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INFLUENCE OF *KIR* IN THE HIV-1/AIDS DISEASE

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Abstract: Introduction: The aim of this study was to investigate NK cell count and the association of *KIR, HLA Class I* and KIR-HLA ligands with HIV infection and progression to AIDS. **Methods:** A total of 99 blood samples were collected from HIV-1 patients and 99 for the control group. The quantification of NK cells was performed by flow cytometry and the definition of the *KIR* and *HLA* genes was performed by PCR-SSOr. *HLA Class I* allele and haplotype frequencies were obtained using Arlequin software, and gene, genotype and haplotype frequencies of *KIR* and KIR-HLA ligands were obtained by direct counting. Statistical analyses were conducted using Chi-square test with Yates correction or Fisher's exact test for categorical variables and the Mann-Whitney test for continuous variables. **Results:** The NK cells count was lower in HIV group compared to controls. *HLA-A*68:02* allele was associated to susceptibility to HIV infection, and *HLA-A*23:01* and *HLA-A*32:01* were protective factor for HIV infection and for AIDS progression, respectively. The KIR3DL1-Bw4-80Ile*hmz* ligand and only the Bw4-80Ile*hmz* ligand group were associated to HIV infection. KIR2DS3/2DL3/C1 and the combination of inhibitory KIR2DL3-C1/3DL2-A3/A11 showed protection to HIV infection. **Conclusions:** The HIV infection interfered with NK cell count. In addition, our results confirmed HLA class I associations with HIV infection and AIDS progression, and suggest the influence of activating and inhibitory KIRs and its HLA class I ligands on course of HIV infection.

Keywords: HIV-1; Genes, MHC Class I; Major Histocompatibility Complex; Polymorphism, Genetic; Receptors, KIR; Killer Cells, Natural.

INTRODUCTION

Human immunodeficiency virus (HIV) is a epidemic infection. This virus has the ability to evade the immune response and cause AIDS. There are approximately 38 million people living with HIV in the world, and this infection has caused more than 40.1 million deaths since the start of the epidemic.[1]

Special attention has been dedicated to the role of the innate immune system in fighting HIV infection, in an effort to better understand the importance of natural killer (NK) cell response in preventing disease progression and fighting infection. NK cells are a heterogeneous population of cytotoxic lymphocytes and producing cytokines. They originate in the bone marrow and represent about 5-15% of total mononuclear cells in blood circulation under normal conditions. The function of NK cells is to provide a first-line defensive response, having the capacity to detect and lyse virus-infected cells without prior sensitization.[2, 3]

These cells can be divided into two subpopulations in healthy individuals: (i) $CD56^{bright}CD16^{neg}$ ($CD56^{bright}$) lymphocytes that have high capacity of productuin of regulatories cytokine and chemokine and (ii) $CD56^{dim}CD16^{neg}$ ($CD56^{dim}$) lymphocytes that have high cytotoxicity activity.[3-6] $CD56^{bright}$ lymphocytes correspond to 5-10% of NK cells and $CD56^{dim}$ to up 90%. The absolute number of circulating NK cells increses during the acute fase of the HIV infection and is restored in the chronic phase, however the distribuition of cells are changed. In chronic phase appears a third lymphocyte subset, $CD56^{neg}$ $CD16^{pos}$ ($CD56^{neg}$), which is rare in healthy individuals and has no cytokine production and secretion capacity that affects the antiviral response of these cells.[7-9]

In addition, NK cell activity depends on the NK cell receptors (NKRs) interation with their ligands in targed cells. These interations generate signals that can inhibit or stimulate the NK activities. Killer Immunoglobulin-

like Receptors (KIR) are one of the NKRs that interact with their HLA ligands (Human Leukocyte Antigen) present in the target cells. These receptors are members of a group of regulatory molecules present on the surface of NK cells, and the balance between NK cell activation and inhibition occurs by binding of KIR with HLA Class I molecules present in all nucleated cells. For effective action on target cells, NK cells depend on their ability to recognize abnormal expression, which may be the reduction or absence of HLA molecules on the surface of these cells, differentiating them from normal or the body's own cells that constitutively express HLA molecules.[3, 8, 10]

KIR genes encode transmenbrane proteins with two (2D) or three (3D) immunoglobulin (Ig)-like domains that can send inhibitory or activating signals. Inhibitory KIRs have long (L) cytoplasmic tails and have the capacity to inhibit cellular activity: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2 and KIR3DL3. Activating KIRs have short (S) cytoplasmic tails and send activating signals: KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5 and KIR3DS1. Two pseudogenes (2DP1 and 3DP1) was also been identified and KIR2DL4 can act as an activating or inhibitory gene.[11, 12]

The KIR-HLA ligands interactions were associated with susceptibility or protection against infectious diseases, including HIV/AIDS. All available evidence indicates that HLA is the most significant molecule in the differential control of HIV in humans.[3, 8] Thus, identifying the behavior of NK cells in terms of their number, associations of HLA and KIR, and investigating other possible associations may help to predict disease progression rate and to design models which can lead to the development of new therapies and vaccines. Therefore, the objective of this study was to investigate NK cell count and the association of KIR, HLA class I and KIR-HLA ligands with HIV infection and progression to AIDS.

MATERIALS AND METHODS

SAMPLE SELECTION

The studied population (N=198) is from the North and Northwest regions of the state of Paraná (22°29'30"-26°42'59"S and 48°02'24"-54°37'38"W), Southern Brazil, and participants were included in the study after signing the informed consent form previously approval by the Committee on Ethics in Research in Human Beings (COPEP) of the State University of Maringá (UEM; number 1022484/2015). This was a cross-sectional study based on convenience (time and location) samples obtained consecutively and serially and data were collected from April to December 2015.

Patients infected with HIV-1 (N=99) were diagnosed and attended at the Specialized Care Service (SAE) for HIV/AIDS in Maringá, Paraná, Brazil, and they were from 29 cities served by 15th Regional Health area of the State of Paraná. Viral load and CD4+/CD8+ were performed at the Teaching and Research Laboratory of Clinical Analysis (Lepac) at the State University of Maringá. Data on patients' epidemiological characteristics (age, gender, viral load, CD4+ and CD8+) were collected in the SAE records. HIV patients were classified into two groups according to the adapted CDC criterion: *i.* AIDS group, composed of 86 individuals with TCD4+ lymphocyte count <350 cells/mm^3; and *ii.* Non-AIDS group, composed of 13 individuals with with TCD4+ lymphocyte count >350 cells/mm^3.[13] The inclusion criteria of patients were over 18 years old, both gender, in using of antiretroviral therapy and negative for hepatitis B and C.

HIV infection was diagnosed according to flowchart six of Technical Manual for the Diagnosis of HIV Infection of the Ministry

of Health.[14] The initial test was performed by the ARCHITECT HIV Ag/Ab Combo kit (Abbott, Wiesbaden, Germany) and for reactive samples the complementary test was performed using the Immunoblot Rapido DPP® HIV 1/2 kit (Institute of Technology in Immunobiologicals, Bio-Manguinhos, Rio de Janeiro-RJ, Brazil). In case of conflicting results, Viral Load for HIV was performed as an additional supplementary test by RealTime HIV kit (Abbott Molecular, Inc., Des Plaines, USA).

The control group comprised 99 individuals non-HIV-1. The negative HIV infection was checked in the Logistical Control of Medicines System (SICLOM) and the Laboratory Tests Control System of the National Network of Lymphocyte Count CD4 +/CD8 + and viral load (SISCEL), both maintained by the Health Ministry. The inclusion criteria of controls were over 18 years old, both gender and negative for hepatitis B and C. Control and patients subjects were the same region and were matched by gender and age.

Due to the differences in the distribution of *HLA* and *KIR* allele frequencies in different populations and ethinic groups, the criterion of non-inclusion was individual of oriental origin. Because the great miscegenation among Brazilians, the studied population was classified according to Probst et al. (2000) and confirmed for our region.[15,16]

NK CELL QUANTIFICATION

NK cell quantitation was performed on peripheral blood collected in 5 mL vacuum tubes containing EDTA by flow cytometry using the Tritest kit CD3 FITC/CD16 + CD56 PE/CD45 PerCP Reagent (BD Biosciences, San Jose, CA) on a FACSCalibur device (Becton-Dickinson, NJ, USA) according to manufacturer's instructions. Results were expressed as cells/mm^3.

