HLA-Bw4 Homozygosity Is Associated with an Impaired CD4 T Cell Recovery after Initiation of Antiretroviral Therapy

Andri Rauch,[1,7] David Nolan,[7] Hansjakob Furrer,[1] Elizabeth McKinnon,[7] Mina John,[7] Simon Mallal,[7] Silvana Gaudieri,[7,8] Huldrych F. Günthard,[2] Patrick Schmid,[3] Manuel Battegay,[4] Bernard Hirschel,[5] Amalio Telenti,[6] Ian James,[7] and the Swiss HIV Cohort Study[a]

[1]Division of Infectious Diseases, University Hospital Bern and University of Bern, [2]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, [3]Division of Infectious Diseases, Cantonal Hospital, St. Gallen, [4]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, [5]Division of Infectious Diseases, University Hospital Geneva, and [6]Institute of Microbiology, University of Lausanne, Switzerland; and [7]Centre for Clinical Immunology and Biomedical Statistics, Murdoch University and Royal Perth Hospital, and [8]Centre for Forensic Science and School of Anatomy and Human Biology, University of Western Australia, Perth, Australia

We assessed the influence of human leukocyte antigen (HLA) alleles *HLA-Bw4* and *HLA-Bw6* on CD4 T cell recovery after starting successful combination antiretroviral therapy in 265 individuals. The median gains in the CD4 T cell count after 4 years were 258 cells/μL for *HLA-Bw4* homozygotes, 321 cells/μL for *HLA-Bw4/Bw6* heterozygotes, and 363 cells/μL for *HLA-Bw6* homozygotes ($P = .01$, compared with *HLA-Bw4* homozygotes). *HLA-Bw4* homozygosity appears to predict an impaired CD4 T cell recovery after initiation of combination antiretroviral therapy.

Human leukocyte antigen (HLA) allelic variation influences the course of HIV infection by shaping adaptive and innate immune responses [1]. HLA genetic variation is most intense in regions involved in antigen-derived peptide binding. However, HLA-B molecules can also be categorized into 2 mutually exclusive HLA-Bw4 and HLA-Bw6 variants defined by amino acid sequence variation at residues 77–83 of the HLA α-1 domain [2]. The HLA-Bw4 variant, defined primarily by the presence at residue 80 of either isoleucine ("80I") or threonine ("80T"), ligates to killer cell Ig-like receptors (KIR) expressed on natural killer cells and selected T cell subsets, whereas the HLA-Bw6 variant ("80N") has no known ligands.

HLA-Bw4 homozygosity is associated with control of the HIV load, protection from AIDS [3], and decreased risk of HIV transmission [4]. The interaction between HLA-Bw4 80I and KIR3DS1 confers protection against progression to AIDS and opportunistic infections [5, 6]. The influence of *HLA-Bw4* and *HLA-Bw6* alleles on recovery of the CD4 T cell count after commencement of combination antiretroviral therapy (cART) is unknown. The aim of this study was to investigate the impact of *HLA-Bw4* and *HLA-Bw6* alleles on CD4 T cell recovery after commencement of successful cART, with incorporation of known or potential predictors of response to cART as covariates.

Methods. Our study included antiretroviral-naive, HIV-infected individuals from the Swiss HIV Cohort Study and the Western Australian HIV Cohort Study (SHCS and WAHIV, respectively). All participants provided informed consent, including consent for genetic testing. The primary analysis was restricted to white men who had received successful, uninterrupted cART, to eliminate the potentially confounding effects of ethnicity, sex, and treatment interruptions. Thereafter, the influence of *HLA-Bw4* and *HLA-Bw6* alleles on CD4 T cell recovery was also assessed in an expanded population that included women and nonwhite participants. Successful cART was defined as treatment that yielded an HIV RNA level <400 copies/mL (i.e., the lower limit of assay detection during most of the study period) within 6 months and with which a suppressed HIV load was maintained throughout the study. Follow-up was censored at the point of the first virologic failure (HIV RNA level, ⩾400 copies/mL) or if cART was stopped for >1 month. Sequenced-based, high-resolution HLA typing was performed for all individuals, and *HLA-Bw4* and *HLA-Bw6* carriage was determined on the basis of sequence variation at residues 77–83.

