From www.bloodjournal.org by guest on May 29, 2017. For personal use only.
Blood First Edition Paper, prepublished online July 20, 2009; DOI 10.1182/blood-2009-04-215186

The Duffy-null state is associated with a survival advantage in leukopenic HIV-infected persons of African ancestry

Hemant Kulkarni[1,2], Vincent C. Marconi[3,4], Weijing He[1,2], Michael L. Landrum[3,4,5], Jason F. Okulicz[3,4], Judith Delmar[3,4], Dickran Kazandjian[6] John Castiblanco[1,2] Seema S. Ahuja[1,2] Edwina J. Wright[7] , Robin A. Weiss[8], Robert A. Clark[1,2], Matthew J. Dolan[4,5] and Sunil K. Ahuja[1,2,9]

[1]Veterans Administration Research Center for AIDS and HIV-1 Infection, South Texas Veterans Health Care System, San Antonio, TX 78229

[2]Department of Medicine, University of Texas Health Science Center, San Antonio, TX 78229

[3]Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

[4]Infectious Disease Service, San Antonio Military Medical Center (SAMMC), Brooke Army Medical Center (BAMC), Fort Sam Houston, TX 78234

[5]Henry M. Jackson Foundation, Wilford Hall United States Air Force Medical Center, Lackland AFB, TX 78236

[6]Department of Medicine, Wilford Hall Medical Center, Lackland AFB, TX

[7]The Alfred Hospital, Melbourne, Victoria Australia 3004, The Burnet Institute, Melbourne Victoria Australia 3004, and Faculty of Medicine, Nursing and Health Sciences, Monash University, Victoria Australia 3800

[8]Division of Infection and Immunity, University College London, London, UK

[9]Departments of Microbiology and Immunology, and Biochemistry, University of Texas Health Science Center, San Antonio, TX 78229

Key words: DARC, chemokine, HIV, leukopenia

[*]To whom correspondence should be addressed: ahujas@uthscsa.edu (S.K.A.)

Copyright © 2009 American Society of Hematology

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

ABSTRACT

Persons of African ancestry, on average, have lower white blood cell (WBC) counts than those of European descent (ethnic leukopenia), but whether this impacts negatively on HIV-1 disease course remains unknown. Here, in a large natural history cohort of HIV-infected subjects we show that although leukopenia (<4,000 WBCs/mm^3 during infection) was associated with an accelerated HIV disease course, this effect was more prominent in leukopenic subjects of European than African ancestry. The African-specific *-46C/C* genotype of Duffy Antigen Receptor for Chemokines (*DARC*) confers the malaria-resisting, Duffy-null phenotype, and we found that the recently described association of this genotype with ethnic leukopenia extends to HIV-infected African Americans (AA). The association of Duffy-null status with HIV disease course differed according to WBC but not CD4+ T cell counts, such that leukopenic but not non-leukopenic HIV+ AAs with *DARC -46C/C* had a survival advantage compared with all Duffy-positive subjects. This survival advantage became increasingly pronounced in those with progressively lower WBC counts. These data highlight that the interaction between *DARC* genotype and the cellular milieu defined by WBC counts may influence HIV disease course, and this may provide a partial explanation of why ethnic leukopenia remains benign in HIV-infected African Americans, despite immunodeficiency.

Running title: Ethnic leucopenia, Duffy-null state and HIV disease course

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Introduction

Leukopenia is observed frequently during HIV-1 infection[1-3]. For example, evaluation of a large number of incarcerated adults revealed that leukopenia correlated strongly with HIV seropositivity, independent of other variables such as sex with a HIV-positive (HIV+) partner, injection drug use, ethnicity, and presence of sexually transmitted diseases[4,5]. Interestingly, in these studies the risk for HIV seropositivity associated with leukopenia and a history of sex with a HIV+ partner were similarly high[4], underscoring that leukopenia is strongly associated with HIV positivity in at-risk individuals and is commonly observed soon after HIV infection. Of note although the *sine qua non* feature of infection with HIV is progressive immunodeficiency related to $CD4^+$ T cell lymphopenia[6], $CD4^+$ T cell counts contribute minimally in quantitative terms to the overall WBC count and their decline during the course HIV infection contributes minimally to this observed leukopenia which is mainly attributable to neutropenia.

However, the impact of leukopenia on HIV disease course is largely undefined. Most of the studies that have examined the frequency or HIV disease-influencing effects of cytopenias have employed cross-sectional study designs (e.g., HIV+ vs HIV^{-1} or HIV+ neutropenics vs HIV+ non-neutropenics[7]). These studies have documented that the prevalence of cytopenias is higher in advanced disease. For example, neutropenia ranges from 0.8% to 13.4% when $CD4^+$ counts are below 250 cells/mm^3 and from 13% to 44% in those with AIDS[1,8]. By contrast there are no prospective studies in natural history cohorts that have examined whether leukopenia impacts on HIV disease course, independent of the known strong relationship between immunodeficiency [reflected by low $CD4^+$ counts or high viral loads (VL)] and either leukopenia or neutropenia[1,2,9].

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

To address this, we determined whether leukopenia impacts on the HIV disease course of subjects in a natural history cohort[10-13]. The large representation of both European Americans (EA) and African Americans (AA) in the study population allowed us to examine whether the impact of leukopenia on disease course differed according to race. This possibility is of particular interest because persons of African ancestry, on average, have significantly lower WBC counts, secondary to lower neutrophil cell counts, than persons of European descent[14-17]. In otherwise healthy individuals, low WBC counts in persons of African ancestry are thought to be both genetically determined and benign, as they have not been associated with an increased incidence of bacterial infection[14]. This has resulted in the designation of this condition as "benign ethnic leukopenia or neutropenia"[14,16,17].

In light of the above, we considered whether differences in WBC counts also result in racial differences in HIV disease outcome. Such an analysis could be confounded by social factors (e.g. access to health care, socioeconomic status). However, our studies were conducted in a well-characterized natural history cohort of HIV infection in which several factors that may confound assessment of race-specific differences in disease progression were minimized (e.g., equal access to health care, similar living standards, and minimal loss to follow-up). In our study cohort, the HIV+ AAs had a slight survival advantage compared with HIV+ EAs[10]. This finding was consistent with the survival advantage observed in the HIV-infected AAs in a much larger segment of the same cohort of HIV+ subjects, of which the group that we studied was a subset[12]. In view of the epidemiological observation that ethnic leukopenia is "benign" in otherwise healthy AAs, a parsimonious explanation for the survival advantage observed in the overall

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

group of HIV-infected AAs compared with EAs was that it may be due to a subset of leukopenic AAs who had a benign disease course compared with their leukopenic HIV+ EA counterparts.

The results of our studies conducted herein affirmed this possibility. This finding and the previously ascribed genotype-phenotype relationships observed for Duffy Antigen Receptor for Chemokines (DARC), a decoy chemokine receptor[18], prompted us to examine whether the associations for *DARC* genotype in HIV-infected subjects may explain in part why leukopenic AAs had a survival advantage compared with leukopenic EAs. DARC is expressed mainly on the surface of red blood cells (RBCs) and endothelial cells, where it binds multiple chemokines that have relevance to both HIV pathogenesis (e.g. RANTES and MCP-1) and neutrophil biology (e.g. GROα)[18,19]. The -46T→C polymorphism is specific to persons of African ancestry[10,20-22], and homozygosity for the -46C allele (*-46C/C* genotype) abrogates DARC expression on erythrocytes[18-20]. Because DARC on RBC serves as a receptor for the malarial protozoa *Plasmodium vivax*[19,20], Duffy-null status (*DARC -46C/C* genotype) on RBC is associated with a selective advantage against this form of malaria. Recent studies revealed two phenotypes associated with the *DARC -46C/C* genotype that had relevance to both leukopenia and HIV pathogenesis. First, admixture mapping and other genetic studies in large non-immunosuppressed cohorts of EAs and AAs demonstrate that over and above its strong link with African ancestry, *DARC -46C/C* is the main genetic basis for ethnic leukopenia[21] and neutropenia[22]; other observational studies substantiate this relationship between *DARC -46C/C* genotype and leukopenia or neutropenia[23,24]. Second, in our study population of HIV+ AAs, those with the *DARC -46C/C* genotype had a slower rate of HIV disease progression than those with *DARC –T/T* or *C/T*[10]. The latter two findings provided the rationale for investigating further

