BRIEF REPORT

Interleukin-10 Promoter Polymorphisms Influence HIV-1 Susceptibility and Primary HIV-1 Pathogenesis

Dshanta D. Naicker,[1,3] Lise Werner,[2] Emil Kormuth,[3] Jo-Ann Passmore,[4] Koleka Mlisana,[2] Salim Abdool Karim,[2] Thumbi Ndung'u,[1,2] and the CAPRISA Acute Infection Study Team

[1]Hasso Plattner Research Laboratory and [2]Centre for the AIDS Programme of Research in South Africa (CAPRISA), Doris Duke Medical Research Institute, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, [3]Department of Genetics, University of KwaZulu-Natal, Pietermaritzburg, and [4]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

Interleukin (IL)–10 directly inhibits human immunodeficiency virus type 1 (HIV-1) replication, but it may also promote viral persistence by inactivation of effector immune mechanisms. Here, we show in an African cohort that individuals with genotypes associated with high IL-10 production at 2 promoter single-nucleotide polymorphisms (−1082 and −592) were less likely to become HIV-1 infected but had significantly higher median plasma viral loads during the acute phase (≤3 months after infection). However, as the infection progressed, the association between genotype and median viral load was reversed. Thus, IL-10 may influence HIV-1 susceptibility and pathogenesis, but effects on the latter may differ according to the infection phase.

The interleukin-10 (IL-10) proximal promoter region contains 2 biallelic polymorphisms at positions −1082 (A to G transition) and −592 (C to A transversion), which are related to levels of IL-10 production [1, 2]. Polymorphisms associated with decreased IL-10 production have been associated with an increased likelihood of human immunodeficiency virus type 1 (HIV-1) acquisition and an accelerated rate of $CD4^+$ T cell decline, particularly in late-stage disease [3, 4], suggesting that high IL-10 production may reduce susceptibility to HIV-1 infection and protect against disease progression. Paradoxically, 2 recent studies of lymphocytic choriomeningitis virus (LCMV) in mice, a model of chronic viral infections, have demonstrated that removal of IL-10 or blockade of the IL-10 pathway may enhance T cell immune responses, resulting in the rapid elimination of virus and the development of antiviral memory T cell responses and culminating in LCMV clearance [5, 6]. The effect of IL-10 on HIV-1 replication in vivo is further complicated by the observation that the cytokine directly inhibits HIV-1 replication in human macrophages [7, 8]. The inhibitory effects of IL-10 on HIV-1 replication may become more pronounced in late stages of disease, when $CD4^+$ lymphocytes are depleted and replication in macrophages and monocytes predominates [9–11].

This study was undertaken to gain better insight into the complex role of IL-10 in HIV-1 infection and pathogenesis. Specifically, we investigated whether genetic polymorphisms in the proximal region of the IL-10 gene promoter contribute to HIV-1 susceptibility and to primary HIV-1 pathogenesis in an African cohort at high risk for infection.

Subjects, materials, and methods. The Centre for the AIDS Programme of Research in South Africa (CAPRISA) acute infection cohort was described in detail elsewhere [12]. This longitudinal cohort study on viral set point and clinical progression in HIV-1 subtype C infection was established in Durban, South Africa, in 2004. The cohort comprised 245 high-risk HIV-negative women who were screened monthly for HIV infection using 2 rapid antibody tests (Determine, Abbott Laboratories; Capillus, Trinity Biotech). Negative or indeterminate samples were subjected to pooled plasma polymerase chain reaction (PCR) testing, and positive pools were deconstructed and individually tested (COBAS AmpliScreen HIV-1 test, Roche Diagnostics). HIV-1 infection was further confirmed in RNA-positive samples with an HIV enzyme immunoassay test (Enzygnost), and a diagnostic nucleic acid test (Roche). $CD4^+$ T cell counts were determined by flow cytometry (Becton Dickinson).

Twenty-eight seroconverters from the HIV-negative cohort and 36 individuals from other seroincidence cohorts were enrolled into the acute infection phase (phase II) of the study. Once enrolled into phase II, participants were seen weekly for

Received 10 October 2008; accepted 12 February 2009; electronically published 17 June 2009.

Potential conflicts of interest: none reported.

