0.05**	1.44	**0.0009**	1.51	**0.001**	1.40	**0.02**

*Genotypes from the STR loci were tested for infection and progression to AIDS considering each locus as a family of tests. The *P* values for STR *IL10-R(−3975)* were significant after Bonferroni correction for the number of alleles at each of these STR loci. Significant associations indicated by boldface were observed for HIV-1 infection (*STR−1140-G*) and for progression to AIDS. The association of the STR *IL10-R(−3975)* with disease progression is the result of a strong linkage disequilibrium between *IL10-R(−3975)* allele *283* (Fig. 2*A*) and the *IL10-5′A* promoter allele. Thus, a haplotype survey of 1,698 human chromosomes (Caucasian), which excluded ambiguous double heterozygotes, showed that 94% of *IL10-5′A*-bearing chromosomes carry an *IL10-R(−3975)283* allele, a significant departure from random expectation for association of included alleles in that haplotype (68%; $P < 0.0001$). The *STR-G(−1140)* is also in strong linkage disequilibrium ($P \leq 0.0001$) with *IL10-5′A* and tracks its effects as well. That the significant signals with *STR−3975-R, STR−1140-G*, and *IL10−592-5′A* follow different genetic models, recessive and dominant, is likely because of incomplete linkage disequilibrium between these polymorphisms.

†The AIDS-delaying influence of *IL10(−1082)-G* SNP was apparent by considering a recessive model where *IL10-(−1082)-G/G* homozygotes were compared with other genotypes in a Cox analysis.

‡The AIDS-delaying influence of *IL10-(−592)-5′A* was observed by considering a dominant model where *IL10-(−592)-+/5′A* and *5′A/5′A* were compared to *IL10(−592)-+/+* genotypes (see Fig. 2 *B–D*).

§The two SNP loci, *IL10(−1082)* and *IL10(−592)*, both show an effect on AIDS progression and are in strong linkage disequilibrium with each other. Thus, in a sample of 3,626 Caucasian chromosomes, three *[−1082 −592]* genotype combinations were never observed: *[G.+]/[G.5′A], [+.G]/[G.5′A]*, and *[G.5′A]/[G.5′A]*, because of the complete absence of the *[G.5′A]* haplotype, as previously reported (42–45).

To determine whether AIDS protection was determined by recessive protection of *IL10−1082-G* or by the dominant susceptible influence of *IL10−592-5′A*, we compared three haplotype groups in Cox relative hazard model analyses: (*i*) those who retained one or two copies of *IL10−592-5′A* (*ii*) those who were homozygous for *IL10−1082-G*; and (*iii*) other patients who contained neither *IL10−592-5′A* nor *IL10−1082G/G* genotypes (referent group). The significant epidemiologic signals were observed with the *IL10−592-5′A*-bearing genotypes (group i; RH = 1.22–1.48, $P = 0.24–0.01$), but not with the *IL10−1082-G/G* homozygotes who lack *IL10−592-5′A* (group ii; RH = 0.66–0.92, $P = 0.71–0.07$) when compared to the referent group. The above three-haplotype-stratified analysis is similar to those used in very complex haplotype analyses (46) but much simpler and implicates *IL10−592-5′A* as the operative SNP in the epidemiologic effects on AIDS progression.

¶The failure to reveal an infection effect of *IL10-5′A* in the face of association with a smaller group of rigorously defined (47) high-risk exposed uninfected patients ($n = 72$; see text), could be because of the difficulty of assessing the extent of HIV-1 exposure in the larger group of HIV-1 antibody negative study participants ($n = 631$ in this table).

with the *IL10-5′A*-bearing genotypes might reflect differences in survival because of better treatments late in the epidemic is unlikely, because clinical data used here were collected from 1978 to 1996, before wide use of highly active antiretroviral therapy (HAART). DNAs were extracted from immortal lymphoblastoid B cell lines established for each patient.

