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Genetic diversity and population structure of *Mycobacterium tuberculosis* in HIV-1-infected compared with uninfected individuals in Burkina Faso

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HIV-1 infection and HIV-1-induced immune deficiency may play a role in selecting particular

Mycobacterium tuberculosis (MTB) strains (i.e. genotypes). We compared 43 MTB isolates obtained from HIV-1-infected patients with 77 MTB isolates obtained from HIV-1-uninfected patients in Burkina Faso, by means of DNA fingerprinting methods (MIRU-VNTR plus spoligotyping). This study suggests a lack of structure of the MTB population caused by HIV-1 infection and a similar genetic diversity of MTB in HIV-1-infected compared with uninfected individuals.

In Africa, the incidence rate of tuberculosis has been increasing since 1990 both in countries with low HIV-1 rates and in countries with high HIV-1 rates [1]. In 2003, the estimated number of new cases of tuberculosis was more than 2 million, with almost 600 000 deaths in the continent [1]. Furthermore, according to the World Health Organization, HIV-1 prevalence in tuberculosis cases caused by *Mycobacterium tuberculosis* (MTB) ranges from 0 to more than 50% in South Africa, 30-40% of deaths occurring in individuals with MTB/HIV-1 co-infection [1]. Tuberculosis infection may be the initial sign of immune dysfunction associated with HIV-1 infection, and active tuberculosis is sometimes considered to be an AIDS-defining illness.

It is widely thought that the immune system plays the role of a selective filter in populations of pathogenic organisms [2]. Our working hypothesis was that the immune system could have an impact on the population structure of MTB. Defects in the immune system caused by HIV-1 infection could allow infections with peculiar MTB genotypes. In order to test this hypothesis, we compared the genetic diversity and population structure of MTB within HIV-1-infected compared with uninfected patients.

Our study was conducted in Burkina Faso, a West African country with a total population of over 13 million people. In 2003, the estimated tuberculosis incidence rate was 163 cases per 100 000 inhabitants [1]. Among the new tuberculosis cases, 23% have been estimated to be associated with HIV-1 infection. In 2001, 120 patients with pulmonary tuberculosis were enrolled in the two major cities of Burkina Faso (Ouagadougou and Bobo-Dioulasso). Of the 120 MTB isolates, 43 were isolated from HIV-1-infected tuberculosis patients and 77 were from HIV-1-uninfected tuberculosis patients. The median age was 34 years (range 15-75). The female to male ratio was 0.4.

For each patient, one MTB strain was genotyped by two genetic markers classically used in molecular epidemiology studies [3], mycobacterial interspersed repetitive unit-variable-number tandem repeat (MIRU-VNTR; 12 loci) and spoligotyping. A high degree of resolution is

gained when data from these two markers are combined [4].

To analyse the genetic variability of the isolates circulating in Burkina Faso, a set of diversity indices, including genotypic diversity and mean genetic diversity (H), was measured [5]. Univariate risk factor analysis was performed calculating odds ratio with 95% confidence intervals. P values of less than 0.05 were considered statistically significant.

In order to identify and quantify the genetic differentiation of MTB according to the presence or absence of HIV-1 co-infection and according to sex, a canonical correspondence analysis (CCA) was carried out using the CANOCO software [6,7]. CCA searches for the multivariate relationships between two data sets (e.g. genotypic and environmental data sets) [6]. Only MTB isolates for which all loci (i.e. MIRU–VNTR and spoligotyping) could be scored were included in the CCA. The significance of the canonical axes was tested with a Monte Carlo permutation test [7]. This also allowed an estimation of the 95% confidence intervals of the centroids of each population.

Genetic differentiation between strains obtained from HIV-1-infected compared with uninfected patients was tested by the calculation of F_{st} (a standardized measure of genetic differentiation among samples) using unbiased estimates over all loci [8]. The F_{st} value ranges between 0 (no differentiation) and 1 (all samples fixed for a different allele). The test was performed by the software F-Stat 2.9 (<http://www.unil.ch/izea/software/fstat.html>).