Presented in part: XVII International AIDS Conference, Mexico City, Mexico, 3–8 August 2008 (abstract WEPDA102).

Financial support: National Institute of Allergy and Infectious Diseases, National Institutes of Health (U19 AI 51794 to CAPRISA 002 study); CAPRISA training fellowship (D.D.N.); Fogarty AIDS International Training and Research Program fellowship (TWO-0023 to D.D.N.); Hasso Plattner Foundation (T.N); South African Department of Science and Technology/National Research Foundation Research Chair in Systems Biology of HIV/AIDS (T.N.).

Reprints or correspondence: Dr. Ndung'u, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Private Bag X7, Congella, Durban 4001, South Africa (ndungu@ukzn.ac.za).

The Journal of Infectious Diseases 2009; 200:448–52

0022-1899/2009/20003-0017$15.00

DOI: 10.1086/600072

3 weeks, then fortnightly for 2 months, then monthly for 10 months, and quarterly thereafter. Thirty-nine participants in the HIV-negative cohort ($n = 245$) were unavailable for follow-up. In the HIV-positive cohort, 8 participants exited the study; 1 refused participation, 2 died accidentally, and 5 had to begin antiretroviral treatment. At the time of assessment, there were a total of 281 individuals in the study, of whom 259 had samples available for assessment. Sixty-four of 259 had acquired HIV-1 infection. Ethics approval was obtained from the ethics committee of the University of KwaZulu-Natal, and participants provided informed consent.

Genotype assessments for the 2 proximal IL-10 promoter single-nucleotide polymorphisms (SNPs; −1082 and −592 relative to the transcription start site) were performed using amplification refractory mutation system PCR, as described elsewhere [13].

The χ^2 test was used to compare allelic frequencies, confirming their fit to Hardy-Weinberg equilibrium. Fisher's exact test was used to analyze the association between HIV status and IL-10 polymorphic variants. Kaplan-Meier survival statistics and Cox proportional hazards model were used to assess the time to HIV-1 infection. Generalized estimating equation (GEE) models were used to compare the longitudinal measurements of viral loads and $CD4^+$ counts between different genotype groups. The GEE models were univariate models, unadjusted for other covariates but taking into account repeated measures. The covariance matrix for the GEE model was unstructured. Haplotype effects were analyzed by fitting the haplotype probabilities as continuous variables to viral load and CD4 counts, using GEE models.

Results. The mutant allele frequencies at SNPs −1082 and −592 in our cohort were 0.33 and 0.32, respectively. Allele frequencies were confirmed by χ^2 tests to be in Hardy-Weinberg equilibrium for the entire cohort and for subgroups analyzed in this study. Linkage disequilibrium estimates between the 2 SNPs were calculated, using Haploview, as $r^2 = 0.12$ and $D' = 0.70$ [14]. The SNPs specified 4 haplotypes, termed 1 (AC), 2 (AA), 3 (GC), and 4 (GA), which occurred at approximate frequencies of 37%, 30%, 29%, and 3%, respectively.

There was no significant difference in the distribution of IL-10 genotypes for the −1082 position between HIV-1–negative and HIV-1–positive groups ($P = .144$) (figure 1*A*). For the 222 participants who were enrolled while HIV negative and who were followed up prospectively, Kaplan-Meier survival analysis of time to HIV-1 seroconversion for genotypes at the −1082 position (figure 1*B*) showed no significant differences in the risk of HIV-1 acquisition between genotype −1082AA and genotype −1082GG or −1082AG. Using the Cox proportional hazards model, if an individual carried the −1082AA genotype the hazard ratio of becoming HIV-1 infected was 2.14 (95% confidence interval [CI], 0.99–4.63; $P = .054$).

However, there was a significant association between HIV-1 status and the IL-10 −592 genotype (figure 1*C*), owing to the large proportion of HIV-positive individuals who carried the −592AA genotype, compared with the other genotypes ($P = .003$). In the analysis of high-risk HIV-1–negative individuals followed up prospectively, those with the −592AA genotype were significantly more likely to become HIV infected than were those with the −592CA or −592CC genotype (figure 1*D*). Using the Cox proportional hazards model, individuals with the −592AA genotype were at increased risk of becoming HIV infected (hazard ratio, 3.0; 95% CI, 1.28–7.08; $P = .012$).