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

the relationships among leukopenia, *DARC* genotype and HIV disease course. The results of these studies highlight that an interaction between *DARC* genotype and the endogenous environment as defined by WBC counts impacts on HIV disease course.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

METHODS

Study cohort

We used *DARC* genotyping data, which we reported on recently[10], derived from HIV-positive subjects from the Department of Defense HIV Natural History Study cohort followed originally at Wilford Hall Medical Center (WHMC) and more recently at the Brooke Army Medical Center, San Antonio, TX. The studied population is the local (San Antonio) site of a prospective multisite observational cohort from the United States Military's HIV Natural History Study. Extensive details of the WHMC cohort have been provided elsewhere and all subjects were followed prospectively from the early stages of their infection[10,11]. The time from diagnosis to entry into the cohort was similar for subjects from different ethnic groups. The genetic and clinical data came from 1,132 subjects representing a total of 7,125.15 person-years of follow up in which a total of 440 (38.9%) deaths occurred. The cohort included 515 (45.5%) subjects with documented seroconversion, and these subjects are referred to here as seroconverters. The proportion of seroconverters across the major ethnic groups was similar (42.1% of EA, 48.4% of AA and 42.0% of other ethnic groups). The clinical characteristics of the subjects are shown in Supplementary Table 1. The research was approved by the Institutional Review Boards of Wilford Hall Medical Center and the University of Texas Health Science Center, San Antonio, TX and informed consent was obtained in accordance with the Declaration of Helsinki.

Study outcomes

We classified the study subjects as those with leukopenia, i.e., a low WBC count (WBC^{low}) and those with a high WBC count (WBC^{high}) according to whether they maintained an average WBC count during HIV disease of $<$ or $\geq$4,000 cells/mm^3, respectively. There were a total of 26,585

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

WBC count measurements on the study subjects during the HIV disease course (16,032 measurements in EAs at a mean of 25.7 measurements per patient; 8,266 measurements in AAs at a mean of 20.8 measurements per patient and 2,287 measurements in other ethnicities at a mean of 22.9 measurements per patient). To assess the influence of genetic determinants on WBC counts during disease we determined the mean of the total WBC counts measured during disease for each patient as that patient's average WBC count measured during disease. The frequency of assessment of WBC counts did not differ significantly according to racial/ethnic group or *DARC -46C/C* genotype (data not shown). Mixed effects models revealed that the time trends of WBC counts during the HIV disease course remained steady in the racial/ethnic group and according to *DARC -46C/C* genotype (Supplementary Fig.1), and for this reason we also defined race/ethnicity-specific tertiles for the average WBC counts. To assess the influence of genotypes on survival, we used time to death as the endpoint.

Genotyping

A single-nucleotide polymorphism in *DARC T-46C* (rs2814778) was genotyped using a TaqMan allelic discrimination assay as described previously[10].

Statistical analyses

All continuous variables were compared across two groups using the nonparametric Mann-Whitney test. For survival analyses, we used Kaplan-Meier plots to compare graphically the survival outcomes. Statistical significance of these differences was assessed by log-rank test as well as univariate and multivariate Cox proportional hazards models after testing the assumption of proportionality of hazards using the Schoenfield residuals. To control for the potential effect

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

of varying lead times, seroprevalent status and antiretroviral therapy (categorized as described previously[11]), we conducted stratified multivariate logistic and Cox regression analyses using the seroconversion status and receipt of antiretoriviral therapy as a stratifying variable. We used generalized linear mixed models to examine the time trends of the WBC counts during disease. This was done since the WBC count measurements were irregularly spaced and not all subjects had the same length of follow-up. Statistical significance was assessed using a two-tailed p-value at a type I error rate of 0.05. Statistical analyses were conducted using the Stata 7.0 (Stata Corp, College Station, TX) statistical software package.

RESULTS

WBC counts and HIV disease course

There was a very high degree of correlation between the initial WBC count at diagnosis (i.e., at entry into cohort) and the average WBC count maintained during HIV disease course (Spearson's rho = 0.7035, p <0.0001, Supplementary Fig. 2). The time to death or AIDS in HIV-infected persons was significantly shorter in those with WBC^{low} than those with WBC^{high} (Fig. 1A and data not shown). As the results were similar whether we used AIDS or death as endpoints, the data presented are only for time to death and average WBC counts.

As the average WBC count during infection was strongly positively correlated with the baseline $CD4^+$ T cell count (Spearman's rho = 0.4340, p <0.0001), one possibility was that WBC^{low} simply tracked the disease-accelerating effects associated with a low $CD4^+$ count. To evaluate this, we investigated whether WBC^{low} vs WBC^{high} had differential impacts on HIV disease course in subjects with a low (<350 cells/mm^3) or high (≥350 cells/ mm^3) baseline/initial $CD4^+$ T cell

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

count, $CD4^+$ count thresholds that in this study population were very strong predictors of rapid vs. slow disease course, respectively[11,25]. These analyses revealed that in subjects who presented with low or high baseline $CD4^+$ counts, WBC^{low} was associated with a significantly shorter survival time than in those with $WBC^{high.}$ (Fig. 1B-C). This suggested that low WBC counts are associated with HIV disease progression independent of the $CD4^+$ T cell count.

Because WBC counts were associated with an accelerated disease course (Fig. 1A-C), it could be surmised that a low WBC count merely reflected a more advanced disease stage in those subjects for whom the date of seroconversion was not known. In this instance, the differential survival outcomes observed for WBC^{low} vs WBC^{high} (Fig. 1A-C) would simply be a reflection of the differences in the disease course of subjects categorized as seroconverters vs seroprevalent individuals. However, this was unlikely to be the case because as compared with those subjects with WBC^{high}, those with WBC^{low} had a faster disease course regardless of whether they were categorized as seroconverting or seroprevalent, or whether the baseline $CD4^+$ count was low or high (Fig. 1D-I).

To test the robustness of these observations, we conducted multivariate Cox proportional hazards regression analyses and found that WBC^{low} was a strong predictor of shorter survival times, independent of baseline $CD4^+$ counts and steady-state VL that in this study population are strong predictors of disease progression[11,25] (Table 1). For example, in the seroconverting subjects, the hazard ratio for time to death for those with WBC^{low} was 1.80 (Table 1). Collectively, these findings (Fig. 1 and Table 1) indicated that a low WBC count is an independent determinant of an accelerated HIV disease course.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Race and leukopenia and HIV infection

As the prevalence of leukopenia is higher in persons of African than European ancestry, we next determined if this phenotype exists in HIV+ individuals. The average WBC counts during disease (Fig. 2A) were significantly lower in HIV-infected AAs (median 4,694 cells/mm^3) than EAs (median 5,100 cells/mm^3) or other ethnicities (median 5,093 cells/mm^3); similarly, the initial WBC count at diagnosis were lower in HIV+ AAs (Supplementary Fig. 3A). The prevalence (%) of leukopenia at time of diagnosis was significantly higher in HIV+ AAs (28%) than in EAs (15%, P=0.0047) or HIV+ persons of other ethnicities (13%, P=$1.7x10^{-10}$) that were represented in the study cohort (Fig. 2B). These data indicated that ethnic leukopenia present in healthy AAs was also present in the setting of HIV infection.