QUANTIFICATION OF VIRAL LOAD FOR HIV-1

The viral load quantification methodology for HIV-1 was performed using the Real Time Polymerase Chair Reaction method using the Abbott RealTime HIV kit, with a detection limit of 40 copies/mL and *m*2000*sp* and *m*2000*rt* automation (Abbott Molecular, Inc., Des Plaines, EUA).

DNA EXTRACTION

Genomic DNA was extracted from buffy coat by the Biopur® DNA extraction kit (Biometrix, Curitiba, Paraná, Brazil). The concentration and quality of DNA samples were analyzed by a NanoDrop® 2000 UV-Vis spectrophotometer (Wilmington, USA). DNA concentration and integrity were confirmed on agarose gel at 2%, and the concentrations were adjusted to 20 ng/μL.

TECHNICAL GENOTYPING

The samples were typed for *KIR* and *HLA-A, B* and *C* genes by the SSOR-PCR methodology (polymerase chain reaction - sequence specific Oligonucleotide Reverse), Luminex technology (One Lambda®, Canoga Park, CA, USA), using the LABType® SSO kit (One Lambda®, Canoga Park, CA, USA) for *KIR* genes and LABType® higher resolution (HD) kit (One Lambda®, Canoga Park, CA, USA) for *HLA-A* and *HLA-B*, according to manufacturer's instructions. For *HLA-C* the LABType® SSO kit (One Lambda®, Canoga Park, CA, USA) was used. When *HLA class I* alleles were not defined using the analysis software, the linkage disequilibrium, allele ligation and allele frequency distribution were used to define them.

STATISTICAL ANALYSIS

Allele and haplotype frequencies for *HLA Class I* were obtained using Arlequin

software 3.5.1.2 (http://cmpg.unibe.ch/software/arlequin35), Population Genetics Lab CMPG[17] and these values were used to calculate relative linkage disequilibrium (LD) between HLA class I haplotypes. Gene, genotype and, haplotype of *KIR* frequencies, and KIR-HLA ligand frequencies were obtained by direct counting. The Arlequin software 3.5.1.2[17] was used for estimating the distribution of *HLA class I* genotype frequencies according to the Hardy-Weinberg (HW) equilibrium. As the presence or absence of KIR genes was analysed, the *KIR2DL2/3* and *KIR3DL1/S1* were used to estimating Hardy-Weinberg equilibrium for *KIR* genes because they segregate as alleles.[18, 19] The haplotype frequencies were estimated using the implementation of the expectation–maximization (EM) algorithm include in the statistical package.

To analyze the influence of KIR with their respective ligands, patients and controls were classified into groups according to their HLA ligands: HLA-A3 or A11, HLA-Bw4 (Bw4-80Ile and Bw4-80Thr) (20, 21) and HLA-C ligand of the C1 or C2 group. [22] The *KIR* haplotype groups (AA, Bx) and genotype ID were obtained from the Allele Frequency Net Database (http://www.allelefrequencies.net/kir6001a.asp). The genotype AA is characterized by the absence of genes *2DL2, 2DL5, 3DS1, 2DS1, 2DS2, 2DS3, 2DS5* and the presence of any of these, genotype is taken as having B.

The Chi-square test with Yates correction or Fisher›s exact test two-tailed, using the 2x2 contingency table, with a confidence interval (CI) of 95%, was performed to comparison between patients and control groups. The risk of HIV infection and the development of AIDS was calculated by determining the odds ratio (OR) for values of P <0.05, using the OpenEpi program version 3.03a (http://www.openepi.com/TwobyTwo/TwobyTwo.htm). The Mann Whitney test was used to compare continuous data using the Bioestat program. [23] P-values less than or equal to 0.05 were considered statistically significant and were corrected by the Bonferroni inequality method for multiple comparisons (Pc). The Pc was not applied for variants that have been previously associated with HIV in any other populations.

RESULTS

The clinical characteristics of the studied subjects are shown in Table 1. HIV and control groups were matched for age and gender.

NK CELL COUNT AND VIRAL LOAD

Data on NK cell count and viral load are shown in Table 2.

The absolute number of NK cells, their percentage in relation to total of lymphocyte, and total lymphocyte count was determined in HIV-1, AIDS and Non-AIDS patients and controls. All of these parameters were lower in HIV patients (266 cells/mm^3, 13.25%; 2.144 cells/mm^3, respectively) compared to the control group (333 cells/mm^3; 14.51%; 2.316 cells/mm^3, respectively). When AIDS group was compared to non-AIDS group, the percentage of NK cells in relation to total lymphocyte was higher in the AIDS group (13.48% *vs.* 7.76%). The absolute number of NK cells and total lymphocyte were similar in the AIDS and Non-AIDS groups. Viral load was detected in 22.2% of patients from the HIV group, 23.2% from AIDS group and in 15.4% of patients from the Non-AIDS group, and there was no statistical difference between groups. All date are shown in Table 2.

HLA ASSOCIATION

The distribution of *HLA-A, HLA-B* and *HLA-C* genotype frequencies was in Hardy-Weinberg equilibrium for HIV (P = 0.74, P = 0.09 and P = 0.63, respectively) and for

control groups (P = 0.58, P = 0.55 and P = 0.68, respectively).

Some alleles and haplotypes were found to be associated with HIV infection (HIV *vs.* controls, Table 3) and were associated to the AIDS (AIDS group *vs.* the Non-AIDS group; Table 4).

When HIV patients were compared to controls, the *HLA-A*23:01* allele frequency was lower in HIV pacients (P = 0.05, OR = 0.26, 95% CI = 0.07 - 0.95) *HLA-A*68:02* allele frequency (P = 0.01, OR = 13.41, 95% CI = 1.47 - Undefined) and *HLA-A*68:01_C*06:02* haplotype frequency (P = 0.03, OR = 11.28, 95% CI = 1.47 - Undefined) were higher in the HIV group. There was no linkage disequilibrium between *HLA-A*68:01* and *HLA-C*06:02* (D' = 0.33, P = 0.21).

*HLA-A*32:01* allele frequency (P = 0.05, OR = 0.20, 95% CI = 0.05 - 0.76), *HLA-B*07:02* allele frequency (P = 0.02, OR = 0.13, 95% CI = 0.03 - 0.56) and the *HLA-B*07:02_C*07:02* haplotype frequency (P = 0.02, OR = 0.13, 95%CI = 0.03 - 0.56) were higher in the Non-AIDS group compared to the AIDS group. There was no linkage disequilibrium between *HLA-B*07:02* and *HLA-C*07:02* (D' = 0.48, P = 0.68)

HLA allele frequencies for all studied population are shown in the Table S1.

KIR AND *HLA* LIGAND ASSOCIATION

The distribution of *KIR* genotype frequencies for the HIV (*KIR2DL2/3* P = 0.82, *KIR3DL1/S1* P = 0.80) and control groups (*KIR2DL2/3* P=0.47, *KIR3DL1/S1* P = 0.77) was according to HWE. To verify the association of the *KIR,* their HLA class I ligands, and KIR-HLA ligands with HIV and progression to AIDS, the following frequency distributions were analyzed in HIV, AIDS, Non-AIDS and control group: *KIR* genes and AA and Bx haplotypes; HLA ligands; KIR-HLA ligands; number of KIR-HLA activating and inhibitory ligands; and *KIR* genotypes. All significant results are shown in Table 5 and all results can be found in Tables S2 – S5.

The gene frequency distributions of *KIR* in the studied groups are shown in Table S2. There were no difference in the distribution of frequencies for *KIR* genes and for AA and Bx haplotypes between HIV, AIDS, Non-AIDS and control groups.

The distribution of frequencies for HLA ligands of KIR are shown in Table S3. The frequency of the Bw4-80Ile ligand in homozygosis (Bw4-80Ile*hmz)*, considering only the *HLA-B* locus, was higher in HIV group compared to controls (P = 0.03, OR = 1.71 95% CI = 1.30 – 2.33) (Table 5).

The frequency distributions of KIR-HLA ligands are shown in Table S4. The frequency of the combination of KIR3DL1 with its Bw4-80Ile*hmz* ligand, considering only *HLA-B* locus, was higher in HIV group than in controls (P = 0.01, OR = 11.01, 95% CI = 1.38 – 87.74) (Table 5). The frequecy of the KIR2DS3 and KIR2DL3 in the presense of the C1 ligand (2DS3/2DL3/C1) was lower in HIV than in controls (P = 0.03, OR = 0.41, 95% CI = 1.38 – 87.74) (Table 5).