Three haplotypes in this cohort had frequencies of >5%: haplotypes 1, 2, and 3. Only haplotypes 2 and 3 showed significant association with HIV-1 status. In normal logistic regression analysis, haplotype 2, predicted to result in low IL-10 production, was associated with a 1.8-fold greater likelihood of being HIV positive (95% CI, 1.21–2.74; $P = .004$). In contrast, haplotype 3 (high IL-10 producer) was associated with a 0.59-fold lower likelihood of HIV acquisition (95% CI, 0.36–0.96; $P = .032$).

For the 64 individuals who became infected with HIV-1, we investigated whether viral load and $CD4^+$ T cell counts differed between the phases of infection according to IL-10 genotypes. At ⩽3 months after infection (the acute phase) and 6–12 months after infection (the early chronic phase), we compared median viral loads and $CD4^+$ T cell counts between the 3 genotypic groups for both SNPs at positions −1082 and −592 (figure 2).

Analysis of the overall change in viral load based on the −1082 genotype (figure 2*A*) shows that individuals with the −1082GG genotype (high IL-10 producer) had significantly higher median viral loads during the acute phase than those with the −1082AA ($P < .001$) or −1082AG ($P = .003$) genotype. During the early chronic phase, however, there was no longer a significant difference between the 3 groups. Analysis of the overall change in $CD4^+$ T cell count (figure 2*B*) showed that during the acute phase, the −1082GG group had the lowest median $CD4^+$ T cell count and that it was significantly different from that in the −1082AA group ($P = .012$). In comparison, during the early chronic phase of infection, the −1082GG group was not significantly different from the other groups.

During the acute phase, the −592CC group (ie, high IL-10 producers) did not have significantly different median viral loads than the −592CA and −592AA groups ($P = .129$ and .059, respectively) (figure 2*C*). During the early chronic phase, the −592AA group (ie, low IL-10 producers) had a significantly higher median viral load than the −592CA and −592CC groups ($P = .002$ and .008, respectively). However, there were no significant differences in $CD4^+$ T cell counts between −592 variants during either the acute or early chronic phase of infection (figure 2*D*).