Race and leukopenia and HIV disease

As ethnic leukopenia is associated with a benign phenotype in HIV-uninfected AAs, we investigated whether this benign phenotype was reflected in a more benign HIV disease course in HIV-infected AAs by evaluating whether the disease course in AAs with WBC^{low} (AA-WBC^{low}) was more similar to those of EAs with WBC^{low} (EA-WBC^{low}) or non-leukopenic AAs (AA-WBC^{high}) and EAs (EA-WBC^{high}). In the overall cohort, before (Fig. 3A) and after stratifying subjects according to their seroconversion status (Fig. 3B-C), the disease course of AA-WBC^{low} and EA-WBC^{low} were dissimilar, whilst the disease course of AA-WBC^{high} and EA-WBC^{high} were similar. Among AA seroconverters, those with WBC^{low} and WBC^{high} had similar rates of disease progression (Fig. 3B). By contrast, among EA seroconverters, those with WBC^{low} had a significantly faster disease course than those with WBC^{high} (Fig. 3B). Among subjects

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

categorized to the seroprevalent group, although AAs with WBC^{low} progressed faster than AAs with WBC^{high} (compare red vs blue survival curves, Fig. 3C) their disease course was slower than EAs with WBC^{low} (compare blue vs black survival curves; Fig. 3C). Hence the hierarchy of disease progression according to ethnicity and WBC status revealed that AAs with WBC^{low} had a survival advantage relative to EAs with WBC^{low}, and this hierarchical relationship was consistent among seroconverters (AA-WBC^{high} = EA-WBC^{high} = AA-WBC^{low} > EA-WBC^{low}) and seroprevalent (AA-WBC^{high} = EA-WBC^{high} > AA-WBC^{low} > EA-WBC^{low}) subjects (Fig. 3).

We undertook further analyses and found that leukopenic EAs had a survival disadvantage compared to leukopenic AAs leukopenia whether leukopenia was defined by contemporary (<4,000 cells/mm^3) versus population-specific cut-offs observed in the study cohort (see Supplementary Fig. 4).

Duffy status, race and leukopenia

The aforementioned data suggested that ethnic leukopenia existed in HIV+ AAs, and that leukopenic HIV+ AAs had a more benign disease course relative to leukopenic EAs. To investigate if there was a genetic basis for this, we determined whether there was any relationship between the aforementioned findings and the recent observation demonstrating that *DARC -46C/C* is a strong genetic basis for benign ethnic leukopenia, attributable to neutropenia, in HIV-negative subjects[21,22].

In our study population, *DARC -46C/C* was present nearly exclusively in AAs (69.1%) compared with EA (0.2%)[10]. Among the hematological parameters available for assessment, *DARC T-46C*

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

genotype (T/T, T/C, C/C), but not race was a strong determinant of the variability in the WBC counts during disease (Table 2) and at entry into the cohort (P=3.9x10^{-12}). Furthermore, among HIV+ AAs, leukopenia at time of diagnosis was three times more prevalent in those with *-46C/C* (36%) than those with *DARC -46 C/T* or *-46 T/T* (11.5%; Fig. 2C). Concordantly, WBC counts were lower in those AAs bearing *DARC -46C/C* than other *DARC* genotypes (Fig. 2D and Supplementary Fig. 3B). Taken together (Table 2 and Fig. 2), our results in HIV+ patients are concordant with those recent findings that the *DARC* genotype and not race was a strong predictor of variability in WBC/neutrophil counts in non-HIV cohorts[21,22].

***DARC-46C/C*, leukopenia and disease course**

Consistent with our previous data[10] in the overall study population and, therein the seroconverting and seroprevalent populations, there was a trend towards a survival advantage for HIV+ AAs with *-46 C/C* compared to HIV+ EAs , or AAs with *DARC -46 C/T* or *T/T* (Fig. 4A-C). However, the survival advantage associated with *-46C/C* was highly dependent on WBC counts as this association was greatly magnified in subjects with WBC^{low} (Fig. 4D-F) and muted in those with WBC^{high} (Fig. 4G-I).

To investigate whether the accentuation of the *DARC* genotype-phenotype associations with leukopenia were meaningful and not artifactual, we determined whether disease course differed according to *DARC* genotype in subjects categorized according to their baseline CD4$^+$ T cell count (< or > 350 cells/mm^3) (Supplementary Fig. 5). These analyses revealed that the disease-influencing phenotype associated with *DARC -46C/C* differed substantially according to the WBC (Fig. 4) but not CD4$^+$ counts (Supplementary Fig. 5).

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

We further explored the relationships between *DARC* genotype, leukopenia, and HIV disease course in HIV+ AAs using univariate, multivariate and other statistical approaches. Univariate models revealed that among those with WBC^{low}, the hazard ratio for survival associated with *DARC -46C/C* was one-third (RH=0.33) of that associated with *DARC -46 C/T* or *T/T* (RH=1; Table 3). In a multivariate model stratified for seroconversion status and receipt of antiretroviral therapy, *DARC -46C/C* was associated with a nearly 80% (RH=0.20) lower risk of progressive disease, independent of other covariates that are strong predictors of disease progression[11] as well as a genetic parameter designated as admixture score[10] (Table 3). Substantiating this, in stepwise Cox regression modeling, the *DARC -46C/C* genotype was retained as a significant predictor of disease progression but only in subjects with a low WBC count (Table 3). In these analyses, an association between *DARC -46C/C* and survival was not observed in subjects with WBC^{high} (Table 3). These data indicate that *DARC -46C/C* is an independent determinant of disease course, but mainly in those with a low WBC count.

***DARC -46C/C*, magnitude of leukopenia and disease course**

The aforementioned genotype-phenotype analyses were conducted with a fixed WBC cut-off of 4,000 cells/mm^3. However, it was plausible that the association between *DARC* genotype with disease progression was quantitatively dependent on the magnitude of leukopenia. We therefore evaluated the hazard ratio for survival associated with *DARC -46C/C* in HIV+ AAs subjects at different thresholds of average WBC counts (Fig. 5). This analysis revealed that with each step-wise decrement in the average WBC count, the hazard ratios for time to death associated with *DARC -46C/C* progressively improved (Fig. 5). This suggested that it was the interaction

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

between *DARC* genotype and WBC counts that actually influenced HIV disease course. This inference was consistent with the results of the interactive logistic regression model that we used to investigate whether (i) leukopenia, (ii) *DARC -46C/C*, and (iii) the interaction between leukopenia and *DARC* genotype were each associated with independent disease-influencing effects (Fig. 5). In this model, among HIV+ AAs, each 500 cell decrement in the average WBC count during disease was associated with a significantly increased risk of progressive HIV disease (RH=1.65; $P=1.8x10^{-6}$; Fig. 5, inset). Notably, this hazard ratio of 1.65 is consistent with that observed for leukopenic AAs (Fig. 3A, RH=1.74 for blue plot). However, although leukopenia *per se* was associated with disease-acceleration in HIV+ AAs, the interaction term between *DARC -46C/C* and WBC counts was significantly associated with a reduced risk of progressive disease (RH=0.74; P=0.019; Fig. 5, inset) rendering the association of *DARC -46C/C* in itself not significant (RH=0.82; P=0.405, Fig. 5, inset).

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

DISCUSSION

In our study population we observed the following relationships for WBC counts during HIV infection. First, a low WBC count during disease was associated with a faster disease progression, independent of known predictors of AIDS development such as the $CD4^+$ T cell count and viral load. Second, although twice as many HIV+ AAs presented with leukopenia than EAs, leukopenic AAs had a slower disease course than leukopenic EAs, whereas the disease course in non-leukopenic EAs and non-leukopenic AAs was similar. This suggested that ethnic leukopenia in HIV-infected AAs may be associated with a benign phenotype, despite HIV-induced immunodeficiency. Additionally, we found that over and above its strong link to African ancestry, *DARC* genotype is a strong predictor of WBC variability during HIV disease. Furthermore, there was a significant survival benefit in *DARC -46C/C* bearing HIV+AAs with low WBCs but not high WBCs. This survival advantage became increasingly pronounced in those with progressively lower WBC counts.

Highlighting that ethnic leukopenia associated with *DARC -46C/C* persists during HIV infection, we found that among HIV+ AAs who presented with leukopenia, 36% possessed *DARC -46C/C* vs 12% had *-46T/C* or *T/T*. The levels of total lymphocytes or $CD4^+$ and $CD8^+$ T cells were similar among AAs with different *DARC* genotypes. Hence, concordant with published reports[21-24], the lower WBC levels found in *-46C/C*-bearing HIV+ AAs we suspect are most likely due to a reduction in neutrophil and possibly, monocyte cell counts.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Our previous data showed that *DARC -46C/C* associated with a slow HIV disease course in the overall AA study population[10]. We now show that this association is evident mainly in those AAs who maintained a low WBC count during disease, suggesting that the genotype-phenotype relationships for the DARC-null state are highly dependent on the cellular milieu. These findings may also help explain the observation that neutropenia, which is found commonly in HIV-infected patients[2,3], was not associated with decreased survival in a large study of African-American HIV-infected women[2].