It was analyzed the number of KIR-HLA class I activating and inhibitory ligands to verify if there is an association with HIV or AIDS development. The combination of KIR2DL3 and KIR3DL2 in the presence of their respective ligands (2DL3-C1/3DL2-A3/A11) had lower frequency in HIV group than controls (P = 0.03, OR = 0.44, 95% CI = 0.21-0.90) (Table 5).

The frequency of *KIR* genotypes was also analyzed and all results are shown in Table S5. The frequency of the genotype Bx ID 73 was higher in HIV group compared to control group (P = 0.05, OR = 9.38, 95% CI = 0.93 – undefined) (Table 5).

DISCUSSION

In HIV-1, NK cells have demonstrated potential viral replication control; during the acute phase of the disease, these cells increase in response to infection[8] and are restored to similar levels of healthy individuals or decrease in the chronic phase of the infection although their cytotoxic activity and the ability to secrete cytokine were reduced.[24, 25] It was proposed that the NK cells subsets ($CD56^{dim}$, $CD56^{bright}$ and $CD56^{neg}$) can influence the total population of NK cells. [7] There was a decrease of $CD56^{dim}$ and $CD56^{bright}$ cells in chronic HIV infection by the increase of $CD56^{neg}$.[9] The $CD56^{neg}$ NK cells have defective in the production and secretion of important immune regulatory cytokines. [3, 7, 9] This NK cells alteration is reversible after antiviral therapy (ART). [26] Our study shows that NK cells were reduced in HIV patients, according to literature[25], although subset of NK cells was not investigated.

The extensive HLA polymorphism can make individuals more resistant or susceptible to inflammatory conditions, autoimmune disease, and infectious diseases, including HIV.[27] HLA is responsible for presenting HIV epitopes for Cytotoxic T Lymphocytes (CTLs) and T CD4+ cells, in addition to interacting with KIR on NK cells and mediating innate immunity against HIV.[8] HLA alleles and haplotypes are involved in delaying or accelerating AIDS progress or even providing resistance to HIV infection.[28]

Our results confirm the association of *HLA-A*68:02* allele with HIV infection. This association was previously observed by Song et al. (2011) in a study evaluating the impact of *HLA Class I* genes in HIV acquisition by serodiscordant heterosexual couples[29], where this allele was found to fail in activate CTLs and provide susceptibility to HIV infection. This allele was also related to the delay AIDS progression in another study.[28] We also found that the *HLA-A*68:01_C*06:02* haplotype was associated to the risk in the HIV infection. The association of *HLA-A*68:01* was not found in literature and *HLA-C*06:02* was associated with protection.[30] Another association observed was between *HLA-A*23:01* and protection against HIV infection. *HLA-A*23:01* belongs to Bw4-80Ile ligand and can bind in the KIR3DL1 and/or 3DS1 in NK cell, and in this condition could play protective effect directing the NK cell for to exercising citotoxic activity. **However,** differently to our results, this allele was previously associated with susceptibility to infection[31], risk for HIV-1 transmission[32], accelerating disease progression to AIDS,[33] and with rapid seroconversion in HIV positive patients.[28]

About to the AIDS progression, although the lower number of non-AIDS patients in this study, the *HLA-A*32:01* was associated to protection against to disease progression, similar to other study where this allele was associated with lower viral load in Caucasians. [34] The protective HLA alleles may present highly conserved HIV-1 epitopes to elicit strongr protective cellular immunity.[33] Besides, this alelle belongs to Bw4-80Ile ligand group of KIR, which serves as a ligand for KIR3DS1 and KIR3DL1 on NK, contributing to the balance between the activation and inhibition of these cells before adaptive imune responses. The NK cells activation provide protection to HIV infection and the inhibition provide susceptibility.[27] Another result that we found is that *HLA-B*07:02* alelle and the *HLA-B*07_C*07* haplotype were protective for HIV progression. These results are not in accordance with the literature that links this alelle and haplotype with susceptibility to disease.[30]

According to KIR and HLA ligands, two important results are highlighted: the KIR3DL1-Bw4-80Ile*hmz* and the Bw4-80Ile*hmz* were susceptibility factors to

HIV infection. In individuals expressing KIR3DL1 and its HLA ligand, Bw4-80Ile, is possible to found more NK cell inhibition,[3] and this factor could provide susceptibility to HIV infection. We were also able to observe susceptibility to HIV infection when Bw4-80Ile was in homozygosis. The NK cells of individuals carrying this combination of KIR3DL1-Bw4 ligand in heterozygosis proved to be more responsive than those carrying two Bw4 copies.[35] It is known that HLA class I ligands, which is codominantly expressed, is downregulated on cell surface by HIV. After the downregulation, Bw4 heterozygous individuals do not present enough Bw4 ligand to inhibit NK cells. In homozygosis, even with the downregulation of HLA-B, a greater number of ligands is expressed on the cell surface and is sufficient to trigger the inhibitory effect of the NK cell contributing to the risk for HIV infection.[35]

In the present study, the KIR2DS3 and KIR2DL3 in the presense of C1 ligand (2DS3/2DL3/C1) and the combination of KIR2DL3 and KIR3DL2 in the presence of their respective ligands (2DL3-C1/3DL2-A3/A11) show a protection to HIV infection. It is known that HIV infected cells downregulate HLA-A, -B by Nef HIV protein, and more recently was demostrated that Vpu HIV protein can do the HLA-C downregulation.[35, 36] It was demonstrated that KIR2DL2 linked to HLA-C allows to HIV scape from NK by its reduced cell function.[33] However, the KIR2DL3 associated with its C1 ligand appears to result in a protective effect in HIV acquisition.[37] In our study the presence of inhibitory KIR2DL3 and activating KIR2DS3 associated with C1 ligands appears to give a protective effect to HIV acquisition. A possible explanation is that KIR2DL3 binds HLA-C with lower affinity resulting in weak NK cell inhibition. In this way a protective effect can be observed in respost to a weak NK cell inhibition.[33, 38] In addition, this protection was achieved in the presence of the activating KIR2DS3 (2DS3/2DL3/C1). This same mechanism can explain the protective relationship found in the combination of KIR2DL3 and KIR3DL2 in the presence of their respective ligands (2DL3-C1/3DL2-HLA-A3/A11).

The non-investigation of NK cells subset and the low number of individuals in the non-AIDS group can be the limitations of the study. We demostratred that NK cells were reduced in HIV patients and further studies could investigate NK cells subsets ($CD56^{dim}$, $CD56^{bright}$ and $CD56^{neg}$) in this pupolation. Also, the association of the KIR and HLA genes with the progression to AIDS could be better investigated by increasing the number of the non-AIDS group.

CONCLUSIONS

NK cell count was lower in the HIV patients in cronic phase of the disease compared to controls. The associations of HLA class I with HIV infection and AIDS confirm the importance of HLA in controlling HIV infection and progression to AIDS through this interation with Cytotoxic T Lymphocytes. Some KIR - HLA class I ligand were associated to susceptibiliy and protection to HIV infection, showing that HLA-Class I can also interact with KIR on NK cells surface and can activate or inhibit this cell and influence the HIV infection outcome. The Bw4-80Ile*hmz* ligand isolated and associated with its KIR3DL1 was associated with susceptibility to HIV infection and the associations KIR2DS3/2DL3/C1 and KIR2DL3-C1/3DL2-A3/A11 show a protective effect.

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REFERENCES

1. UNAIDS - Global HIV & AIDS statistics - 2021 fact sheet. (n.d.). Retrieved September 18, 2022 from https://www.unaids.org/en/resources/fact-sheet.

2. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331(6013):44-9.

3. Mikulak J, Oriolo F, Zaghi E, Di Vito C, Mavilio D. Natural killer cells in HIV-1 infection and therapy. AIDS (London, England). 2017;31(17):2317-30.

4. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001;22(11):633-40.

5. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. J Immunol. 1986;136(12):4480-6.

6. Lanier LL, Phillips JH, Hackett J, Jr., Tutt M, Kumar V. Natural killer cells: definition of a cell type rather than a function. J Immunol. 1986;137(9):2735-9.

7. Mavilio D, Lombardo G, Benjamin J, Kim D, Follman D, Marcenaro E, et al. Characterization of CD56–/CD16+ natural killer (NK) cells: A highly dysfunctional NK subset expanded in HIV-infected viremic individuals. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(8):2886-91.