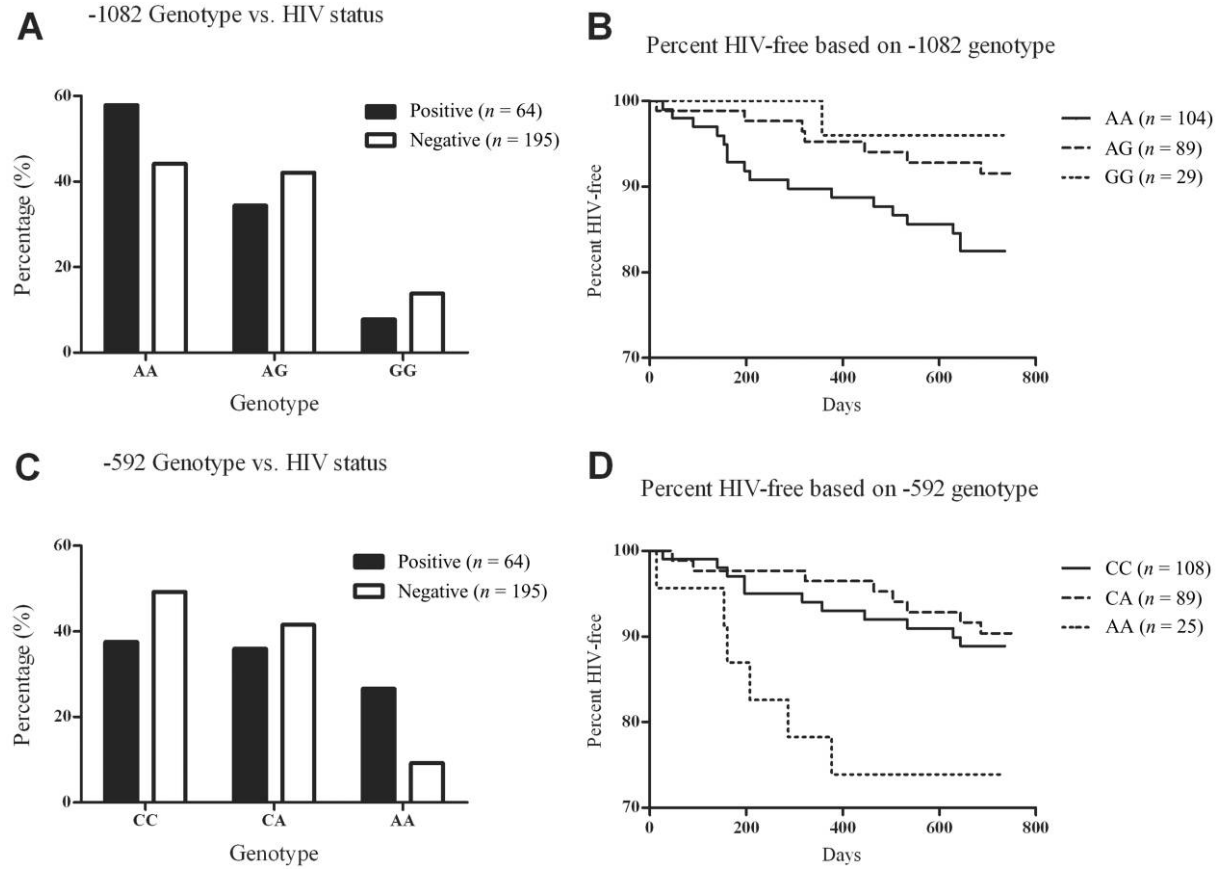

Figure 1. The influence of interleukin (IL)–10 promoter polymorphisms on human immunodeficiency virus type 1 (HIV-1) susceptibility. *A,* Association of −1082 genotype and HIV status. The distribution of genotypes for the −1082 position, based on HIV status, showed no significant association between IL-10 genotypes and HIV-1 status. *B,* Kaplan-Meier graph, analyzing time to infection, showing no significant differences between −1082 genotypes, although there was a trend toward higher likelihood of infection for the −1082AA genotype. *C,* Distribution of genotypes for the −592 position, based on HIV status, showing a significant association between IL-10 genotype and HIV status, because of the large number of HIV-positive individuals in the −592AA group ($P = .003$). *D,* Kaplan-Meier graph of time to infection showing that individuals with the −592AA genotype were significantly more likely to become HIV infected ($P = .012$).

During the acute phase of infection, haplotype 2 trended toward a negative effect on viral load ($P = .081$), whereas haplotype 3 had a significantly positive effect ($P < .001$). However, during the early chronic phase, haplotype 2 had a significantly positive effect on viral load ($P < .001$), and the positive effect of haplotype 3 was no longer significant ($P = .493$).

Discussion. Recently, the immunoregulatory cytokine IL-10 was identified as playing a key role in suppressing antiviral immune responses, leading to viral persistence [5, 6]. Neutralization of IL-10 has also been shown to result in enhanced T cell immune responses and viral clearance in the LCMV model of chronic viral infection [15]. However, 2 HIV-1 natural history studies of genetic polymorphisms in the IL-10 promoter appear to run counter to these recent findings by demonstrating that IL-10 promoter genetic polymorphisms associated with higher IL-10 production attenuate $CD4^+$ T cell loss and are protective against disease progression, albeit in long-term follow-up cohorts [5, 6].

In our South African cohort, carriers of the −592AA genotype were more likely to become HIV-1 infected. Our results are in agreement with those of Shin et al. [4], who demonstrated in a North American cohort that carriers of the −592A allele were at higher risk of HIV acquisition than individuals carrying the wild-type allele. These results generally suggest that IL-10 promoter polymorphisms linked to low IL-10 production are associated with increased HIV-1 susceptibility, although the effect of the −592 SNP appears to be stronger than that of the −1082 SNP, because the trend for the latter SNP did not reach statistical significance. These results are reinforced by the observation that lower IL-10 producer haplotypes were significantly more likely to become HIV-1 infected.