The basis for the association between *DARC -46C/C* and survival advantage during HIV disease remains unknown, but we offer the following possible explanations. First, it can be argued that the association of *DARC -46C/C* with survival advantage in leukopenic HIV+ AAs represents a tautology. As *DARC -46C/C* is African-specific and associated with leukopenia, it follows that the association between *DARC -46C/C* and disease protection in leukopenic AAs was merely a reflection of the association between *-46C/C* with a low WBC count in AAs. Although this argument has merit, it does not account for the following observations which make this line of reasoning implausible: (i) not all leukopenic HIV+ AAs possessed *DARC -46C/C*, and conversely, not all subjects with *DARC -46C/C* were leukopenic; and (ii) leukopenic AAs with *DARC -46C/T* or *-T/T* had an accelerated disease course which was similar to the disease course of leukopenic EAs. This suggested that *DARC -46C/C* may offset the detrimental effects associated with leukopenia in AAs. Supporting the latter inference, were the results of statistical analyses showing that among HIV+ AAs it was the interaction between *DARC -46C/C* and WBC counts, and not *DARC* genotype alone that was associated with a survival advantage (Fig. 5, inset). This dependence of *DARC* genotype on WBC counts for full expression of its phenotypic

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

effects on disease was also highlighted by the observation that the survival advantage associated with *DARC -46C/C* was augmented with increasing degrees of leukopenia.

A second possibility is that *DARC -46C/C* may be merely a surrogate for other unknown genotypes which influence HIV disease course in the setting of leukopenia. Because WBC counts are a genetically-related phenotype, it follows that subjects with leukopenia may be enriched for polymorphisms in other genes that confer a low WBC count trait. In this setting the cellular milieu defined by genetically-determined leukocyte count profiles and/or genes that track with leukopenia (which may include *DARC -46C/C*) may serve as a modifier of HIV disease. Although we cannot exclude this possibility, we outline below several observations that together favor a role for DARC in HIV pathogenesis.

Why does the *DARC -46C/C* genotype specifically associate with a survival advantage in those HIV-infected AAs who have a low WBC count? Indeed, that leukopenia could be 'good' for a patient appears on the surface to be counter intuitive. We speculate that what we have observed in the context of HIV infection may reflect an evolutionarily adaptive mechanism to limit both inflammatory response and cell entry, to an ancestral pathogen such as *P. vivax*. The *DARC* locus shows complex genetic signatures of positive natural selection[26,27], and it has been suggested that the *-46C* allele has arisen under the selective pressure of an ancestral form of malaria that also used DARC, but was more lethal than *P. vivax,* which is generally not associated with mortality and is no longer very common in Africa[20]. Interestingly, neutropenia is commonly observed during malaria and is more common in infection with *P. vivax* than *P. falciparum*[28,29], and there is clinical and experimental evidence that neutrophils contribute to the adverse outcomes

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

following malarial infection[30-34]. Furthermore, a genome-wide analysis of the host response to malaria also implicates neutrophil-mediated pathways in underlying malarial pathogenesis[35]. Hence, one possibility is that the protection against neutrophil-mediated inflammation during infection by a lethal ancestral form of malaria may have also promoted the selection of the Duffy-null trait.

Studies suggest that ethnic leukopenia/neutropenia may be related to a reduced ability to mobilize neutrophils from the marginated granulocyte pool[36]. Supporting this, studies in mouse models show that DARC expression on both RBC and endothelial cells influences neutrophil migration[37,38]. Also, *DARC -46C/C* may associate with impaired hematopoiesis because some of the chemokines that bind to DARC (e.g. GROα[39]) regulate many hematopoietic responses, including survival, proliferation, migration, and homing[40]. Supporting this possibility, healthy subjects of African ancestry have reduced numbers of bone marrow progenitor cells than Caucasians[41]. Although the precise mechanism underlying ethnic leukopenia is unknown, given the importance of neutrophils in inflammation, the reduced numbers of circulating neutrophils associated with *DARC -46C/C* may lead to dampened inflammatory responses, and this may explain in part the "benign" nature of ethnic leukopenia. In contrast, the HIV-associated leukopenia/neutropenia observed in EAs or AAs who bear at least one *DARC -46T* allele is associated with a less favorable outcome possibly because this leukopenia occurs within the context of the Duffy-positive state and hence originates from a different mechanism which is not associated with a "benign" leukopenia.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Additional data suggest a link between *DARC -46C/C* and inflammatory responses relevant to HIV pathogenesis. Studies demonstrate a key role for circulating lipopolysaccharide (LPS), a marker of microbial translocation, and inflammation in the pathogenesis of HIV infection, especially acute infection[42]. In this respect it is notable that following administration of LPS (endotoxin) to healthy individuals, compared with Duffy-positive Caucasians, Duffy-null individuals of African descent had significantly lower levels of total blood chemokine levels (derived from plasma levels, red blood cell– and neutrophil-associated MCP-1, GROα, and IL-8, ligands of DARC)[24]. This may have relevance to HIV pathogenesis because prior studies had found that plasma levels of MCP-1, a chemokine that associates with higher HIV viral load *in vivo* and increased viral replication *in vitro*[43], are lower in Duffy-null subjects[44]. Additionally, following administration of LPS, compared with Duffy-positive Caucasians, Duffy-null subjects had significantly lower levels of three biomarkers of *in vivo* thrombin generation - TAT, F1+2 and D-dimer - suggesting that the Duffy-null state associates with reduced tissue factor-triggered activation of coagulation pathways[45]. This may also be relevant to HIV pathogenesis as Kuller et al demonstrated recently that the degree of HIV-induced activation of coagulation pathways, as assessed by D-dimer levels, has an adverse effect on all-cause mortality among HIV+ patients with relatively preserved $CD4^+$ counts[46]. Hence, future studies are warranted to determine whether the increased survival in HIV-infected AAs associated with the Duffy-null/low WBC profile is a result of attenuated coagulation responses and chemokine- and neutrophil-mediated inflammation.

The importance of neutrophil-mediated inflammation has been highlighted in simian models of Simian Immunodeficiency Virus (SIV) infection. For example, Elbim et al[47] showed that the

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

degree of neutropenia during acute infection correlates with viral load, and the differential sensitivity of neutrophils to apoptosis may distinguish pathogenic versus nonpathogenic SIV infections of nonhuman primates. Further affirming a possible role of neutrophils during the acute phases of lentiviral infection are the studies of Li et al[48]. They used a vaginal transmission model of SIV infection in rhesus macaque and found that after viral inoculation of SIV, infected cells in the cervix were generally in areas of inflammation containing IL-8-positive cells[48]. Notably, IL-8 is a potent chemoattractant of neutrophils and application of glycerol monolaurate, an inhibitor of IL-8, protected these animals from infection[48].

There may be additional mechanisms by which DARC influences HIV pathogenesis. In a previous study[10], we found that in addition to binding to HIV-suppressive chemokines, HIV also binds to erythrocyte DARC *in vitro*, and that RBC-bound HIV can be displaced by chemokines and transferred in *trans* to CD4+ T cells[10]. Thus, we had speculated that the binding of fewer HIV particles to DARC-null RBCs may lead to a slower disease course.

In summary, prior results associated the DARC-null state with malaria resistance, and the present data suggest that an intricate interaction between *DARC -46C/C* genotype and leukopenia associates with survival advantage in HIV-infected AAs. Thus, further evaluation of the intersection of *DARC* genotype, the cellular milieu, as reflected by the WBC count, and HIV disease outcome may provide new insights into pathogenesis. Of broad relevance, accounting for the modifier effects of the cellular environment (e.g., as reflected in the present study by genetically-determined leukocyte parameters) may provide greater insights into genotype-

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

phenotype relationships for DARC and other genes during HIV infection as well as other diseases.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Acknowledgements: We thank Duane Hospenthal, Clint Murray, Brain Agan and the anonymous reviewers for their critical feedback.

Author Contribution: S.K.A., H.K. and M.J.D conceptualized the research, analyzed the data and wrote the manuscript; V.C.M., M.L.L., J.F.O., J.D., J.C., and M.J.D contributed substantially to clinical cohort development, conceptual ideas, data analyses and preparation of the manuscript. W.H., D.K., S.S.A., E.J.W., R.W., and R.A.C. contributed conceptual ideas and immensely to manuscript preparation.