Genotype Assessment. A screen for new polymorphisms within the five coding exons of IL10 was undertaken by dHPLC and sequence analysis (36) of 45 Caucasians and 45 African Americans.

No additional coding single nucleotide polymorphisms (SNPs) were detected in the screens. Primers for SNP polymorphisms were: IL10-SNP−592 site (311 base pairs), IL10-SNP−592-A: 5′-TACTCTTACCCACTTCCCCC-3′ and IL10-SNP−592-Z: 5′-TGAGAAATAATTGGGTCCCC-3′; IL10-SNP−1082 site (193 base pairs), IL10-SNP−1082-A: 5′-CACTACTAAGGCTCCTTTGGG-3′ and IL10-SNP−1082-Z: 5′-CCTGGATTAAATTGGCCTT-3′. Primers for STR polymorphisms were: IL10-G site, IL10-G-A: 5′-CAACCCAACTGGCTCCC-3′ and IL10-G-Z: 5′-ATGGAGGCTGGATAGGAGGT-3′; and IL10-R site, IL10-R-A: 5′-CCCTCCAAAATCTATTTGCATA-3′ and IL10-R-Z: 5′-CTCATCAAGAAGCCCAAAGC-3′.

Electrophoretic Mobility-Shift Assay. Nuclear extracts were prepared as described previously (37).

Single-stranded oligonucleotides were commercially synthesized (Life Technologies, Rockville, MD) to span −604 to −581 of the *IL-10* promoter as follows: 5′-GACCCCGCCTGT**C**CTGTAGGAAGC-3′ (*IL10-5′C*); and 5′-GACCCCGCCTGT**A**CTGTAGGAAGC-3′ (*IL10-5′A*). The SP-1-binding site in HIV-1 LTR was GGGAGGCGTGGCCTGGGCGGACTGGGGAGTGGCGA. Detailed methods are available at lgd.nci.nih.gov.

Results and Discussion

Candidate Gene Screening with STRs. One STR, *IL10-G(−1140)*, a CA repeat 1 kb upstream of the *IL10* gene (Fig. 1), showed a weak association with sensitivity to HIV-1 infection ($P = 0.03$, 10 df for 11 allele locus), whereas all other loci showed no significant associations with infection (Table 1); a summary of *IL10*-linked STR association with HIV-1 infection is in Table 2. For disease progression rate association tests, four loci revealed significant associations (*IL10-R−3975*, $P = 0.003$; and three STRs within 1 cM of *CCR5* and *CCR2*, ref. 18, AFMB362WB9, $P = 0.05$; GAAT12D11, $P = 0.0009$; and STRL-33, $P = 0.05$). On correction for multiple tests (17–19, 48, 49), *IL10-R−3975* and *GAAT12D1* retained statistical significance (Table 1). A survival analysis illustrating the association of *IL10-R(−3975)-283/283* homozygosity with relatively rapid progression to AIDS is presented in Fig. 2*A*.

The observed infection and disease progression association with STRs tightly linked to *CCR5* (Table 1) was not unexpected given previously described *CCR5-Δ32* genetic restriction on HIV-1 infection and progression plus the strong linkage disequilibrium of the two adjacent STRs, *AFMB362wB9* and *GATT12D11* (4, 12, 50), but an *IL10* effect was not anticipated. The *IL10*-associated signal with linked STRs for both HIV-1 infection and the rate of AIDS progression among infected patients (Table 1; Fig. 2*A*) was studied further by screening for new and previously identified SNPs within the coding exons and promoter region (42, 38, 51) of the *IL10* gene (Fig. 1*A*).