We also investigated the influence of IL-10 proximal promoter polymorphisms on primary HIV-1 pathogenesis. Generally, we found that higher-producing IL-10 polymorphisms were associated with high viral load during the acute infection phase. However, as infection progressed toward the chronic

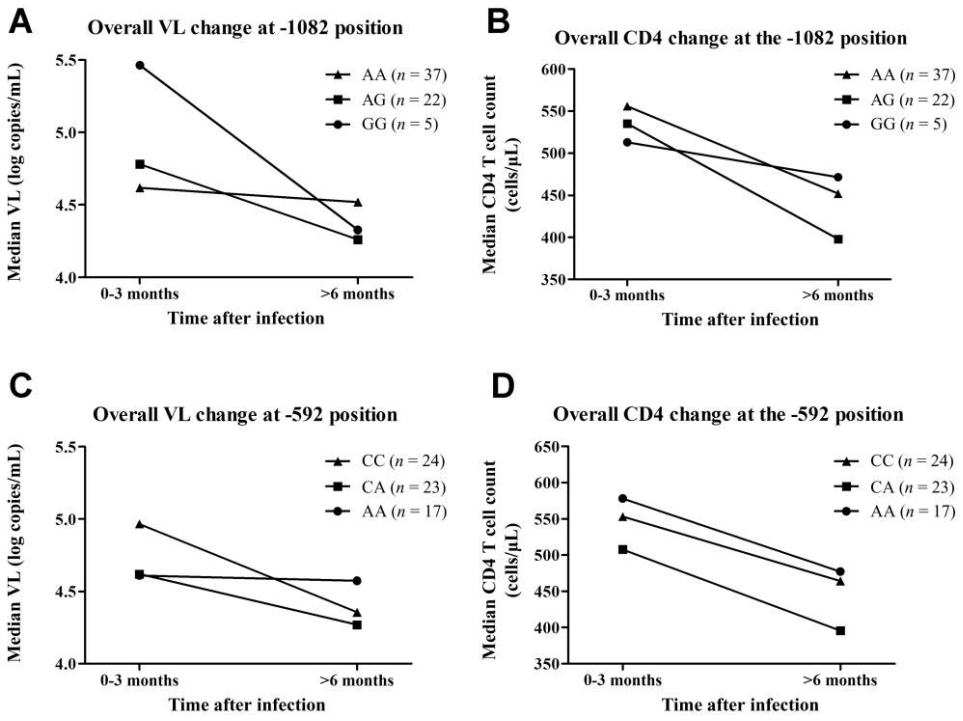

Figure 2. Influence of interleukin (IL)–10 promoter polymorphisms on primary human immunodeficiency virus type 1 (HIV-1) pathogenesis. *A,* Overall change in viral load (VL) over time, based on −1082 genotype. During the first 3 months after infection, the median viral load of the −1082GG group was significantly higher than those of the −1082AA and −1082AG groups ($P < .001$ and $P = .003$, respectively). After 6 months, there was no significant difference in median viral loads between the −1082GG group and either the −1082AA ($P = .758$) or −1082AG ($P = .280$) group. *B,* Overall change in CD4$^+$ T cell count over time, by −1082 genotype. During the first 3 months after infection, the −1082GG group had a significantly lower median CD4$^+$T cell count than the −1082AA group ($P = .012$). After 6 months, the median CD4$^+$ T cell count in the −1082GG group was not significantly different from that in the −1082AA or −1082AG group ($P = .274$ and $P = .474$, respectively). *C,* Overall change in viral load over time, based on −592 genotype. During the first 3 months after infection, the median viral load in the −592CC group was not significantly different from that in the −592AA or −592CA group ($P = .059$ and $P = .129$, respectively). After 6 months, the median viral load in the −592AA group was significantly different from those in the −592CC and −592CA groups ($P = .008$ and $P = .002$, respectively). *D,* Overall change in CD4$^+$ T cell count over time, by −592 genotype. There was no significant difference in median CD4$^+$ T cell count between groups at either the acute or the early chronic phase of infection.

phase, differences in viral load between the genotype groups either disappeared or reversed. Thus, during the acute phase of HIV-1 infection, our data are consistent with recent data in the LCMV model, in which IL-10 was shown to be a key player in inhibiting effector immune responses, leading to increased viral replication and persistence [5, 6] . However, as infection proceeded toward the chronic phase, we observed that viral load differences between genotypes disappeared, or a polymorphism associated with increased IL-10 production was also associated with lower plasma viral load and increased CD4$^+$ T cell count. We interpret this result to suggest that, as HIV-1 infection progresses to a chronic state, IL-10 may have a protective role against CD4$^+$ T cell loss and disease progression. This is in agreement with previous findings demonstrating that IL-10 promoter polymorphisms linked with increased IL-10 production are protective against CD4$^+$ T cell loss and disease progression [3, 4]. We acknowledge that our data need to be interpreted cautiously, given the small sample size of this study and the short follow-up period of the cohort. It is also obvious that mechanistic studies are needed to investigate the role of IL-10 in immune activation and HIV-1 replication in different phases of HIV-1 infection.