Competing interests: None

Financial Disclosure: This work was supported by the Veterans Administration (VA) Center on AIDS and HIV infection of the South Texas Veterans Health Care System, and a MERIT (R37046326) and other awards (AI043279 and MH069270) from the NIH to S.K.A. S.K.A. is also supported by a VA MERIT award and is a recipient of the Elizabeth Glaser Scientist Award, the Burroughs Wellcome Clinical Scientist Award in Translational Research, and the Doris Duke Distinguished Clinical Scientist Award. Support for the DoD HIV Natural History Study cohort and staff involved in this work was provided by the Infectious Disease Clinical Research Program (IDCRP) of the Uniformed Services University of the Health Sciences (USUHS), of which the HIV Natural History Study is a component. The IDCRP is a Department of Defense tri-service program executed through USUHS and the Henry M. Jackson Foundation for the

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Advancement of Military Medicine (HJF), in collaboration with HHS/NIH/NIAID/DCR through Interagency Agreement HU0001-05-2-0011.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

References

1. Kaslow RA, Phair JP, Friedman HB, et al. Infection with the human immunodeficiency virus: clinical manifestations and their relationship to immune deficiency. A report from the Multicenter AIDS Cohort Study. Ann Intern Med. 1987;107:474-480.
2. Levine AM, Karim R, Mack W, et al. Neutropenia in human immunodeficiency virus infection: data from the women's interagency HIV study. Arch Intern Med. 2006;166:405-410.
3. Farber BF, Lesser M, Kaplan MH, Woltmann J, Napolitano B, Armellino D. Clinical significance of neutropenia in patients with human immunodeficiency virus infection. Infect Control Hosp Epidemiol. 1991;12:429-434.
4. D'Angelo LJ, Getson PR, Luban NL, Gayle HD. Human immunodeficiency virus infection in urban adolescents: can we predict who is at risk? Pediatrics. 1991;88:982-986.
5. Altice FL, Marinovich A, Khoshnood K, Blankenship KM, Springer SA, Selwyn PA. Correlates of HIV infection among incarcerated women: implications for improving detection of HIV infection. J Urban Health. 2005;82:312-326.
6. Henry WK, Tebas P, Lane HC. Explaining, predicting, and treating HIV-associated CD4 cell loss: after 25 years still a puzzle. Jama. 2006;296:1523-1525.
7. Hermans P, Sommereijns B, Van Cutsem N, Clumeck N. Neutropenia in patients with HIV infection: a case control study in a cohort of 1403 patients between 1982 and 1993. J Hematother Stem Cell Res. 1999;8 Suppl 1:S23-32.
8. Murphy MF, Metcalfe P, Waters AH, et al. Incidence and mechanism of neutropenia and thrombocytopenia in patients with human immunodeficiency virus infection. Br J Haematol. 1987;66:337-340.
9. Servais J, Nkoghe D, Schmit JC, et al. HIV-associated hematologic disorders are correlated with plasma viral load and improve under highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2001;28:221-225.
10. He W, Neil S, Kulkarni H, et al. Duffy antigen receptor for chemokines mediates trans-infection of HIV-1 from red blood cells to target cells and affects HIV-AIDS susceptibility. Cell Host Microbe. 2008;4:52-62.
11. Dolan MJ, Kulkarni H, Camargo JF, et al. CCL3L1 and CCR5 influence cell-mediated immunity and affect HIV-AIDS pathogenesis via viral entry-independent mechanisms. Nat Immunol. 2007;8:1324-1336.
12. Silverberg MJ, Wegner SA, Milazzo MJ, et al. Effectiveness of highly-active antiretroviral therapy by race/ethnicity. Aids. 2006;20:1531-1538.
13. Crum-Cianflone N, Hullsiek KH, Marconi V, et al. Trends in the incidence of cancers among HIV-infected persons and the impact of antiretroviral therapy: a 20-year cohort study. Aids. 2009;23:41-50.
14. Haddy TB, Rana SR, Castro O. Benign ethnic neutropenia: what is a normal absolute neutrophil count? J Lab Clin Med. 1999;133:15-22.
15. Freedman DS, Gates L, Flanders WD, et al. Black/white differences in leukocyte subpopulations in men. Int J Epidemiol. 1997;26:757-764.
16. Grann VR, Bowman N, Joseph C, et al. Neutropenia in 6 ethnic groups from the Caribbean and the U.S. Cancer. 2008;113:854-860.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

17. Hsieh MM, Everhart JE, Byrd-Holt DD, Tisdale JF, Rodgers GP. Prevalence of neutropenia in the U.S. population: age, sex, smoking status, and ethnic differences. Ann Intern Med. 2007;146:486-492.
18. Rot A. Contribution of Duffy antigen to chemokine function. Cytokine Growth Factor Rev. 2005;16:687-694.
19. Hadley TJ, Peiper SC. From malaria to chemokine receptor: the emerging physiologic role of the Duffy blood group antigen. Blood. 1997;89:3077-3091.
20. Langhi DM, Jr., Bordin JO. Duffy blood group and malaria. Hematology. 2006;11:389-398.
21. Nalls MA, Wilson JG, Patterson NJ, et al. Admixture mapping of white cell count: genetic locus responsible for lower white blood cell count in the Health ABC and Jackson Heart studies. Am J Hum Genet. 2008;82:81-87.
22. Reich D, Nalls MA, Kao WH, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet. 2009;5:e1000360.
23. Grann VR, Ziv E, Joseph CK, et al. Duffy (Fy), DARC, and neutropenia among women from the United States, Europe and the Caribbean. Br J Haematol. 2008.
24. Mayr FB, Spiel AO, Leitner JM, et al. Duffy antigen modifies the chemokine response in human endotoxemia. Crit Care Med. 2008;36:159-165.
25. Kulkarni H, Agan BK, Marconi VC, et al. CCL3L1-CCR5 genotype improves the assessment of AIDS Risk in HIV-1-infected individuals. PLoS ONE. 2008;3:e3165.
26. Hamblin MT, Thompson EE, Di Rienzo A. Complex signatures of natural selection at the Duffy blood group locus. Am J Hum Genet. 2002;70:369-383.
27. Tournamille C, Blancher A, Le Van Kim C, et al. Sequence, evolution and ligand binding properties of mammalian Duffy antigen/receptor for chemokines. Immunogenetics. 2004;55:682-694.
28. Rodriguez-Morales AJ, Sanchez E, Arria M, et al. White blood cell counts in Plasmodium vivax malaria. J Infect Dis. 2005;192:1675-1676; author reply 1676-1677.
29. Jadhav UM, Singhvi R, Shah R. Prognostic implications of white cell differential count and white cell morphology in Malaria. J Postgrad Med. 2003;49:218-220; discussion 221.
30. Chen L, Zhang Z, Sendo F. Neutrophils play a critical role in the pathogenesis of experimental cerebral malaria. Clin Exp Immunol. 2000;120:125-133.
31. Senaldi G, Vesin C, Chang R, Grau GE, Piguet PF. Role of polymorphonuclear neutrophil leukocytes and their integrin CD11a (LFA-1) in the pathogenesis of severe murine malaria. Infect Immun. 1994;62:1144-1149.
32. Mohammed AO, Elghazali G, Mohammed HB, et al. Human neutrophil lipocalin: a specific marker for neutrophil activation in severe Plasmodium falciparum malaria. Acta Trop. 2003;87:279-285.
33. Hemmer CJ, Vogt A, Unverricht M, Krause R, Lademann M, Reisinger EC. Malaria and bacterial sepsis: similar mechanisms of endothelial apoptosis and its prevention in vitro. Crit Care Med. 2008;36:2562-2568.
34. Huy NT, Trang DT, Kariu T, et al. Leukocyte activation by malarial pigment. Parasitol Int. 2006;55:75-81.
35. Griffiths MJ, Shafi MJ, Popper SJ, et al. Genomewide analysis of the host response to malaria in Kenyan children. J Infect Dis. 2005;191:1599-1611.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