IL10 SNPs. No variants were observed in a dHPLC and sequencing screen of 90 individuals across five exons (Fig. 1*A*); however, three single nucleotide variants were detected within the upstream promoter region: an A–G transition at position −1082, a C–T transition at position −819, and a C–A transversion of

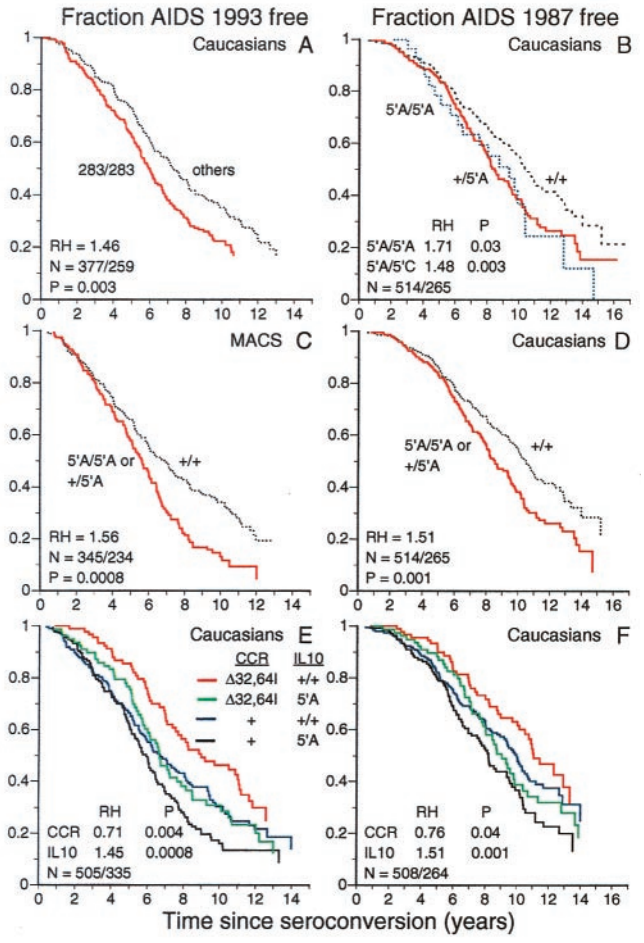

Fig. 2. Survival analysis of STR and SNP variants of *IL10* in AIDS cohorts. Number of patients and number of events (*n*), *P* value (*P*) and RH based on the Cox proportional hazards model are given. (*A*) Individuals homozygous for *IL10-R(−3975)−283/283* compared to those bearing any other STR allele genotypes in progression to AIDS-1993 among Caucasians. (*B*) Kaplan–Meier survival curves demonstrating time to AIDS-1987 were examined from combined Caucasian cohorts based on *IL10-5′−592A* (abbreviated *IL10-5′A*), genotypes: *IL10-+/+*; *IL10-+/5′A*, and *IL10-5′A/5′A*. (*C*) Dominant model *IL10-+/+* vs. *IL10-+/5′A* plus *5′A/5′A* tested in Multicenter AIDS Cohort Study cohort for time to AIDS-1993. (*D*) Same as *C* for combined Caucasian cohorts to AIDS-1987. (*E* and *F*) Kaplan–Meier survival curve demonstrating the interactive influence of dominant susceptible *IL10-5′A*-bearing genotypes and *CCR2-64I*- or *CCR5-Δ32*-bearing genotype dependence of time to AIDS-1993 (*E*) and AIDS-1987 (*F*) in combined Caucasian cohorts. Numbers of patients/AIDS events, *P* value (*P*) and RH based on the Cox proportional hazards model are given. Cox models are based on combined analysis that considered *IL10-5′A* and *CCR5-Δ32/CCR2-64I* effects together. RH and *P* values represent analyses where *CCR5/2* protective genotypes are assessed in a Cox model with *IL10* genotypes treated as covariables and vice versa.

position −592 measured from the transcription start site (designated *IL10-5′−592A* and abbreviated *IL10-5′A*; Fig. 1*A*). Because sites −819 and −592 were found to occur in complete linkage disequilibrium association with each other (see also refs. 42, 43), we genotyped two *IL10* promoter SNP variants, positions −592 (tracking −819) and −1082, for association with HIV-1 infection or disease progression. These two variants were particularly interesting, because several previous reports have demonstrated quantitative difference in *IL10* transcription and/or expression determined by alternative alleles in stimulated peripheral blood mononuclear cells (42, 44, 45, 51). The less common variants at both SNP sites showed significant associa-