Based on combined analysis by spoligotyping and the MIRU–VNTR methods, mean genetic diversity H and genotypic diversity were not significantly different for HIV-1-infected patients compared with HIV-1-uninfected patients [(HIV-1-infected individuals, $H=0.2$ versus HIV-1-uninfected individuals, $H=0.17$; $P=0.26$), (genotypic diversity of HIV-1-infected, $31/43=0.72$ versus genotypic diversity of HIV-1-uninfected, $57/77=0.74$, $P=0.81$)].

Figure 1 shows estimates for genetic variability in the MTB population when classified by patient HIV-1 infection status and sex, with the ellipses representing the 95% confidence intervals around these estimates. The overall Monte Carlo permutation test on the first four CCA axes is not significant ($P=0.20$) indicating no structure in the different MTB samples. No genotypic differentiation between MTB patient status and sexes could be detected, and these results are consistent with F_{st} estimates (see below). A similar analysis comparing the MTB population structures from the two Burkina Faso sampling locations (i.e. Ouagadougou and Bobo-Dioulasso) also found no evidence for genetic

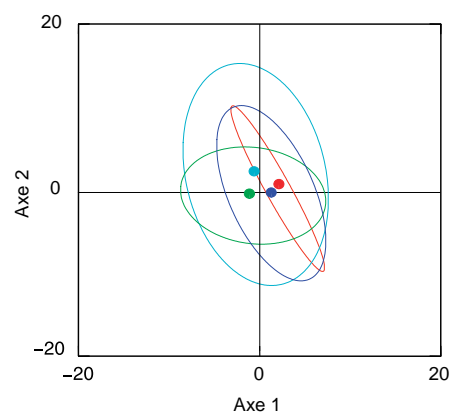


Fig. 1. Graphical representation of the results of a canonical correspondence analysis of the *Mycobacterium tuberculosis* genotypes according to a patient's infection status and sex. ○ F+, HIV-1-infected female; ○ F-, HIV-1-uninfected female; ○ H+, HIV-1-infected male; ○ H-, HIV-1-uninfected male. Centroid dots for each sample are surrounded by ellipses representing 95% confidence intervals of estimates.

differentiation among MTB strains ($P=0.63$). Furthermore, genetic differentiation (F_{st}) calculated by means of combined genetic data did not indicate any differentiation between the two populations, HIV-1-infected versus uninfected patients ($F_{st}=0.0001$, $P=0.56$).

Since the development of fingerprinting techniques of MTB strains, a debate exists concerning the genetic divergence of strains circulating in HIV-1-infected and in HIV-1-uninfected patients [9]. It has been speculated that HIV/AIDS patients constitute a specific niche for MTB, in which less virulent strains could multiply without selection pressure provided by a competent immune system [9]. The present results do not agree with these hypotheses. A lack of structure in the MTB population based on HIV-1 status was found and the genetic diversity of MTB was similar for HIV-1-infected and uninfected individuals in Burkina Faso. This latter result is in agreement with those obtained by Yang *et al.* [10] suggesting a similar risk of infection with a defined MTB strain for HIV-1-infected and uninfected individuals.

Three hypothesis could explain the lack of genetic differentiation between these two groups of MTB strains: (i) HIV-1-infected patients were not at an advanced stage of disease and still have a sufficiently efficient immune system to select infecting MTB; (ii) HIV-1-infected patients with AIDS developed symptomatic tuberculosis after reactivation of an endogenous MTB strain acquired while the patient was still immunocompetent; and (iii) the relative immune competence of the majority of tuberculosis patients could select MTB populations with a high level of virulence (or fitness), mitigating the

probability of encountering less virulent/fit genotypes by immunocompromised patients.

Studies such as this should be replicated and extended before one can conclude that HIV-1-infected patients should be considered as a niche for MTB strains with particular virulence or fitness characteristics.