36. Phillips D, Rezvani K, Bain BJ. Exercise induced mobilisation of the marginated granulocyte pool in the investigation of ethnic neutropenia. J Clin Pathol. 2000;53:481-483.
37. Lee JS, Wurfel MM, Matute-Bello G, et al. The Duffy antigen modifies systemic and local tissue chemokine responses following lipopolysaccharide stimulation. J Immunol. 2006;177:8086-8094.
38. Pruenster M, Mudde L, Bombosi P, et al. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. Nat Immunol. 2009;10:101-108.
39. Fukuda S, Bian H, King AG, Pelus LM. The chemokine GRObeta mobilizes early hematopoietic stem cells characterized by enhanced homing and engraftment. Blood. 2007;110:860-869.
40. Broxmeyer HE. Chemokines in hematopoiesis. Curr Opin Hematol. 2008;15:49-58.
41. Rezvani K, Flanagan AM, Sarma U, Constantinovici N, Bain BJ. Investigation of ethnic neutropenia by assessment of bone marrow colony-forming cells. Acta Haematol. 2001;105:32-37.
42. Douek D. HIV disease progression: immune activation, microbes, and a leaky gut. Top HIV Med. 2007;15:114-117.
43. Weiss L, Si-Mohamed A, Giral P, et al. Plasma levels of monocyte chemoattractant protein-1 but not those of macrophage inhibitory protein-1alpha and RANTES correlate with virus load in human immunodeficiency virus infection. J Infect Dis. 1997;176:1621-1624.
44. Jilma-Stohlawetz P, Homoncik M, Drucker C, et al. Fy phenotype and gender determine plasma levels of monocyte chemotactic protein. Transfusion. 2001;41:378-381.
45. Mayr FB, Spiel AO, Leitner JM, et al. Racial differences in endotoxin-induced tissue factor-triggered coagulation. J Thromb Haemost. 2009;7:634-640.
46. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008;5:e203.
47. Elbim C, Monceaux V, Mueller YM, et al. Early divergence in neutrophil apoptosis between pathogenic and nonpathogenic simian immunodeficiency virus infections of nonhuman primates. J Immunol. 2008;181:8613-8623.
48. Li Q, Estes JD, Schlievert PM, et al. Glycerol monolaurate prevents mucosal SIV transmission. Nature. 2009.
49. He W, Marconi VC, Castiblanco J, et al. Response: Association of Duffy Antigen Genotype with HIV-AIDS Susceptibility. Cell Host Microbe. 2009;5:In press.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Tables

Table 1. Association of low WBC counts during disease with rapid rate of HIV disease progression, independent of baseline $CD4^+$ T cell count and steady-state viral load (VL) in the entire study population (all subjects) or subjects categorized as seroconverters or seroprevalent.

	All Subjects	**Seroconverters**	**Seroprevalent**
Average WBC count <4000 cells/mm^3	1.70	1.80	1.64
	(1.35 – 2.14)	(1.17 – 2.76)	(1.24 – 2.17)
	<0.0001	0.007	<0.0001
Baseline $CD4^+$ T cell count <350 cells/mm^3	2.50	2.08	2.68
	(1.90 – 3.30)	(1.20 – 3.59)	(1.94 – 3.70)
	<0.0001	0.009	<0.0001
Steady-state viral load ≥20,000 copies/ml	4.54	4.27	4.64
	(3.43 – 6.01)	(2.70 – 6.75)	(3.24 – 6.65)
	<0.0001	<0.0001	<0.0001

Results are from full multivariate Cox proportional hazards models showing relative hazards (95% CI) for time-to-death and P value.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Table 2. Two-way analyses of variance of hematological parameters as outcomes predicted by race/ethnicity and *DARC T-46C* genotypes.

	Race/Ethnicity		***DARC T-46C***	
	F	*P*	F	*P*
Average WBC count	1.08	0.3724	17.43	<0.0001
Hemoglobin	0.72	0.6073	0.24	0.7896
Platelet count	1.18	0.3160	0.77	0.4653
Lymphocyte count	0.83	0.5288	0.10	0.9091
$CD4^+$ T cell count	0.81	0.5438	0.80	0.4515
$CD8^+$ T cell count	0.50	0.7774	0.39	0.6783

F, Snedecor's F estimated using ANOVA; p, significance value

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Table 3. Association of *DARC -46C/C* with disease progression in HIV+ African Americans.

Group	RH (95% CI) P		
	Univariate	**Full model**	**Final model**
All subjects	0.77	0.66	
	(0.55 – 1.09)	(0.40 – 1.09)	---
	0.146	0.102	
Average WBC <4000	0.33	0.20	0.22
	(0.19 – 0.60)	(0.07 – 0.60)	(0.08 – 0.64)
	<0.001	0.004	0.005
Average WBC ≥4000	0.84	1.18	
	(0.54 – 1.31)	(0.62 – 2.27)	---
	0.441	0.617	

Covariates for the full multivariate model are baseline $CD4^+$ T cell count; cumulate $CD4^+$ T cell count (cCD4), a parameter that reflects the changes in $CD4^+$ counts over disease course and is a very strong predictor of disease progression[11]; steady-state viral load; and an admixture score derived from 11 genetic markers that was highly predictive of self reported ethnicity (>98% accuracy)[10] and was also strongly correlated with the degree of admixture as assessed by the first principal component (obtained by EIGENSOFT program) of 96 ancestry informative markers[49]. Both full model and final step-wise model have been adjusted for seroconversion and receipt of antiretroviral therapy, as defined previously[11]. Results depicted are relative hazards (95% CI) for time-to-death and P value. RH =1 represents those with *DARC* -46C/T or T/T.

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

FIGURE LEGENDS

Figure 1. Association of low WBC counts during disease with survival in HIV-infected subjects.

Each panel shows Kaplan-Meier (KM) survival plots for subjects with an average WBC count during disease that was less than (orange) or ≥4000 cells/mm^3 (purple). The KM plots are for all subjects (left column), and those with baseline CD4$^+$ T cell counts of <350 (middle column) or ≥350 (right column) cells/mm^3 in the entire cohort (top row), and subjects categorized as seroconverter (middle row) and seroprevalent (bottom row) component of the cohort. RH, relative hazards (reference RH = 1 represents those with WBC counts of ≥4000 cells/mm^3); CI, confidence interval; P, significance value estimated using Cox proportional hazards modeling. n, number of subjects

Figure 2. WBC counts according to race/ethnicity and *DARC -46C/C* genotype.

Panels A show box-and-whisker plots depicting the distribution of the average of the total WBC counts during disease, in the major ethnic groups represented in the study population. AA, African Americans; EA, European Americans; OT, other ethnicities. Numbers at the top are p-values obtained by Mann-Whiney tests for the indicated comparisons. N, number of subjects. Panel B shows the proportion of subjects with leukopenia (initial WBC count <4000 cells/mm^3) in the indicated racial/ethnic groups. Vertically-oriented numbers are significance values obtained by Pearson's chi-square test. Panel C shows the proportion of AA (n=397) possessing the indicated *DARC* T-46C genotype (122 subjects with T/C or T/T and 275 subjects with C/C) who were leukopenic at presentation. The significance value for the comparison was obtained

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

using the Pearson's chi-square test. In panel D, the bar charts depict the mean (vertical bars) and 95% confidence intervals (error bars) for the average WBC counts during disease in the indicated racial/ethnic groups according to their *DARC T-46C* genotype. Numbers at the top are p-values obtained by Mann-Whiney tests for the indicated comparisons. N, number of subjects *, too few subjects (1 EA and 3 others) for whom the vertical bars are not shown.

Figure 3. Differential effects of low WBC counts on HIV disease course in HIV-infected EA and AA. KM plots depict survival curves computed according to race/ethnicity and average WBC count in the entire cohort (A), and HIV+ subjects categorized as seroconverter (B) and seroprevalent (C). RH, relative hazards (reference RH = 1 represents AAs with WBC counts of ≥4000 cells/mm^3); CI, confidence interval; P, significance values obtained by Cox proportional hazards modeling; n, number of subjects.