8. Martin MP, Carrington M. Immunogenetics of HIV disease. Immunol Rev. 2013;254(1):245-64.

9. Alter G, Teigen N, Davis BT, Addo MM, Suscovich TJ, Waring MT, et al. Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. Blood. 2005;106(10):3366-9.

10. Vivier E, Romagne F. Good news, bad news for missing-self recognition by NK cells: autoimmune control but viral evasion. Immunity. 2007;26(5):549-51.

11. Finton KA, Strong RK. Structural insights into activation of antiviral NK cell responses. Immunol Rev. 2012;250(1):239-57.

12. Carrington M, Norman P. The KIR gene cluster. 2003.

13. Ministério da Saúde - Critérios de definição de casos de AIDS em adultos e crianças. (2004). Retrieved fom http://bvsms.saude.gov.br/bvs/publicacoes/criterios_definicao_AIDS_adultos_criancas.pdf.

14. Ministério da Saúde - Manual Técnico para Diagnóstico da Infecção pelo HIV em Adultos e Crianças. (2018). Retrieved from: file:///C:/Users/Elvis/Downloads/manual_tecnico_hiv_27_11_2018_web%20(1).pdf.

15. Reis PG, Ambrosio-Albuquerque EP, Fabreti-Oliveira RA, Moliterno RA, de Souza VH, Sell AM, et al. HLA-A, -B, -DRB1, -DQA1, and -DQB1 profile in a population from southern Brazil. Hla. 2018;92(5):298-303.

16. Probst CM, Bompeixe EP, Pereira NF, de ODMM, Visentainer JE, Tsuneto LT, et al. HLA polymorphism and evaluation of European, African, and Amerindian contribution to the white and mulatto populations from Parana, Brazil. Hum Biol. 2000;72(4):597-617.

17. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular ecology resources. 2010;10(3):564-7.

18. Trundley A, Frebel H, Jones D, Chang C, Trowsdale J. Allelic expression patterns of KIR3DS1 and 3DL1 using the Z27 and DX9 antibodies. Eur J Immunol. 2007;37(3):780-7.

19. Uhrberg M, Parham P, Wernet P. Definition of gene content for nine common group B haplotypes of the Caucasoid population: KIR haplotypes contain between seven and eleven KIR genes. Immunogenetics. 2002;54(4):221-9.

20. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh Steven GE. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Research. 2014;43(D1):D423-D31.

21. Ayo CM, Frederico FB, Siqueira RC, Brandao de Mattos CC, Previato M, Barbosa AP, et al. Ocular toxoplasmosis: susceptibility in respect to the genes encoding the KIR receptors and their HLA class I ligands. Sci Rep. 2016;6:36632.

22. Alves HV, de Moraes AG, Pepineli AC, Tiyo BT, de Lima Neto QA, Santos TDS, et al. The impact of KIR/HLA genes on the risk of developing multibacillary leprosy. PLoS Negl Trop Dis. 2019;13(9):e0007696.

23. Ayres M. BioEstat: Aplicações estatísticas nas áreas das ciências bio-médicas (Software). Version 5.3. 2009.

24. Brunetta E, Hudspeth KL, Mavilio D. Pathologic natural killer cell subset redistribution in HIV-1 infection: new insights in pathophysiology and clinical outcomes. J Leukoc Biol. 2010;88(6):1119-30.

25. Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination. J Leukoc Biol. 2008;84(1):27-49.

26. Mavilio D, Benjamin J, Daucher M, Lombardo G, Kottilil S, Planta MA, et al. Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(25):15011-6.

27. Goulder PJR, Walker BD. HIV and HLA class I: an evolving relationship. Immunity. 2012;37(3):426-40.

28. Bardeskar NS, Mania-Pramanik J. HIV and host immunogenetics: unraveling the role of HLA-C. Hla. 2016;88(5):221-31.

29. Song W, He D, Brill I, Malhotra R, Mulenga J, Allen S, et al. Disparate Associations of HLA Class I Markers with HIV-1 Acquisition and Control of Viremia in an African Population. PLoS ONE. 2011;6(8):e23469.

30. Peterson TA, Kimani J, Wachihi C, Bielawny T, Mendoza L, Thavaneswaran S, et al. HLA class I associations with rates of HIV-1 seroconversion and disease progression in the Pumwani Sex Worker Cohort. Tissue Antigens. 2013;81(2):93-107.

31. Olvera A, Pérez-Álvarez S, Ibarrondo J, Ganoza C, Lama JR, Lucchetti A, et al. The HLA-C* 04: 01/KIR2DS4 gene combination and human leukocyte antigen alleles with high population frequency drive rate of HIV disease progression. AIDS. 2015;29(5):507-17.

32. MacDonald KS, Fowke KR, Kimani J, Dunand VA, Nagelkerke NJ, Ball TB, et al. Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. J Infect Dis. 2000;181(5):1581-9.

33. Crux NB, Elahi S. Human Leukocyte Antigen (HLA) and Immune Regulation: How Do Classical and Non-Classical HLA Alleles Modulate Immune Response to Human Immunodeficiency Virus and Hepatitis C Virus Infections? Front Immunol. 2017;8:832.

34. Fellay J, Ge D, Shianna KV, Colombo S, Ledergerber B, Cirulli ET, et al. Common genetic variation and the control of HIV-1 in humans. PLoS Genet. 2009;5(12):e1000791.

35. Bernard NF. Natural killer (NK) cell receptor-HLA ligand genotype combinations associated with protection from HIV infection: investigation of how protective genotypes influence anti HIV NK cell functions. AIDS Res Ther. 2017;14(1):38.

36. Apps R, Del Prete GQ, Chatterjee P, Lara A, Brumme ZL, Brockman MA, et al. HIV-1 Vpu Mediates HLA-C Downregulation. Cell Host Microbe. 2016;19(5):686-95.

37. Zwolinska K, Blachowicz O, Tomczyk T, Knysz B, Gasiorowski J, Zalewska M, et al. The effects of killer cell immunoglobulin-like receptor (KIR) genes on susceptibility to HIV-1 infection in the Polish population. Immunogenetics. 2016;68(5):327-37.

38. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science. 2004;305(5685):872-4.

TABLES

		HIV N=99	AIDS N=86	Non-AIDS N=13	Control N=99
Age	Mean	43.9	45.3	35.1	44.4
	SD	± 11.4	± 11.0	± 10.3	± 15.4
		n (%)	n (%)	n (%)	n (%)
Gender	Male	47 (47.5)	41 (47.7)	6 (46.2)	47 (47.5)
	Female	52 (52.5)	45 (52.3)	7 (53.8)	52 (52.5)

N: number of individuals; SD: Standard Deviation

HIV, AIDS and Non-AIDS were were matched for age and gender with control (P>0.05).

Table 1. Characteristics of studied subjects: HIV, AIDS, Non-AIDS and Control groups, according to age and sex.

Variables	Groups			
	HIV	AIDS	Non-AIDS	Control
	N = 99 (%)	N = 86 (%)	N = 13 (%)	N = 99 (%)
NK lymphocytes (median):				
Cells/mm^3	266 [a]	269	184	333 [a]
%	13.25 [a]	13.48 [b]	7.76 [b]	14.51 [a]
Total lymphocytes (median)	2.144 [a]	2.143	2.392	2.316 [a]
Viral load (copies/mL):				
Detected	22 (22.2)	20 (23.2)	2 (15.4)	NP
Undetected or < detection limit	77 (77.8)	66 (76.8)	11 (84.6)	NP

N = total number of individuals. NP = Not performed.

[a] P<0.05 comparing HIV *vs.* Control

[b] P<0.05 comparing AIDS *vs.* Non-AIDS

Table 2. NK cell count and viral load in HIV, AIDS, Non-AIDS and control groups.

Alleles and haplotypes *HLA Class I*	Groups				
	HIV N = 99	Control N = 99	*P*	OR	CI
	n (%)	n (%)			
A*23:01	3 (1.5)	11 (5.5)	0.05	0.26	0.07 – 0.95
A*68:02	6 (3.0)	0	0.01	13.41	1.47 – U
A*68:01_C*06:02	5 (2.5)	0	0.03	11.28	1.19 – U

*Only significant results are shown.

N = total number of subjects; n: number of alleles or haplotypes; *P*: *P*-value; OR: odds ratio; CI: confidence interval; U: undefined. Only significant results are shown. The *Pc* was not applied because these variants that have been previously associated with HIV in any other populations.