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Table 3. Survival analysis for progression to three AIDS outcomes among seroconverters as a function of *IL10-5′A*/*5′A* or +/*5′A* vs. *IL10*-+/+ genotype

	Time* interval, years	AIDS-1993					AIDS-1987					Death				
		n/event	RHu	*P* value	adjRH†	*P* value	*n*/event	RHu	*P* value	adjRH†	*P* value	*n*/event	RHu	*P* value	adjRH†	*P* value
All races	All	766/462	1.28	**0.01**	1.37	**0.002**	769/332	1.43	**0.002**	1.44	**0.002**	769/253	1.32	**0.03**	1.32	**0.03**
	0–5		1.06	0.66	1.17	0.26		1.26	0.24	1.25	0.27		1.04	0.86	1.05	0.82
	>5		1.54	**0.002**	1.58	**0.0009**		1.52	**0.003**	1.54	**0.002**		1.49	**0.01**	1.48	**0.02**
Caucasians	All	511/336	1.44	**0.0009**	1.45	**0.0008**	514/265	1.51	**0.001**	1.51	**0.001**	514/213	1.40	**0.02**	1.41	**0.02**
	0–5		1.23	0.21	1.27	0.15		1.21	0.42	1.26	0.33		1.05	0.85	1.09	0.75
	>5		1.63	**0.0009**	1.61	**0.001**		1.65	**0.0007**	1.63	**0.001**		1.57	**0.007**	1.56	**0.008**
Multicenter	All	345/234	1.56	**0.0008**	1.57	**0.0007**	348/186	1.52	**0.005**	1.53	**0.004**	348/147	1.34	**0.08**	1.36	**0.06**
AIDS Cohort	0–5		1.28	0.17	1.30	0.15		1.19	0.49	1.22	0.43		1.05	0.87	1.07	0.81
Study	>5		1.96	**0.0005**	1.96	**0.0005**		1.74	**0.003**	1.74	**0.003**		1.55	**0.04**	1.57	**0.03**
Multicenter	All	157/100	1.34	0.17	1.39	0.13	157/78	1.49	0.10	1.56	0.10	157/65	1.46	0.15	1.45	0.17
Hemophilia	0–5		0.97	0.95	1.15	0.75		1.15	0.83	1.39	0.62		0.95	0.95	1.33	0.74
Cohort Study	>5		1.48	0.11	1.50	0.10		1.56	0.09	1.54	0.10		1.53	0.13	1.49	0.16
African	All	220/102	0.92	0.69	1.22	0.42	220/53	1.08	0.81	1.12	0.73	220/31	1.01	0.81	1.07	0.87
Americans‡	0–5		0.74	0.24	0.93	0.80		1.48	0.37	1.31	0.57		1.02	0.96	0.96	0.94
	>5		1.42	0.37	1.93	0.13		0.77	0.54	0.96	0.93		1.25	0.74	1.32	0.69

Statistical analysis. Cox models (15, 16) were used for calculating relative hazards and *P* values for separate and combined cohorts and racial groups, as we have previously reported (5, 6). Nonsignificant 2- and 3-year windows in the initial infection period were grouped together and later times considered separately in further Cox analyses of AIDS outcomes (14, 35). A strict and conservative Bonferroni correction of the 120 *P* values presented would mean that only those of $P \leq 0.00042$ are significant, which is coincidentally equivalent to the smallest observed *P* value ($P = 0.0005$; Multicenter AIDS Cohort Study, AIDS-1993 >5-year portion of the analysis). Because the tests were not independent, the observed statistical associations seem robust. Eighteen of the AIDS progression *P* values are lower than 0.002 (some by an order of magnitude), and overall, 45 are $P \leq 0.05$ and 30 are $P \leq 0.01$.

*Time was not taken into account in all models and was included as an interaction term by separating 0–5 and >5 years in the models (except death, where 0–6 and >6 years was used).