Increases in the CD4 T cell count after commencement of cART were estimated by flexible piece-wise linear profiles using mixed-effects regression, with use of S-Plus software, version 7.0 (Insightful Corp). In these analyses, the individual-specific and population-average time trends in increases in the CD4 T cell count are modeled (on the square-root scale) by continuous, piece-wise linear functions, with change points at every 12 months. CD4 T cell counts at the time of cART initiation (i.e., the baseline CD4 T cell count) influence increases in the CD4 T cell count after commencement of cART [7]; therefore,

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[a] Members of the Swiss HIV Cohort Study are listed at the end of the text.

Reprints or correspondence: Dr. Andri Rauch, Klinik und Poliklinik für Infektiologie, Inselspital PKT2B, 3010 Bern, Switzerland (andri.rauch@insel.ch).

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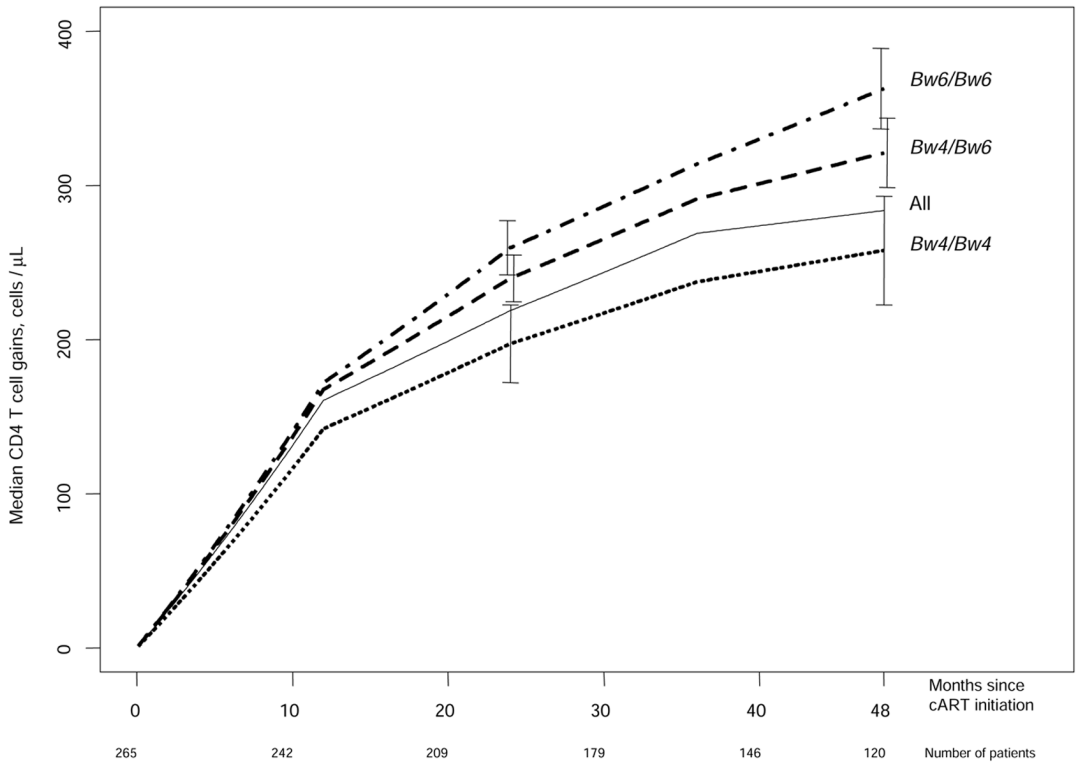