Table 3. *HLA class I* allele and haplotype frequencies in the HIV patients and controls*.

Alleles and haplotypes *HLA Class I*	Groups		*P*	OR	CI
	AIDS N = 86	Non-AIDS N = 13			
	n (%)	n (%)			
A*32:01	6 (3.48)	4 (15.38)	0.05	0.20	0.05 – 0.76
B*07:02	4 (2.32)	4 (15.38)	0.02	0.13	0.03 – 0.56
B*07_C*07	4 (2.32)	4 (15.38)	0.02	0.13	0.03 – 0.56

*Only significant results are shown.

N = total number of subjects; n: number of alleles or genotypes; *P*: *P*-value; OR: odds ratio; CI: confidence interval. Only significant results are shown. The *Pc* was not applied because these variants have been previously associated with HIV in any other populations.

Table 4. *HLA class I* allele and haplotype frequencies in the AIDS and Non-AIDS groups*.

	Groups					
	HIV N = 99	Control N = 99	p	OR	CI	*P*c
	n (%)	n (%)				
Bw4-80Ile*hmz* [a]	10 (10.1)	2 (2.0)	0.03	1.71	1.30 – 2.33	NA
3DL1-Bw4-80Ile*hmz*[a]	10 (10.1)	1 (1.0)	0.01	11.01	1.38 – 87.74	NA
2DS3/2DL3/C1	11 (11.1)	23 (23.2)	0.03	0.41	0.19 – 0.90	NA
2DL3-C1/3DL2-A3/A11	14 (14.4)	27 (27.3)	0.03	0.44	0.21 – 0.90	NA
Bx ID73	4 (4.0)	0	0.05	9.37	0.93 – U	>0.05

N = total number of subjects; n: number of haplotypes/genotypes/KIR-ligants; *P*: *P*-value; OR: odds ratio; CI: confidence interval; U: undefined; *P*c: *P*-value after Bonferroni correction; NA: not applied.

[a]Bw4-80Ile*hmz* KIR ligand considering only *HLA* locus B.

Table 5. Significant associations of KIR and their HLA ligands in HIV infection compared to control group.

SUPPLEMENTARY INFORMATION

HLA de class I Alleles	Individuals Groups			
	HIV	AIDS	Non-AIDS	Control
	N = 99	N = 86	N = 13	N = 99
	n (%)	n (%)	n (%)	n (%)
A*01:01	19 (9.6)	17 (9.9)	2 (7.7)	16 (8.1)
A*01:02	1 (0.5)	1 (0.6)	0	1 (0.5)
A*02:01	47 (23.7)	39 (22.7)	8 (30.8)	45 (22.7)
A*02:02	4 (2)	2 (1.2)	2 (7.7)	1 (0.5)
A*02:05	3 (1.5)	3 (1.7)	0	3 (1.5)
A*02:11	1 (0.5)	1 (0.6)	0	1 (0.5)
A*02:22	1 (0.5)	1 (0.6)	0	0
A*02:33	1 (0.5)	1 (0.6)	0	0
A*03:01	14 (7.1)	10 (5.8)	4 (15.4)	22 (11.1)
A*03:02	2 (1.0)	2 (1.2)	0	0
A*11:01	10 (5.0)	10 (5.8)	0	11 (5.5)
A*23:01	3 (1.5)[a]	3 (1.7)	0	11 (5.5)[a]
A*24:02	13 (6.6)	12 (7.0)	1 (3.8)	24 (12.1)
A*24:03	1 (0.5)	1 (0.6)	0	1 (0.5)
A*25:01	1 (0.5)	1 (0.6)	0	3 (1.5)
A*26:01	8 (4.0)	7 (4.1)	1 (3.8)	6 (3.0)
A*29:02	5 (2.5)	5 (2.9)	0	8 (4.0)
A*30:01	11 (5.5)	11 (6.4)	0	8 (4.0)
A*30:02	6 (3.0)	6 (3.5)	0	1 (0.5)
A*30:04	1 (0.5)	1 (0.6)	0	1 (0.5)
A*31:01	11 (5.5)	10 (5.8)	1 (3.8)	9 (4.5)
A*32:01	10 (5.0)	6 (3.5)[c]	4 (15.4)[c]	8 (4.0)
A*33:01	2 (1.0)	2 (1.2)	0	2 (1.0)
A*33:03	3 (1.5)	3 (1.7)	0	1 (0.5)
A*34:01	0	0	0	1 (0.5)
A*34:02	1 (0.5)	1 (0.6)	0	1 (0.5)
A*36:01	1 (0.5)	1 (0.6)	0	0
A*66:20	0	0	0	1 (0.5)
A*68:01	11 (5.5)	10 (5.8)	1 (3.8)	8 (4.0)
A*68:02	6 (3.0)[b]	4 (2.3)	2 (7.7)	0[b]
A*69:01	1 (0.5)	1 (0.6)	0	3 (1.5)
A*74:01	0	0	0	1 (0.5)
B*07:02	8 (4.0)	4 (2.3)[d]	4 (15.4)[d]	8 (4.0)
B* 07:05	2 (0.5)	2 (1.2)	0	2 (1.0)
B* 07:14	1 (0.5)	1 (0.6)	0	0
B* 08:01	9 (4.5)	9 (5.2)	0	10 (5.0)
B* 13:02	4 (2.0)	4 (2.3)	0	3 (1.5)

B* 14:01	0	0	0	3 (1.5)
B* 14:02	6 (3.0)	4 (2.3)	2 (7.7)	9 (4.5)
B* 14:03	0	0	0	1 (0.5)
B* 15:01	6 (3.0)	6 (3.5)	0	9 (4.5)
B* 15:03	1 (0.5)	1 (0.6)	0	1 (0.5)
B* 15:04	2 (1.0)	1 (0.6)	1 (3.8)	3 (1.5)
B* 15:08	0	0	0	1 (0.5)
B* 15:10	2 (1.0)	1 (0.6)	1 (3.8)	1 (0.5)
B* 15:15	0	0	0	1 (0.5)
B* 15:16	1 (0.5)	0	1 (3.8)	0
B* 15:17	2 (1.0)	2 (1.2)	0	1 (0.5)
B* 15:18	2 (1.0)	2 (1.2)	0	1 (0.5)
B* 15:20	1 (0.5)	0	1 (3.8)	1 (0.5)
B* 18:01	12 (5.5)	11 (6.4)	1 (3.8)	9 (4.5)
B* 27:05	3 (1.5)	2 (1.2)	1 (3.8)	6 (3.9)
B* 35:01	12 (6.1)	10 (5.8)	2 (7.7)	12 (6.1)
B* 35:02	7 (3.5)	6 (3.5)	1 (3.8)	8 (4.0)
B* 35:03	3 (1.5)	2 (1.2)	1 (3.8)	5 (2.5)
B* 35:04	1 (0.5)	1 (0.6)	0	1 (0.5)
B* 35:05	2 (1.0)	2 (1.2)	0	1 (0.5)
B* 35:08	2 (1.0)	2 (1.2)	0	5 (2.5)
B*35:19	0	0	0	1 (0.5)
B* 37:01	4 (2.0)	3 (1.7)	1 (3.8)	4 (2.0)
B* 38:01	3 (1.5)	3 (1.7)	0	3 (1.5)
B* 39:01	1 (0.5)	1 (0.6)	0	3 (1.5)
B* 39:02	1 (0.5)	1 (0.6)	0	0
B* 39:03	0	0	0	1 (0.5)
B* 39:05	1 (0.5)	1 (0.6)	0	0
B* 39:06	0	0	0	2 (1.0)
B* 39:13	1 (0.5)	1 (0.6)	0	0
B* 40:01	2 (1.0)	2 (1.2)	0	2 (1.0)
B* 40:02	3 (1.5)	3 (1.7)	0	5 (2.5)
B* 40:04	1 (0.5)	1 (0.6)	0	1 (0.5)
B* 41:01	3 (1.5)	2 (1.2)	1 (3.8)	1 (0.5)
B* 41:02	1 (0.5)	1 (0.6)	0	0
B* 42:01	4 (2.0)	3 (1.7)	1 (3.8)	2 (1.0)
B* 42:02	1 (0.5)	1 (0.6)	0	0
B* 44:02	13 (6.6)	11 (6.4)	2 (7.7)	8 (4.0)
B* 44:03	12 (6.1)	10 (5.8)	2 (7.7)	13 (6.6)
B* 45:01	5 (2.5)	5 (2.9)	0	3 (1.5)
B* 49:01	5 (2.5)	5 (2.9)	0	7 (3.5)
B* 50:01	2 (1.0)	2 (1.2)	0	6 (3.0)