†Relative hazards were shown as RHu from unadjusted models and as adjRH from adjusted models, in which the effects of the previously described *CCR5-Δ32* and *CCR2-64I* genotypes were taken into account. The effects of *IL10* and *CCR* (*CCR5-Δ32* and *CCR2-64I*) appear additive, because, in the Cox analyses, interaction terms were not significant ($P = 0.53–0.87$) for the four outcomes in Caucasians.

‡*IL10-5′A* AIDS accelerating effects were most apparent in Caucasian and mixed ethnic group analyses. The less significant *P* values in African Americans are likely a consequence of fewer patients ($n = 220$ seroconverters), fewer AIDS cases after 5 years of infection (15 AIDS-1987 and 5 AIDS deaths), and the relative recency of the recruitment of the AIDS Link to the Intravenous Experience cohort, which comprises 70% of the African American seroconverters (5, 27). This interpretation is supported by high relative hazards (at >5 years); RH = 3.41 and 1.93 for CD4 < 200 and AIDS-1993, respectively, the two earliest AIDS end points.

tions with one or more AIDS outcomes by using four endpoint AIDS definitions (14, 35) (Table 2). Alleles at the four loci (two STRs and two SNPs, each within 4,000 base pairs on chromosome 1q31-32) were also found to be in strong linkage disequilibrium with each other, and haplotype analysis narrowed the focus of the genetic epidemiological association signal to the SNP allele at position −592, termed *IL10-5′A* (Table 2 legend).

The *IL10-5′A* Variant Accelerates Progression to AIDS in Late Infection. The allele frequencies of *IL10-5′A* in four ethnic groups were as follows: Caucasians, 0.236 ($n = 2{,}208$ study participants); African Americans, 0.400 ($n = 937$); Hispanics, 0.327 ($n = 153$); and Asians, 0.600 ($n = 47$). Allele frequencies of the most common or wild-type allele (*IL10-5′−592C*, abbreviated *IL10*-+ here) is equal to 1 minus the *IL10-5′A* frequencies for each ethnic group. An influence of the *IL10-5′A* on HIV-1 infection was seen in a categorical analysis of allele and genotype frequency distributions among 377 HIV-1-infected seroconverter patients plus a group of 72 high-risk exposed uninfected (HREU) individuals (those with extremely high-risk sexual practices) (12, 13, 47) but was not seen in a comparison to a collection of all uninfected individuals (Table 2). (We selected the well-characterized Multicenter AIDS Cohort Study seroconverters to avoid the previously described frailty bias of certain cohorts and of seroprevalents that are nonrandomly depleted of very rapid progressors to AIDS) (32, 33). A diminution in *IL10-5′A* alleles ($P = 0.04$) and *IL10-5′A*-bearing genotypes in HREU (31.0% among HREU individuals compared to 45.1% among infected patients; odds ratio = 1.75; Fisher's exact test, $P = 0.03$), suggested that the *IL10-5′A* allele was associated with increased risk for HIV-1 infection.

A role for *IL10-5′A* in disease progression among 769 HIV-1-infected seroconverters was demonstrated by survival analyses by using the Cox proportional hazards model (15, 16) (Fig. 2 *B–D*; Table 3). For combined and individual cohort analyses, heterozygous *IL10*-+/5′A and homozygous *IL10-5′A*/*5′A* patients were indistinguishable in the rates of progression to 4 AIDS end points (Fig. 2*B*). However, a highly significant acceleration to AIDS progression was apparent among HIV-infected Caucasians of genotypes *IL10-5′A*/*5′A* or *IL10*-+/*5′A* compared to *IL10*-+/+ homozygotes for every AIDS outcome ($P = 0.0009–0.05$, Table 3). This susceptible effect was also apparent when the protective effects of other AIDS restriction alleles, *CCR2-64I* and *CCR5-Δ32*, were considered as covariables in adjusted Cox analyses (Table 3).