Figure 1. Estimated median increases in the CD4 T cell count after initiation of combination antiretroviral therapy (cART). Patients in the *HLA-Bw4* homozygote group had lower median increases in the CD4 T cell count than did those in the *HLA-Bw6* homozygote group ($P = .01$) after cART initiation. The number of patients refers to the number of individuals with uninterrupted and successful cART for the respective follow-up times. The changes in the CD4 T cell count from baseline were standardized to the median baseline CD4 T cell count of the combined cohort (i.e., 250 cells/μL). *Solid line,* median increases in the CD4 T cell count of 849 HIV-infected individuals (All) from the Swiss HIV Cohort Study, irrespective of *HLA-Bw4* and *HLA-Bw6* status; *bars,* SEs estimated from the mixed model.

our models allowed the slopes of the trend-line segments to depend on baseline CD4 T cell counts in addition to other covariate information (*CCR5Δ32* carriage, age, and cART regimen). For comparisons, estimated changes in the CD4 T cell count from baseline were standardized to the median baseline CD4 T cell count (i.e., 250 cells/μL).

Results. One hundred seventy-eight participants in SHCS and 87 participants in WAHIV were included in the study. The distribution of *HLA-Bw4* homozygotes *(Bw4/Bw4),* heterozygotes *(Bw4/Bw6),* and *HLA-Bw6* homozygotes *(Bw6/Bw6)* was similar among study participants (16%, 48%, and 36%, respectively) and among 1551 healthy individuals from the Western Australian Bone Marrow Registry (13%, 46%, and 40%, respectively; $P = .3$, by the χ^2 test) and did not differ significantly between SHCS and WAHIV participants.

The *HLA-Bw4* homozygote, *HLA-Bw4/Bw6* heterozygote, and *HLA-Bw6* homozygote groups did not differ significantly with regard to the median CD4 T cell count before cART initiation (248, 240, and 273 cells/μL, respectively; $P = .5$, by analysis of variance). The median HIV RNA levels at baseline were similar for the *HLA-Bw4* homozygote, *HLA-Bw4/Bw6* heterozygote, and *HLA-Bw6* homozygote groups (4.8, 4.8, and 4.9 $\log_{10}$ copies/mL, respectively). Of the 265 participants, 195 (74%) had received uninterrupted, successful cART for >2 years, and 120 (45%) had received it for >4 years (figure 1). The 3 groups did not differ with regard to the duration of follow-up ($P = .8$, by analysis of variance). A median of 12 measurements of the CD4 T cell count (interquartile range, 8–16) were analyzed per patient.

After 4 years of successful cART, the median increase in the CD4 T cell count in the *HLA-Bw4* homozygote group was 258 cells/μL, which was smaller than the increase in the *HLA-Bw6* homozygote group (363 cells/μL; $P = .01$) (table 1). The differences between the *HLA-Bw4* and *HLA-Bw6* homozygote groups remained statistically significant after adjustment for age, carriage of *CCR5Δ32*, and commencement of cART that included a protease inhibitor ($P = .02$). When we excluded individuals with the HLA alleles that are most consistently associated with protective effects (*HLA-B*57* and *HLA-B*27* [1, 8, 9]) or detrimental effects (*HLA-B*35P(x)* and *HLA-B* ho-

Table 1. Median increases in the CD4 T cell count after 2 and 4 years of successful, uninterrupted combination antiretroviral therapy.

Characteristic	No. of patients	Median increase in the CD4 T cell count (IQR[a])		*P*	
		2 Years	4 Years	Unadjusted	Adjusted[b]
All patients	265	241 (133–361)	325 (194–473)		
HLA allele group					
Bw4/Bw4	42	197 (96–311)	258 (135–397)	Reference	Reference
Bw6/Bw4	127	240 (134–359)	321 (191–469)	.10	.08
Bw6/Bw6	96	**260 (151–381)**	**363 (227–515)**	.01	.02
CCR5Δ32					
Not present	199	242 (135–362)	336 (202–487)	Reference	Reference
Present[c]	51	236 (130–356)	290 (162–436)	.34	.39
Age					
⩾40 years	119	222 (118–339)	299 (173–442)	Reference	Reference
<40 years	146	255 (147–376)	346 (214–495)	.10	.06
Protease inhibitors in regimen					
No	115	240 (133–359)	336 (205–485)	Reference	Reference
Yes	150	241 (134–361)	316 (187–462)	.86	.88
*HLA-B*57*					
Not present	237	243 (136–364)	329 (197–479)	Reference	Reference
Present	28	**220 (115–337)**	**296 (167–441)**	.06	.04
*HLA-B*27*					
Not present	244	238 (131–358)	326 (193–477)	Reference	Reference
Present	21	267 (157–390)	333 (199–484)	.77	.75
*HLA-B*35P(x)*[d]					
Not present	247	242 (135–362)	325 (191–477)	Reference	Reference
Present	18	224 (119–343)	353 (215–508)	.83	.90
HLA-B status					
Heterozygous	249	238 (131–358)	325 (192–475)	Reference	Reference
Homozygous	16	291 (178–417)	353 (217–507)	.61	.39