B* 50:02	0	0	0	1 (0.5)
B* 51:01	16 (9.6)	14 (8.1)	2 (7.7)	8 (4.0)
B* 51:07	0	0	0	2 (1.0)
B* 52:01	2 (1.0)	2 (1.2)	0	3 (1.5)
B* 53:01	6 (3.0)	6 (3.5)	0	2 (1.0)
B* 55:01	4 (2.0)	4 (2.3)	0	3 (1.5)
B* 57:01	7 (3.5)	7 (4.1)	0	7 (3.5)
B* 57:03	3 (1.5)	2 (1.2)	1 (3.8)	2 (1.0)
B* 58:01	5	5 (2.9)	0	2 (1.0)
B*58:02	0	0	0	1 (0.5)
B*78:01	1 (0.5)	1 (0.6)	0	0
B*78:02	1 (0.5)	1 (0.6)	0	0
B*81:01	1 (0.5)	1 (0.6)	0	3 (1.5)
C*01:02	8 (4.0)	7 (4.1)	1 (3.8)	8 (4.0)
C*02:02	8 (4.0)	8	0	8 (4.0)
C*03:02	1 (0.5)	1 (0.6)	0	2 (1.0)
C*03:03	8 (4.0)	7 (4.1)	1 (3.8)	8 (4.0)
C*03:04	9 (4.5)	8 (4.7)	1 (3.8)	9 (4.5)
C*04:01	36 (18.2)	30 (17.4)	6 (23.1)	38 (19.2)
C*05:01	18 (9.1)	15 (8.7)	3 (11.5)	10 (5.0)
C*06:02	21 (10.6)	20 (11.6)	1 (3.8)	19 (9.6)
C*06:03	0	0	0	1 (0.5)
C*07:01	27 (13.6)	25 (14.5)	2 (7.7)	28 (14.1)
C*07:02	14 (7.1)	10 (5.8)	4 (15.4)	13 (6.6)
C*07:04	3 (1.5)	3 (1.7)	0	2 (1.0)
C*08:01	0	0	0	1 (0.5)
C*08:02	6 (3.0)	4 (2.3)	2 (7.7)	10 (5.0)
C*12:02	1 (0.5)	1 (0.6)	0	3 (1.5)
C*12:03	5 (2.5)	5 (2.9)	0	11 (5.6)
C*14:02	4 (2.0)	3 (1.7)	1 (3.8)	3 (1.5)
C*14:03	0	0	0	1 (0.5)
C*15:02	6 (3.0)	4 (2.3)	2 (7.7)	7 (3.5)
C*15:04	0	0	0	1 (0.5)
C*15:05	1 (0.5)	1 (0.6)	0	1 (0.5)
C*16:01	9 (4.5)	9 (5.2)	0	7 (3.5)
C*16:02	1 (0.5)	1 (0.6)	0	1 (0.5)
C*17:01	11 (5.5)	9 (5.2)	2 (7.7)	5 (2.5)
C*18:01	1 (0.5)	1 (0.6)	0	1 (0.5)

N = subject number; n: allele number;

For HIV x Control: [a] For HLA-A*23:01 (P = 0.05. OR = 0.26. 95% CI = 0.07-0.95); [b] For HLA-A*68:02 P = 0.01. OR = 13.41. 95% CI = 1.47 - undefined. For AIDS x Non-AIDS: [c] For HLA-A*32:01 (P = 0.05. OR = 0.19. 95% CI = 0.52-0.75); [d] For HLA-B*07:02 (P = 0.02. OR = 0.13. 95% CI = 0.03-0.56).

Table S1. *HLA class I* allele frequency distributions for HIV, AIDS, Non-AIDS and control groups.

Genes	HIV N = 99 n (%)	AIDS N = 86 n (%)	Non-AIDS N = 13 n (%)	Controls N = 99 n (%)
Inhibitory *KIR*				
KIR2DL1	97 (97.98)	84 (97.67)	13 (100)	95 (95.96)
KIR2DL2	57 (57.98)	49 (56.98)	8 (61.54)	53 (53.54)
KIR2DL3	88 (88.89)	77 (89.53)	11 (84.62)	92 (92.93)
KIR2DL4*+	98 (98.99)	85 (98.84)	13 (100)	99 (100)
KIR2DL5	58 (58.59)	49 (56.98)	9 (69.23)	59 (59.60)
KIR3DL1	90 (90.91)	80 (93.02)	10 (76.92)	94 (94.95)
KIR3DL2+	99 (100)	86 (100)	13 (100)	99 (100)
KIR3DL3+	99 (100)	86 (100)	13 (100)	98 (98.99)
Activating *KIR*				
KIR2DS1	48 (48.48)	40 (46.51)	8 (61.54)	38 (38.38)
KIR2DS2	55 (55.56)	48 (55.81)	7 (53.85)	54 (54.55)
KIR2DS3	24 (24.24)	21 (24.42)	3 (23.08)	35 (35.35)
KIR2DS4	88 (88.89)	79 (91.86) [a]	9 (69.23) [a]	94 (94.95)
KIR2DS5	45 (45.45)	38 (44.19)	7 (53.85)	38 (38.38)
KIR3DS1	46 (46.46)	38 (44.19)	8 (61.54)	38 (38.38)
Pseudogene *KIR*				
KIR2DP1	97 (97.98)	84 (97.67)	13 (100)	96 (96.97)
KIR3DP1+	98 (98.99)	85 (98.84)	13 (100)	99 (100)
haplotypes				
AA	24 (24.2)	22 (25.6)	2 (15.4)	27 (27.3)
BX	75 (75.8)	64 (74.4)	11 (84.6)	72 (72.7)

**KIR2DL4*: activator or inhibitor; +*KIR2DL4. KIR3DL2. KIR3DL3* and *KIR3DP1*: framework genes;

N: total number of subjects; n: number of individuals with the indicated *KIR* gene.

Non significant difference was observed in the distribution of *KIR* genes and haplotypes in the studied groups.

Table S2. *KIR* gene frequency distributions for HIV, AIDS, Non-AIDS and controls.

HLA Ligands	HIV N = 99 n (%)	AIDS N = 86 n (%)	Non-AIDS N = 13 n (%)	Control N = 99 n (%)
A*03/A*11	24 (24.2)	20 (23.3)	4 (30.8)	32 (32.3)
Bw4 80 Ile	56 (56.6)	48 (55.8)	8 (61.5)	62 (62.6)
Bw4 80 The	34 (34.3)	28 (32.6)	6 (46.2)	32 (32.3)
Bw4	76 (76.8)	65 (75.6)	11 (84.6)	76 (76.8)
C1	74 (74.7)	65 (75.6)	9 (69.2)	80 (80.8)
C2	78 (78.8)	68 (70.1)	10 (76.9)	73 (73.7)
C1/C1	21 (21.2)	18 (20.9)	3 (23.1)	26 (26.3)
C2/C2	25 (25.2)	21 (24.4)	4 (30.8)	19 (19.2)
C1/C2	53 (53.5)	47 (54.7)	6 (46.2)	54 (54.5)
A*03	16 (16.1)	12 (14.0)	4 (30.8)	21 (21.2)
A*11	10 (10.1)	10 (11.6)	0 (0)	11 (11.1)
Bw4Bw4	30 (30.3)	26 (30.2)	4 (30.8)	36 (36.4)
Bw4Bw4 80Ile	20 (20.2)	19 (22.1)	1 (7.7)	22 (22.2)
Bw4Bw4 80The	2 (2.0)	2 (2.3)	0 (0)	2 (2.0)
Bw4Bw4 80 Ile (A)	2 (2.0)	2 (2.3)	0 (0)	5 (5.1)
Bw4Bw4 80 Ile (B)	10 (10.1) [a]	10 (11.6)	0 (0)	2 (2.0) [a]

N: number of individuals; n: number of individuals with the HLA ligands.

(A): Considering only locus A; (B) considering only locus B;

For HIV x Control: Bw4Bw4 80Ile (B) [a] (*P* = 0.03. OR = 1.74. 95% CI = 1.30-2.34).