The patterns of AIDS acceleration illustrated in Fig. 2 *B–D* suggest a greater survival difference in *IL10-5′A*-bearing genotypes in later, as compared to earlier, stages of HIV-1 infection. A formal analysis of time dependence in Table 3 applies a relative hazards model partitioned into early progressors (0–5 years after seroconversion) and late/slow progressors (≥5 years after seroconversion). A partitioned time interval approach with combined and separate cohorts is also presented. These analyses show that acceleration to AIDS associated with *IL10-5′A*-bearing genotypes was more pronounced starting approximately 5 years after HIV-1 exposure, whereas the *IL10-5′A* allele has no significant effect on AIDS progression in the initial 5 years after infection.

The tendency of *IL10-5′A*-bearing genotypes to progress to AIDS rapidly was also apparent in a defined disease category analysis (Fig. 3), which allows the addition of seroprevalent patients (those who enter the cohorts already HIV-1 positive) to the slow/nonprogressor category, because avoiding AIDS for longer

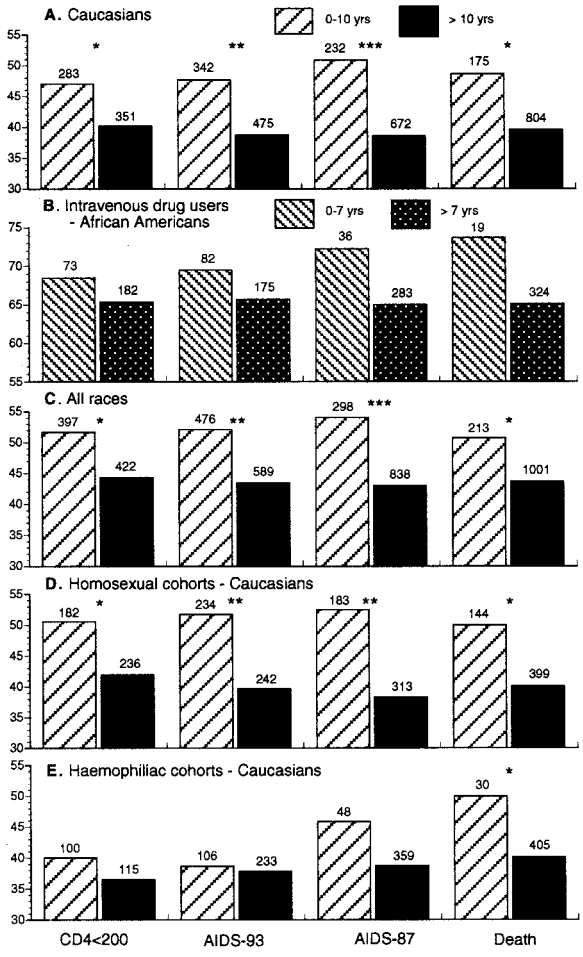

Fig. 3. Frequency of *IL10-+/5′A* or *5′A/5′A* genotypes in defined categorical analysis. Only seroconverters were included in earlier categories to avoid a survivor's frailty bias (32, 33). A 10-year cutoff in Caucasians and a 7-year cutoff in African Americans were chosen so that seroprevalents from a variety of cohorts would still be included, and failures in seroconverters before that time could be considered. The numbers above bars are the total number of patients in each category. (*A*) All Caucasians; (*B*) intravenous drug users (AIDS Link to the Intravenous Experience); (*C*) combined ethnic groups; (*D*) Homosexual cohorts (Multicenter AIDS Cohort Study and San Francisco City Clinic Study); and (*E*) hemophiliac cohorts (Multicenter Hemophiliac Cohort Study and Hemophilia Growth and Development Study). *P* values are marked as follows: *, <0.05, *, <0.01, and ***, <0.001.