NOTE. The changes in the CD4 T cell count from baseline were standardized to the median baseline CD4 T cell count of the combined cohort (i.e., 250 cells/μL). Increases in the CD4 T cell count that differ significantly from the reference group are shown in boldface font. IQR, interquartile range.

[a] IQRs were estimated from the mixed model.

[b] *P* values adjusted for *CCR5Δ32*, age, and use of a protease inhibitor in the initial combination antiretroviral therapy regimen.

[c] Results of CCR5 typing were not available for 15 individuals.

[d] *HLA-B*3502, -B*3503, -B*3504,* and *-B*5301.*

mozygosity [10]) on untreated HIV infection, the lower increases in the CD4 T cell count for the *HLA-Bw4* homozygote group persisted (it was 100 cells/μL lower than that for the *HLA-Bw6* homozygote group; $P = .03$ in unadjusted analyses).

When female patients and nonwhite patients were included ($n = 368$), the median increase in the CD4 T cell count for the *HLA-Bw4* homozygote group after 4 years of successful cART was 88 cells/μL smaller than that for the *HLA-Bw6* homozygote group (unadjusted and adjusted *P* values were .04 and .06, respectively) and 86 cells/μL smaller than that for the *HLA-Bw4/Bw6* heterozygote group (unadjusted and adjusted *P* values were both .04).

Carriage of the HLA-Bw4 80T variant was associated with impaired recovery of the CD4 T cell count ($P = .04$, compared with Bw6/Bw6), whereas this effect was less pronounced for carriage of the HLA-Bw4 80I variant and was not statistically significant. Because only 9 individuals had *HLA-Bw4* homozygosity without carriage of HLA-Bw4 80T, we could not assess the relative importance of *HLA-Bw4* homozygosity versus HLA-Bw4 80T carriage. In comparison with all SHCS participants (i.e., including 849 patients who did not have HLA typing results but who had received uninterrupted, successful cART), the median increases in the CD4 T cell count in our sample ($n = 265$) were lower for the *HLA-Bw4* homozygote group and higher for the heterozygote group and the *HLA-Bw6* homozygote group (figure 1).

There was no significant difference between the SHCS and WAHIV populations in terms of trends in the increase in the CD4 T cell count ($P = .7$) or with regard to the *HLA-Bw4* effects ($P = .6$). The estimated rates of change for differential effects of *HLA-Bw4* homozygosity, compared with *HLA-Bw6* homozygosity, on recovery of the CD4 T cell count were similar in the SHCS and WAHIV populations (-0.045 vs. -0.042 square root CD4 T cell counts/month). Age was the only parameter with a significantly different effect ($P = .04$) on recovery of the CD4 T cell count between the SHCS and WAHIV cohorts.

The unexpected association of *HLA-Bw4* homozygosity with impaired recovery of the CD4 T cell count led us to assess whether the impact of *HLA-Bw4* homozygosity on CD4 T cell dynamics before commencement of cART in our population differed from that in previous reports [3]. In 95 SHCS participants with known dates of HIV seroconversion, the hazard of developing AIDS or reaching a CD4 T cell count <500 cells/μL was significantly lower in the *HLA-Bw4* homozygote group (hazard ratio, 0.36; $P = .005$, by Cox regression), confirming the protective role of *HLA-Bw4* homozygosity in untreated HIV infection.