Table S3. *HLA* class I (*KIR* ligands) frequency distributions for HIV, AIDS, Non-AIDS and controls.

KIR – HLA ligands	HIV N = 99 n (%)	AIDS N = 86 n (%)	Non-AIDS N = 13 n (%)	Controls N = 99 n (%)
***2DL1*/C2+**	76 (76.77)	66 (76.74)	10 (76.92)	69 (69.70)
***2DL1/C2*-**	21 (21.21)	18 (20.93)	3 (23.08)	26 (26.26)
***2DL1*/C1C2+**	52 (52.53)	46 (53.49)	6 (46.15)	51 (51.52)
***2DL1*/C2C2+**	24 (24.24)	20 (23.26)	4 (30.77)	18 (18.18)
***2DL2*/C1+**	44 (44.44)	38 (44.19)	6 (46.15)	45 (45.45)
***2DL2*/C1-**	13 (13.13)	11 (12.79)	2 (15.38)	8 (8.08)
***2DL2*/C1C2+**	32 (32.32)	29 (33.72)	3 (23.08)	31 (31.31)
***2DL2*/C1C1+**	12 (12.12)	9 (10.47)	3 (23.08)	14 (14.14)
***2DL3*/C1+**	65 (65.66)	58 (67.44)	7 (53.85)	74 (74.75)
***2DL3*/C1-**	23 (23.23)	19 (22.09)	4 (30.77)	18 (18.18)
***2DL3*/C1C2+**	45 (45.45)	40 (46.51)	5 (38.46)	49 (49.49)
***2DL3*/C1C1+**	20 (20.20)	18 (20.93)	2 (15.38)	25 (25.25)
***3DL1*/Bw4+**	68 (68.69)	59 (68.60)	9 (69.23)	72 (72.73)
3DL1/ Bw4 80Ile	53 (53.54)	46 (53.49)	7 (53.85)	58 (58.59)
3DL1/Bw4 80Tre	23 (23.23)	23 (26.74)	5 (38.46)	24 (24.24)
***3DL1*/Bw4Bw4+**	28 (28.28)	26 (30.23)	2 (15.38)	36 (36.36)
***3DL1*/Bw4-**	22 (22.22)	21 (24.41)	1 (7.69)	22 (22.22)
***3DL2*/A3/A11+**	24 (24.24)	20 (23.26)	4 (30.77)	32 (32.32)
***2DS1*/C2+**	36 (36.36)	30 (34.88)	6 (46.15)	26 (26.26)
***2DS1*/C2-**	12 (12.12)	10 (11.62)	2 (15.38)	10 (10.10)
***2DS1*/C1C2+**	22 (22.22)	19 (22.09)	3 (23.08)	17 (17.17)
***2DS1*/C2C2+**	14 (14.14)	11 (12.79)	3 (23.08)	9 (9.09)
***2DS2*/C1+**	42 (42.42)	37 (43.02)	5 (38.46)	44 (44.44)
***2DS2*/C1-**	13 (13.13)	11 (12.79)	2 (15.38)	10 (10.10)
***2DS2*/C1C2+**	33 (33.33)	30 (34.88)	3 (23.08)	30 (30.30)
***2DS2*/C1C1+**	9 (9.09)	7 (8.14)	2 (15.38)	14 (14.14)
***2DS3/C1*+**	18 (18.18)	17 (19.77)	1 (7.69)	26 (26.26)
***2DS3/C1*-**	6 (6.06)	4 (4.65)	2 (15.38)	9 (9.09)
***2DS3/C1C1*+**	5 (5.05)	4 (4.65)	1 (7.69)	8 (8.08)
***2DS3/C1C2*+**	13 (13.13)	13 (15.12)	0 (0)	18 (18.18)
***3DS1*/Bw4+**	35(35.35)	29 (33.72)	6 (46.15)	31 (31.31)

3DS1/ Bw4 80Ile	23 (23.23)	20 (23.26)	3 (23.08)	27 (27.27)
3DS1/Bw4 80Tre	18 (18.18)	13 (15.12)	5 (38.46)	10 (10.10)
***3DS1*/Bw4-**	11 (11.11)	9 (10.47)	2 (15.38)	7 (7.07)
***3DS1*/Bw4Bw4+**	13 (13.13)	10 (11.62)	3 (23.08)	15 (15.15)
2DS5/C2+	34 (34.34)	29 (33.72)	5 (38.46)	29 (29.29)
2DS5/C2-	11 (11.11)	9 (10.47)	2 (15.38)	9 (9.09)
2DS5/C2C2+	12 (12.12)	10 (11.62)	2 (15.38)	10 (10.10)
2DS5/C1C2+	22 (22.22)	19 (22.09)	3 (23.08)	19 (19.19)
***2DL2/2DL2*/C1+**	9 (9.09)	7 (8.14)	2 (15.38)	6 (6.06)
***2DL2/2DL2*/C1-**	2 (2.02)	2 (2.32)	0 (0)	1 (1.01)
***2DL2/2DL2*/C1C1+**	1 (1.01)	0 (0)	1 (7.69)	1 (1.01)
2DL2/2DL2/C1C2+	8 (8.08)	7 (8.14)	1 (7.69)	5 (5.05)
2DL3/2DL3/C1+	30 (30.30)	27 (31.39)	3 (23.08)	35 (35.35)
2DL3/2DL3/C1-	12 (12.12)	10 (11.62)	2 (15.38)	11 (11.11)
2DL3/2DL3/C1C1+	9 (9.09)	9 (10.47)	0 (0)	12 (12.12)
2DL3/2DL3/C1C2+	21 (21.21)	18 (20.93)	3 (23.08)	23 (23.23)
2DL2/2DL3/C1+	35 (35.35)	31 (36.04)	4 (30.77)	39 (39.39)
2DL2/2DL3/C1-	11 (11.11)	9 (10.47)	2 (15.38)	7 (7.07)
***2DL2/2DL3*/C1C1+**	11 (11.11)	9 (10.47)	2 (15.38)	13 (13.13)
***2DL2/2DL3*/C1C2+**	24 (24.24)	22(25.58)	2 (15.38)	26 (26.26)
***2DS2/2DL2*/C1+**	40(40.40)	35 (40.69)	5 (38.46)	42 (42.42)
***2DS2/2DL2*/C1-**	13 (13.13)	11 (12.79)	2 (15.38)	8 (8.08)
***2DS2/2DL2*/C1C1+**	9 (9.09)	7 (8.14)	2 (15.38)	14 (14.14)
***2DS2/2DL2*/C1C2+**	31 (31.31)	28 (32.55)	3 (23.08)	28 (28.28)
***2DS2+ /2DL2-*/C1+**	2 (2.02)	2 (2.32)	0 (0)	2 (2.02)
2DS2- /2DL2+ /C1+	4 (4.04)	3 (3.49)	1 (7.69)	3 (3.03)
2DS1/2DL1/C2+	35 (35.35)	29 (33.72)	6 (46.15)	25 (25.25)
2DS1/2DL1/C2-	12 (12.12)	10 (11.62)	2 (15.38)	12 (12.12)
2DS1/2DL1/C2C2+	13 (13.13)	10 (11.62)	3 (23.08)	9 (9.09)
2DS1/2DL1/C1C2+	22 (22.22)	19 (22.09)	3 (23.08)	16 (16.16)
2DS1+ /2DL1- / C2+	1 (1.01)	1 (1.16)	0 (0)	1 (1.01)
2DS1- /2DL1+ / C2+	41(41.41)	37 (43.02)	4 (30.77)	44 (44.44)
2DS2/2DL3/C1+	33 (33.33)	30 (34.88)	3 (23.08)	38 (38.38)