periods (i.e., >10 years) is informative whether the interval of infection is precise or is greater than the time period since enrollment (4–8). The frequency of AIDS acceleration genotype *IL10-5′A/5′A* or *IL10-+/5′A* was considerably higher than *IL10-+/+* in patients who progress to AIDS 0–10 years after seroconversion vs. those who avoid AIDS for 10 or more years (Caucasian $P = 0.0007$–0.05). Replication of this effect is also apparent when different risk groups (hemophiliac, homosexual) or different ethnic categories (Caucasian American, African American) are examined separately (Fig. 3). The frequency of the dominant AIDS-accelerating *IL10-5′A* allele is common (frequency, 0.23–0.6 in various ethnic groups; see above), and 25–30% of patients who avoid AIDS for 10 years or longer do so as a consequence of their *IL10-+/+* genetic protection (attributable risk computation; refs. 52, 53). The results of both the survival (Table 3) and defined disease category analyses (Fig. 3) affirm a strong dominant *IL10-5′A* association with more rapid progression to different AIDS outcomes, particularly in the later stages of HIV-1 infection.

***IL10-5′A, CCR5-Δ32,* and *CCR2-64I* in HIV-1/AIDS.** The AIDS-accelerating effects for *IL10-5′A* were compared to the protective effects of *CCR5-Δ32* and *CCR2-64I* in delaying AIDS (4, 5, 10, 12, 13). Because the protective effects of *CCR2-64I* and *CCR5-Δ32* are dominant, genetically independent, and equivalent in their influence on AIDS progression (5), we combined *CCR5* and *CCR2* protective genotypes (*CCR5-+/Δ32*, *CCR2-+/64I*, and *CCR264I/64I*) and examined survival analyses of patients in combined cohorts for *IL10-5′A* acceleration of AIDS and for *CCR2/5* protection (Fig. 2 *E* and *F*). Separations of genotype-specific survival curves are gradual, and the strength of the *IL10-5′A* effects is equivalent to (if not slightly greater than) but in opposite directions of the *CCR2/5* effects (compare Figs. 2 *C* and *D* to 1; ref. 5). Perhaps more illuminating is the analysis of *CCR5, CCR2*, and *IL10* genotypes together (Fig. 2 *E* and *F*) where patients protected by *CCR5-Δ32* or *CCR2-64I* with the *IL10-+/+* (also protective) genotype avoid AIDS considerably longer than do *CCR5-Δ32* or *CCR2-64I* protected patients carrying the *IL10-5′A* accelerating genotypes (red vs. green lines: relative hazard (RH) = 0.62, $P = 0.02$; RH = 0.65, $P = 0.05$ for AIDS-1993 and AIDS-1987, respectively). Similarly, *IL10-5′A*-accelerating genotypes carrying wild-type (susceptible) *CCR2-+* and *CCR5-+* alleles (black lines, Fig. 2 *E* and *F*) progress more rapidly than any genotypic group including *IL10-+/+* plus *CCR5-+* or *CCR2-+* (i.e., *CCR2/5* susceptible) genotypes (black vs. blue lines: RH = 1.38, $P = 0.02$; RH = 1.47, $P = 0.01$ for AIDS-1993 and AIDS-1987, respectively). These patterns demonstrate the cumulative effects in cohort populations of these genetic influences.

***IL10-5′A* Mediates Differential Nuclear-Binding Activity.** The DNA sequence surrounding the *IL10-5′A* variant (positions −604 to −581) was examined for homology to recognized binding sites for known transcription factors (41) and was shown to include motifs specific for SP-1 and avian erythroblastosis virus (ETS) family-binding sites (Fig. 1*A*). The *IL10-+* sequence lacks the ETS motif, whereas the *IL10-5′A* retains it, posing a potential molecular mechanism for increased IL10 production in peripheral blood mononuclear cells of *IL10-+/+* homozygotes compared to *IL10-5′A/5′A* homozygotes (51). To resolve potential allele distinctions *in vitro*, DNA sequence oligonucleotides representing *IL10-+* and *IL10-5′A* (−604 to −581, approximately 11 base pairs on either side of SNP-592) were synthesized and tested for specific DNA-binding protein recognition by using the electrophoretic mobility-shift assay with nuclear extracts from phytohemagglutinin-stimulated primary human peripheral blood T lymphocytes (37). Two specific binding complexes were resolved by using *IL10-+*-specific oligonucleotides, but only one of these bound to *IL10-5′A*-specific oligonucleotides (Fig. 1*B*). Specificity for the binding was demonstrated by the fact that competitive binding of cold (100-fold excess) allele-specific oligonucleotides eliminated complex formation completely. Cold synthetic SP-1 oligonucleotide effectively competed with and eliminated the faster migrating complex, implicating SP-1 as binding both *IL10* promoter alleles. A synthetic oligonucleotide designed from the ETS core consensus sequence (41) did not compete for either complex formation, indicating that nuclear proteins other than ETS family members may be involved in the formation of the *IL10-+*-binding complex.