Discussion. *HLA-Bw4* homozygosity was associated with an impaired recovery of the CD4 T cell count after commencement of cART. This contrasts with the protective effects of *HLA-Bw4* homozygosity during untreated HIV infection [3]. There was a trend towards poorer CD4 T cell recovery in patients in the *HLA-Bw4/Bw6* heterozygote group. Additional studies are warranted to determine the contribution of dominant or recessive effects of *HLA-Bw4/Bw6* on recovery of the CD4 T cell count. Carriage of *HLA-B*57,* which has been consistently associated with protective effects in untreated HIV infection [11, 12], tended to correlate with an impaired recovery of the CD4 T cell count during cART. This is in line with recent observations that described a trend toward poorer post-cART survival [13] and an impaired CD4 T cell recovery in the initial years of cART [14] in individuals carrying *HLA-B*5701.*

The reasons for the differing effects of *HLA-Bw4* and *HLA-Bw6* alleles before and after commencement of cART remain unclear. The slower decrease in the CD4 T cell count and the lower rate of AIDS-related events before commencement of cART could lead to a delay in cART initiation, and a longer duration of untreated HIV infection may cause a more profound and unrecognized depletion of T cell subsets [15]. In this case, the slower decrease in the CD4 T cell count in individuals with favorable genotypes before receipt of cART could lead to a slower recovery of the CD4 T cell count after receipt of cART. Because <20% of the study participants had known dates of HIV seroconversion, we could not assess whether patients in the *HLA-Bw4* homozygote group had been infected for a longer period. Alternatively, the unfavorable effect of *HLA-Bw4* homozygosity could point toward immunogenetic mechanisms (e.g., interactions between natural killer cells and T cells) that may play an important role in CD4 T cell homeostasis during cART. It remains to be determined whether the genetic association shown in this study can be explained by further analysis of patients' immunogenetic characteristics. Genetic variation in HLA alleles has a profound influence on the effectiveness of cellular immune responses, on viral escape and reversion, and on the impact of escape mutations on viral fitness in untreated HIV infection [1]. However, given the dramatic impact of cART on HIV replication, we anticipate that these mechanisms are not major determinants of CD4 T cell recovery and that they do not explain the influence of host genetic factors observed in our study.

Protective genotypes in untreated HIV infection (*CCR5Δ32* deletion and *HLA-B*57)* were associated with slightly lower increases in the CD4 T cell count after commencement of cART. Although these effects were not statistically significant, this observation is in line with the finding that some genotypes with favorable effects before receipt of cART may be associated with opposite effects after receipt of cART.

The lower increases in the CD4 T cell count persisted when we excluded individuals who carried the HLA alleles most consistently associated with protective or detrimental effects. Nevertheless, we cannot exclude that effects of a small number of constituent HLA alleles within the *HLA-Bw4* and *HLA-Bw6* groups and not broader differences between the *HLA-Bw4* and *HLA-Bw6* motifs per se explain the different biological effects. Because of the extreme polymorphism at the HLA-B locus and the multitude of additional important genetic determinants in HIV infection, the effects of single HLA alleles would only be evident in very large cohorts. With the exception of the effect of age, there were no significant differences between participants from the SHCS and WAHIV cohorts, suggesting that our findings are representative for similar populations.

Our findings and previous studies from the SHCS [16] suggest that a substantial minority of HIV-infected patients demonstrate slow and/or incomplete recovery of the CD4 T cell count that cannot be sufficiently predicted by the current pretreatment clinical assessment. Because of the favorable effects of *HLA-Bw4* homozygosity before commencement of cART, it could be erroneously anticipated that *HLA-Bw4* homozygotes recover CD4 T cells particularly well.

This study adds to the growing knowledge about host genetic factors that influence the efficacy and tolerability of cART [13, 17]. It is uncertain at this stage whether the impaired recovery of the CD4 T cell count among patients with *HLA-Bw4* homozygosity is associated with diminished recovery of functional cellular immune responses or with higher morbidity after cART initiation. Additional studies are warranted to clarify whether individualizing therapy for HIV infection on the basis of the

host's genetic factors would optimize the timing and the efficacy of cART.

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