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CD8 T-cell subsets and viral load in the cerebrospinal fluid of therapy-naive HIV-infected individuals

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Central nervous system involvement is common in HIV infection. We determined the relationship between T-cell subsets and viral load in the cerebrospinal fluid (CSF) of therapy-naive, asymptomatic HIV-infected individuals. A shift from naive to effector-memory CD8 T cells was observed in the CSF of HIV-infected individuals compared with controls. The HIV load strongly correlated with CD8 T cells in CSF. Effector-memory CD8 T cells were positively and effector CD8 T cells were negatively associated with viral replication in CSF.

HIV-1 is a neurotropic virus that causes damage to the central nervous system (CNS) by a number of mechanisms [1]. The role of the adaptive immune system in controlling HIV infection of the CNS is still poorly understood. Invasion of T cells, in particular CD8 T cells, seems to be associated with viral infection of the parenchyma, suggesting that these cells are crucial in virus control [2]. Little is known, however, about the phenotypes of T cells in the CNS of HIV-infected therapy-naive individuals and their relationship to viral infection.

Twenty individuals with early-stage disease (all Caucasians; Centers for Disease Control and Prevention classifications A1, A2, B1, B2), who had never received antiretroviral therapy, and 53 HIV-negative patients affected by other neurological diseases were included in the study. The study was approved by the ethics committee of the Heinrich Heine University. All patients gave written informed consent.

Aliquots of 8–15 ml CSF were obtained by lumbar puncture. The median CSF cell number was 15.5 cells/ μ l (range 0.5–34) in HIV-infected donors and 25.75 cells/ μ l (range 1–229) in controls. CSF and blood cells were analysed by flow cytometry as described [3]. The following monoclonal antibodies were used: the combination of CD3-FITC (clone SK7*), CD4-APC (clone RPA-T4*), and CD8-PerCP (clone SK1*) for global T-cell analysis, and the combination of CD28-FITC (clone CD28.2[#]), CD8-PerCP (clone SK1*), CD45RO-APC (clone UCHL1*), and CD45RA-PE (clone ALB11[#]) for CD8 T-cell subset analysis (*BD Biosciences, San Jose, California, USA; [#]Immunotech, Marseille, France). At least 5000 events were acquired for each staining. The HIV load in the CSF and blood was determined in all HIV-infected individuals by reverse transcription polymerase chain reaction (Versant 3.0; Bayer Diagnostics, Tarrytown, New York, USA).