***IL10-5′A* Function in HIV-1/AIDS.** In sum, the genetic influence of *IL10* SNP alleles on HIV-1 infection and AIDS progression was first detected by observing epidemiologic association of two STR loci within 4 kb of the *IL10* gene [*IL10-G(−1140)* and *IL10-R(−3975)*; Fig. 1*A*] with different outcomes after HIV-1 exposure and HIV-1 infection (Table 1). *IL10-R(−3975)−283/283* homozygotes are associated with relatively rapid progression to AIDS after infection with HIV-1 (Fig. 2*A*), and this STR allele, *IL10-R(−3975)−283*, is tracking an *IL10* promoter SNP allele (*IL10-5′A*) by strong positive linkage disequilibrium in human popula-

tions. *IL10-5′A*-bearing individuals (*IL10-5′A/5′A* or *+/5′A*) also progress much more rapidly to AIDS end points than patients homozygous for the more common wild-type allele (*IL10-+/+*) (Fig. 2 *C–F*; Table 3). *IL10-5′A* may also enhance HIV-1 infection insofar as the frequency of *IL10-5′A* alleles and genotypes is greater among infected individuals than among high-risk exposed uninfected patients. The facilitation of HIV-1 disease progression by *IL10-5′A* is consistent with the possibility that differential promoter allele affinity for transcription factors (such as ETS, which is present in *IL10-5′A* but not in *IL10-+*) (Fig. 1*B*) plays a role in diminished transcription and IL10 production observed for *IL10-5′A* (44, 51). Decreased IL10 could contribute to accelerating HIV-1 replication and the appearance of late-stage X4 or T-tropic HIV-1 strains, a scenario consistent with more pronounced AIDS acceleration effects in later stages (>5 years) of infection (Table 3).

In addition to demonstrated inhibitory activity on macrophage growth and on cytokine secretion from T-helper cells (54, 55), IL10 also inhibits HIV-1 replication in macrophages (56, 57), and serum IL10 concentrations are elevated in AIDS patients, particularly in later stages of disease (58). High levels of IL10 were detected among AIDS patients with non-Hodgkin's B-lymphoma compared to HIV-1-infected individuals without lymphoma (59). Studies showing that elevated *IL10* mRNA is seen in slower progressors to AIDS (60) plus the knowledge that IL10 inhibits macrophage and HIV-1 proliferation (56, 57) all suggest that it can stem HIV-1 spread, possibly by limiting activated macrophages available for HIV-1 replication. This interpretation gains support from the observation that HIV-1-infected patients with declining CD4 cell counts also show a decline in IL10 production by peripheral blood cells (61). The data reported here suggest that the *IL10-5′A* promoter allele, which is associated with diminished IL10 production (44, 51) and failure to bind a putative nuclear transcription factor (Fig. 1*B*), can accelerate AIDS progression likely through facilitation of viral replication in infected patients. AIDS acceleration mediated by an *IL10-5′A* promoter variant offers support for targeted immunotherapeutic strategies that would retard the deadly progression to AIDS by mimicking or enhancing the natural inhibitory role of the IL10 cytokine.

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