In conclusion, we propose a model for the role of IL-10 in HIV-1 susceptibility and disease progression, whereby high levels of IL-10 protect against HIV-1 infection, possibly by reducing immune activation and counteracting inflammatory processes that increase the pool of susceptible cells, and low levels of IL-10 may have the opposite effects. In acute HIV-1 infection, high IL-10 levels may promote viral replication by dampening innate and adaptive effector immune responses in a manner similar to that described for the LCMV model [5, 6]. Furthermore, we propose that, in chronic HIV-1 infection—particularly during the late stages of disease—IL-10 may be protective by reducing immune activation and inhibiting HIV-1 replication in macrophages. Given the importance of understanding the role of immune activation and dysregulation

in driving HIV-1 infection and pathogenesis, and considering that IL-10 has been reported as an integral player in these processes, the model proposed here warrants experimental scrutiny.

Acknowledgments

We thank the study participants and CAPRISA clinical and laboratory staff for providing specimens. We give special acknowledgments to the following members of the CAPRISA Acute Infection Study team: Carolyn Williamson, Lynn Morris, Clive Gray, Winston Hide, and Francois van Loggerenberg.

References

1. Kamali-Sarvestani E, Kiany S, Gharesi-Fard B, Robati M. Association study of IL-10 and IFN-gamma gene polymorphisms in Iranian women with preeclampsia. J Reprod Immunol **2006**; 72:118–26.
2. Lan Q, Zheng T, Rothman N, et al. Cytokine polymorphisms in the Th1/Th2 pathway and susceptibility to non-Hodgkin lymphoma. Blood **2006**; 107:4101–8.
3. Erikstrup C, Kallestrup P, Zinyama-Gutsire RB, et al. Reduced mortality and CD4 cell loss among carriers of the interleukin-10 -1082G allele in a Zimbabwean cohort of HIV-1-infected adults. AIDS **2007**; 21: 2283–91.
4. Shin HD, Winkler C, Stephens JC, et al. Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. Proc Natl Acad Sci USA **2000**; 97:14467–72.
5. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. Nat Med **2006**; 12:1301–9.
6. Ejrnaes M, Filippi CM, Martinic MM, et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med **2006**; 203:2461–72.
7. Ancuta P, Bakri Y, Chomont N, Hocini H, Gabuzda D, Haeffner-Cavaillon N. Opposite effects of IL-10 on the ability of dendritic cells and macrophages to replicate primary CXCR4-dependent HIV-1 strains. J Immunol **2001**; 166:4244–53.
8. Wang Y, Rice AP. Interleukin-10 inhibits HIV-1 LTR-directed gene expression in human macrophages through the induction of cyclin T1 proteolysis. Virology **2006**; 352:485–92.
9. Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections. Science **1997**; 276:1857–61.
10. Swingler S, Mann A, Jacque J, et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat Med **1999**; 5:997–103.
11. Verani A, Gras G, Pancino G. Macrophages and HIV-1: dangerous liaisons. Mol Immunol **2005**; 42:195–212.
12. van Loggerenberg F, Mlisana K, Williamson C, et al. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 Acute Infection Study. PLoS ONE **2008**; 3:e1954.
13. Opdal SH, Opstad A, Vege A, Rognum TO. IL-10 gene polymorphisms are associated with infectious cause of sudden infant death. Hum Immunol **2003**; 64:1183–9.
14. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics **2005**; 21:263–5.
15. Brooks DG, Lee AM, Elsaesser H, McGavern DB, Oldstone MB. IL-10 blockade facilitates DNA vaccine-induced T cell responses and enhances clearance of persistent virus infection. J Exp Med **2008**; 205: 533–41.