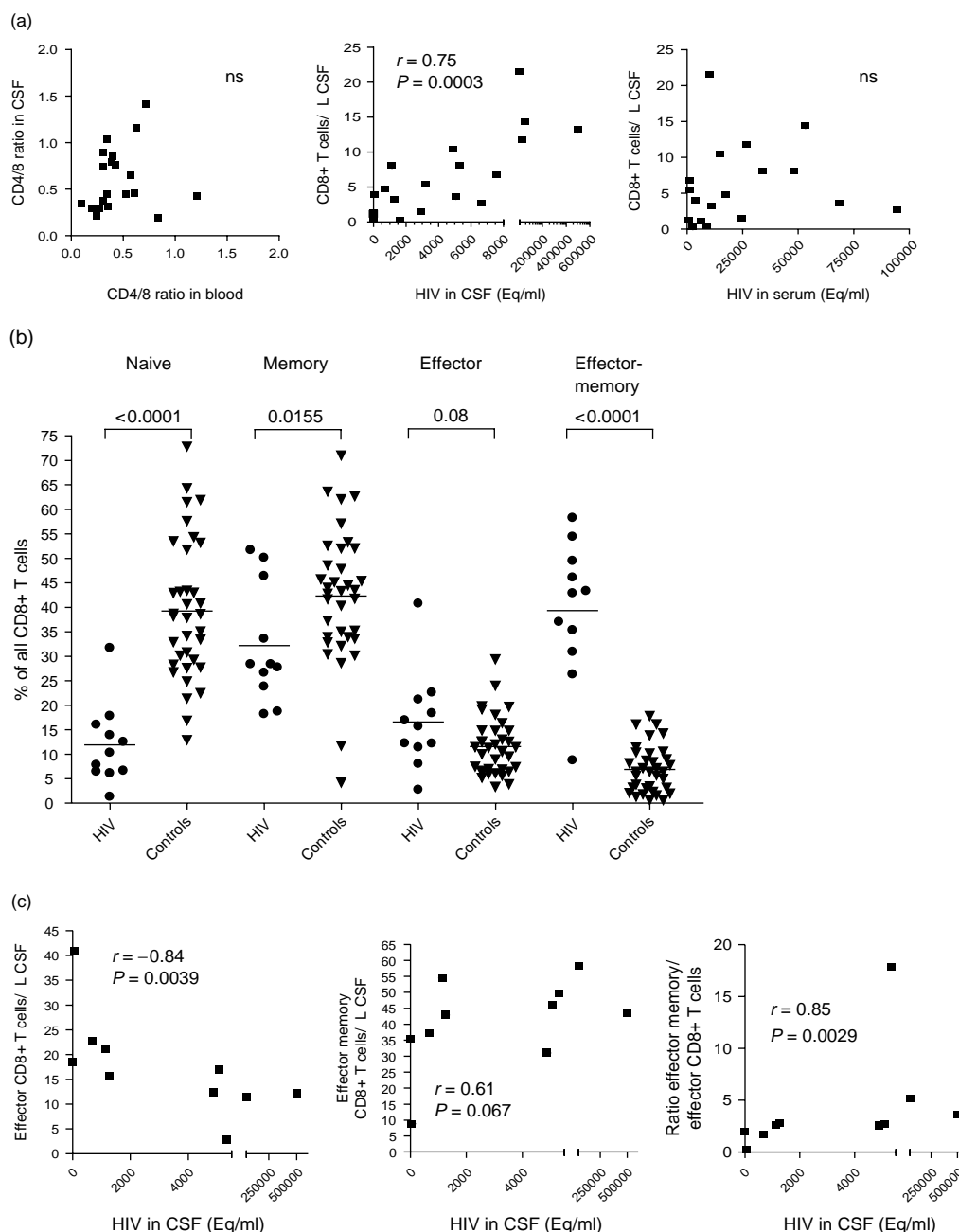


Fig. 1. CD8 T-cell subsets in the cerebrospinal fluid of therapy-naive HIV-infected individuals. (a) Correlation of CD4/CD8 ratios in blood and cerebrospinal fluid (CSF; left) and CD8 T cells in the CSF with HIV load in the CSF (middle) and blood (right) of therapy-naive asymptomatic HIV-infected individuals. Spearman’s rank test was used to determine r and P values. (b) Comparison of naive (CD45RA+CD28+), memory (CD45RO+CD45RA–CD28+), and effector (CD45RO+CD45RA–CD28–), and effector-memory (CD45RA+CD28–) CD8 T cells in therapy-naive HIV patients and HIV-negative controls. P values, determined by t -test, are displayed. (c) Correlation of effector-memory, effector CD8 T cells, and the effector-memory/effector ratio with CSF HIV load. Spearman’s rank test was used to determine r and P values.

HIV-infected therapy-naive individuals had lower CD4/CD8 ratios in the CSF (mean 0.604, range 0.19–1.41) and blood (mean 0.46, range 0.1–1.21) than controls. CD4/CD8 ratios in the CSF and blood did not correlate in HIV-infected individuals (Fig. 1a). We observed a weak correlation between HIV load in the CSF and blood of those patients ($r=0.4726$,

$P=0.0476$) but a strong correlation between the number of CD8 T cells and viral burden in the CSF (Fig. 1a). A weak correlation was also established between HIV load and CD4 T cells in the CSF ($r=0.46$, $P=0.036$). We found no correlation between HIV load in blood and any T-cell subset in the CSF including CD8 T cells (Fig. 1a).