Figure 4. Rate of HIV disease progression in HIV+ EAs, and HIV+ AAs who are Duffy-null (*DARC -46C/C*) or -positive (*DARC -46C/T or T/T*). Each panel shows KM survival plots for three groups of HIV-infected subjects: EA (blue), AAs possessing *DARC -46 C/C* (pink) and AAs possessing *DARC -46 C/T* or *T/T* (green). The KM plots are for all subjects (left column), and those with baseline $CD4^+$ T cell counts of <4,000 (middle column) or ≥4,000 (right column) cells/mm^3 in the entire cohort (top row), and those subjects categorized as seroconverter (middle row) and seroprevalent (bottom row). RH, relative hazards (reference RH = 1 represents AAs with *DARC -46C/C*); CI, confidence interval; P, significance value estimated using Cox proportional hazards modeling, n, number of subjects. Note, whether *DARC -46C/T* or *T/T* was associated with disease-acceleration in leukopenic seroconverting AAs could not be evaluated as

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

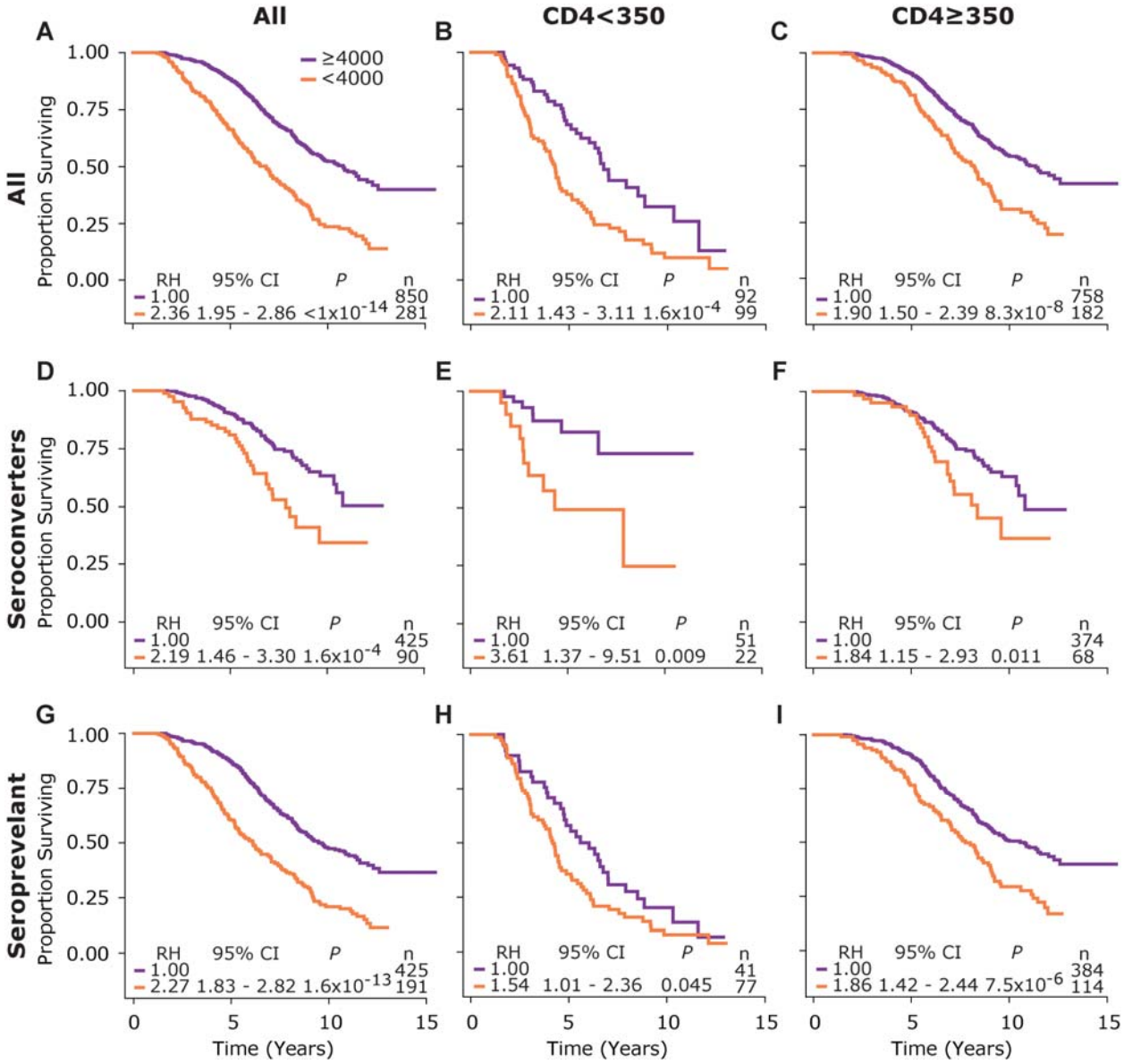
in this group there were only four subjects who did not have *-46C/C* and no death events had occurred (green survival curve in panel E).

Figure 5. Relationship between decrements in the average WBC counts and the hazard ratios for time to death associated with the *DARC -46C/C* genotype in HIV-infected AAs. The plot shows the hazard ratios (y-axis) for rate of progression to death estimated using Cox proportional hazards modeling in subjects with average WBC counts below the indicated cutpoints (x-axis). The hazard ratios were estimated over the range of WBC counts shown on the x-axis for every 50 cell count change. The reference group is those subjects who possess *DARC -46T/T* or *T/C* (RH = 1). Inset depicts data showing an interactive effect of low total WBC count on the survival advantage associated with the *DARC -46C/C* genotype in HIV+ AAs. RH, relative hazards (reference RH = 1 represents AAs with *DARC -46T/T or T/C*); CI, confidence interval; P, significance value estimated using Cox proportional hazards modeling with indicated parameters (a, b, and c).

From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

FIGURES

Figure 1.



From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Figure 2.