2DS2/2DL3/C1-	11 (11.11)	9 (10.47)	2 (15.38)	9 (9.09)
2DS2/2DL3/C1C1+	8 (8.08)	7 (8.14)	1 (7.69)	13 (13.13)
2DS2/2DL3/C1C2+	25 (25.25)	23 (26.74)	2 (15.38)	25 (25.25)
2DS2+ / 2DL3- / C1+	9 (9.09)	9 (10.47)	0 (0)	6 (6.06)
2DS2- / 2DL3+ / C1+	32 (32.32)	28 (32.55)	4 (30.77)	36 (36.36)
2DS3/2DL3/C1+	11 (11.11) [a]	11 (12.79)	0 (0)	23 (23.23) [a]
2DS3/2DL3/C1-	5 (5.05)	3 (3.49)	2 (15.38)	8 (8.08)
2DS3/2DL3/C1/C1+	4 (4.04)	4 (4.65)	0 (0)	7 (7.07)
2DS3/2DL3/C1/C2+	7 (7.07)	7 (8.14)	0 (0)	16 (16.16)
2DS3+/2DL3- /C1+	7 (7.07)	6 (6.98)	1 (7.69)	3 (3.03)
2DS3- /2DL3+ /C1+	54 (54.54)	47 (54.65)	7 (53.85)	51 (51.51)
3DL1/3DS1/Bw4+	28 (28.28)	24 (27.91)	4 (30.77)	27 (27.27)
3DL1/3DS1/Bw4/Bw4+	11 (11.11)	9 (10.47)	2 (15.38)	12 (12.12)
3DL1/3DS1 Bw4 80Ile	20 (20.20)	18 (20.93)	2 (15.38)	23 (23.23)
3DL1/3DS1/Bw4 80Tre	13 (13.13)	9 (10.47)	4 (30.77)	10 (10.10)
***3DL1/3DS1/*Bw4-**	10 (10.10)	9 (10.47)	1 (7.69)	6 (6.06)
2DS2/2DL2/2DL3/C1+	31 (31.31)	28 (32.55)	3 (23.08)	36 (36.36)
2DS2/2DL2/2DL3/C1-	11 (11.11)	9 (10.47)	2 (15.38)	7 (7.07)
2DS2/2DL2/2DL3/C1C1+	8 (8.08)	7 (8.14)	1 (7.69)	13 (13.13)
2DS2/2DL2/2DL3/C1C2+	23 (23.23)	21 (24.42)	2 (15.38)	23 (23.23)
2DS2+ /2DL2- /2DL3+ /C1+	2 (2.02)	2 (2.32)	0 (0)	2 (2.02)
2DS2+ /2DL2+ /2DL3- /C1+	9 (9.09)	7 (8.14)	2 (15.38)	6 (6.06)
2DS2- /2DL2+ /2DL3+ /C1+	4(4.04)	3 (3.49)	1 (7.69)	3 (3.03)
2DS2- /2DL2- /2DL3+ /C1+	28 (28.28)	25 (29.07)	3 (23.08)	33 (33.33)
3DL1/Bw4Bw480Ile (B)	10 (10.1)	10 (11.62)	0 (0)	1 (1.0)

N: number of individuals; n: number of individuals with the *KIR* genes their HLA ligands. (B) considering only locus B;

For HIV x Controle: [a] For *2DS3/2DL3/C1+* (P = 0.03. OR = 0.41. 95% CI = 0.19-0.90); [b] For 3DL1/Bw4Bw480Ile (B) (P = 0.01. OR = 11.01. 95% CI = 1.38-87.74).

Table S4. *KIR* genes and their respective HLA ligands frequency distributions for HIV, AIDS, non-AIDS and controls.

		KIR genes																		
Hapl.	**Gen. ID**	*3DL1*	*2DL1*	*2DL3*	*2DS4*	*2DL2*	*2DL5*	*3DS1*	*2DS1*	*2DS2*	*2DS3*	*2DS5*	*2DL4*	*3DL2*	*3DL3*	*2DP1*	*3DP1*	**HIV N =99 n (%)**	**AIDS N =86 n (%)**	**Non-AIDS N =13 n (%)**	**Control N =99 n (%)**
AA	1	■	■	■	■								■	■	■	■	■	24 (24.2)	22 (25.6)	2 (15.4)	27 (27.3)
Bx	**2**	■	■	■	■		■	■	■			■	■	■	■	■	■	11 (11.1)	9 (10.5)	2 (15.4)	12 (12.1)
Bx	**3**	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	8 (8.1)	8 (9.3)	0 (0)	4 (4.0)
Bx	**4**	■	■	■	■	■				■			■	■	■	■	■	13 (13.1)	11 (12.8)	2 (15.4)	11 (11.1)
Bx	**5**	■	■	■	■	■	■			■	■		■	■	■	■	■	3 (3.0)	3 (3.5)	0 (0)	9 (9.1)
Bx	6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5 (5.1)	5 (5.8)	0 (0)	8 (8.1)
Bx	**7**	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	3 (3.0)	2 (2.3)	1 (7.7)	3 (3.0)
Bx	8	■	■	■	■		■	■	■		■		■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	0 (0)
Bx	**9**	■	■	■	■	■	■		■	■		■	■	■	■	■	■	3 (3.0)	2 (2.3)	1 (7.7)	1 (1.0)
Bx	**10**	■	■	■	■					■			■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	0 (0)
Bx	**12**	■	■	■	■		■	■	■	■		■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**13**	■	■	■	■	■	■	■		■	■		■	■	■	■	■	2 (2.0)	1 (1.2)	1 (7.7)	1 (1.0)
Bx	**18**	■	■	■	■	■	■	■	■			■	■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	0 (0)
Bx	**19**	■	■	■	■	■							■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	0 (0)
Bx	**20**	■	■	■	■	■	■					■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**21**	■	■	■	■	■	■			■		■	■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	1 (1.0)
Bx	35	■	■	■	■		■		■			■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**36**	■	■	■	■		■			■	■		■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	2 (2.0)
Bx	**48**	■	■	■	■		■			■		■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**51**	■	■	■	■	■	■				■		■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)

Bx	**58**	■	■	■	■	■	■	■	■	□	■	■	■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	1 (1.0)
Bx	**68**	□	■	■	□	■	■	■	■	■	□	■	■	■	■	■	■	3 (3.0)	3 (3.5)	0 (0)	1 (1.0)
Bx	**69**	□	■	■	□	□	■	■	■	□	□	■	■	■	■	■	■	3 (3.0)	2 (2.3)	1 (7.7)	0 (0)
Bx	**70**	□	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**71**	■	■	□	■	■	■	□	□	■	■	□	■	■	■	■	■	3 (3.0)	3 (3.5)	0 (0)	3 (3.0)
Bx	72	■	□	□	■	■	□	□	□	■	□	□	■	■	■	□	■	1 (1.0)	1 (1.2)	0 (0)	2 (2.0)
Bx	**73**	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	■	4 (4.0)[a]	4 (4.7)	0 (0)	0 (0)[a]
Bx	75	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**76**	■	□	□	■	■	■	■	■	■	□	■	■	■	■	□	■	1 (1.0)	1 (1.2)	0 (0)	1 (1.0)
Bx	**81**	□	■	□	□	■	■	■	■	■	■	■	■	■	■	■	■	1 (1.0)	0 (0)	1 (7.7)	0 (0)
Bx	**150**	□	■	■	■	□	■	■	■	□	■	■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**159**	□	■	■	□	■	■	■	■	■	■	□	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	**167**	□	■	■	□	■	■	■	■	□	□	■	■	■	■	■	■	1 (1.0)	0 (0)	1 (7.7)	0 (0)
Bx	**342**	■	□	□	■	■	■	□	□	■	■	□	■	■	■	■	■	1 (1.0)	0 (0)	1 (7.7)	0 (0)
Bx	**417**	■	■	□	□	■	■	■	■	■	□	■	■	■	■	■	■	1 (1.0)	0 (0)	1 (7.7)	0 (0)
Bx	**449**	■	■	■	□	■	■	■	□	■	■	■	■	■	■	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
Bx	481	■	■	■	□	□	□	□	□	□	□	■	■	■	■	■	■	1 (1.0)	1 (1.2)	0 (0)	0 (0)
Bx	**NAS0**	□	■	■	□	■	■	□	■	■	□	□	□	■	■	■	□	1 (1.0)	1 (1.2)	0 (0)	0 (0)
Bx	**NAS1**	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	■	0 (0)	0 (0)	0 (0)	1 (1.0)
		Number of *KIR* genotypes																28	24	11	27

Hapl: Haplotype group; Gen. ID: Genotype ID assigned by the Allele Frequencies Net Database (March 2020); NAS: Not yet Assigned ID; black box = gene detected; white box = gene absent. no detected; N: number of individuals; n: number of individuals with genotype ID.

[a] (P = 0.05. OR = 9.38. 95% CI = 0.93 - undefined. for **Genotype ID 73** HIV patients: 4.0% *vs.*. 0.0% controls).

Table S5. *KIR* genotype frequency distribution for HIV, AIDS, Non-AIDS and controls.