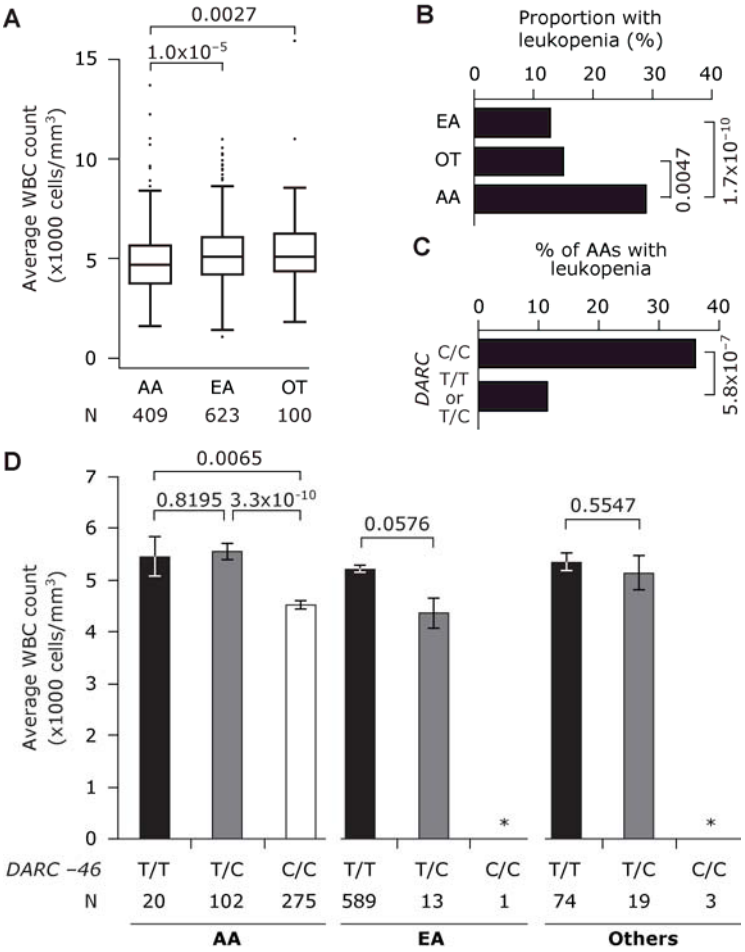
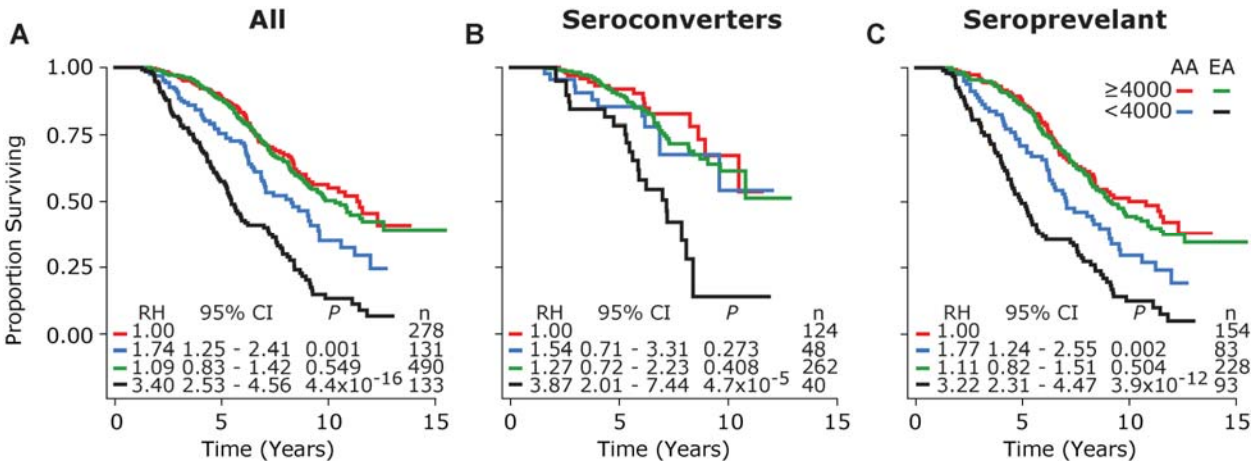
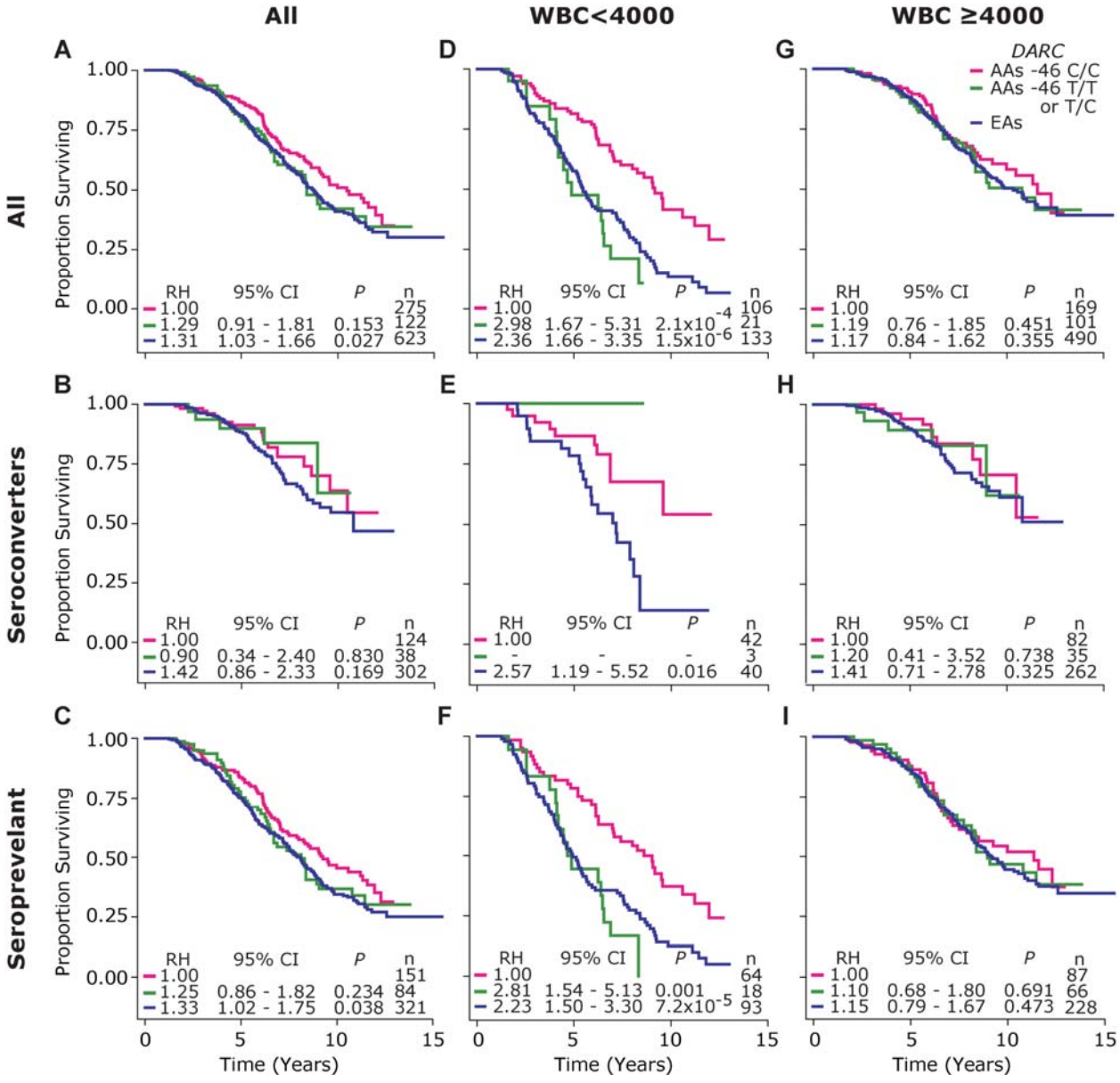


Figure 3.



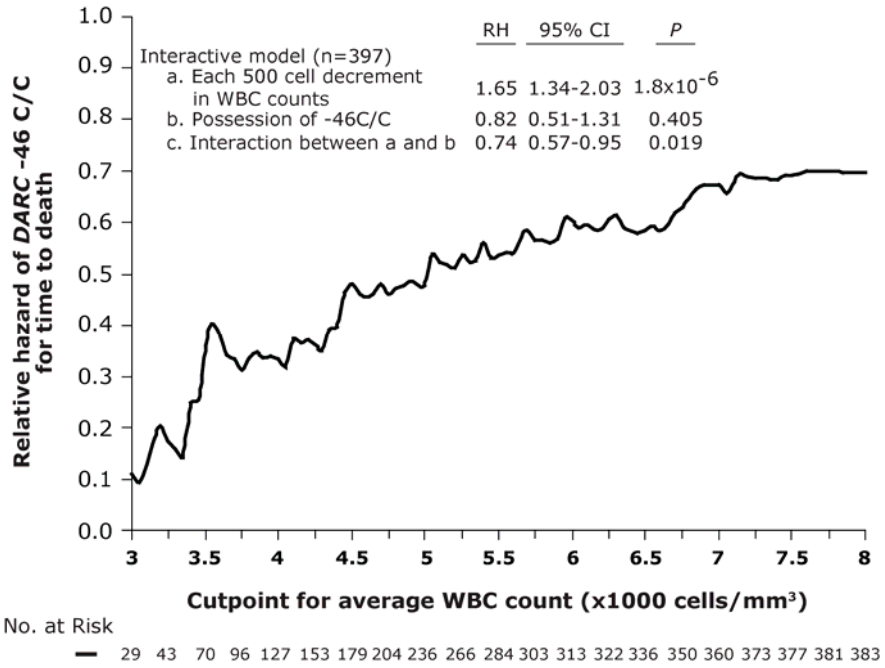
From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Figure 4.



From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Figure 5.



From www.bloodjournal.org by guest on May 29, 2017. For personal use only.

Prepublished online July 20, 2009;
doi:10.1182/blood-2009-04-215186

The Duffy-null state is associated with a survival advantage in leukopenic HIV-infected persons of African ancestry

Hemant Kulkarni, Vincent C. Marconi, Weijing He, Michael L. Landrum, Jason F. Okulicz, Judith Delmar, Dickran Kazandjian, John Castiblanco, Seema S. Ahuja, Edwina J. Wright, Robin A. Weiss, Robert A. Clark, Matthew J. Dolan and Sunil K. Ahuja

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.
Copyright 2011 by The American Society of Hematology; all rights reserved.