

# HLA-Bw4-B\*57 and Cw\*18 alleles are associated with plasma viral load modulation in HIV-1 infected individuals in Salvador, Brazil

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## ABSTRACT

Host genetic factors play an important role in mediating resistance to HIV-1 infection and may modify the course of infection. HLA-B alleles (Bw4 epitope; B\*27 and B\*57) as well as killer cell immunoglobulin-like receptors have been associated with slow progression of HIV-1 infection. **Objective:** To evaluate the association between serological epitopes HLA-Bw4 and HLA-Bw6 and prognostic markers in AIDS. **Methods:** 147 HIV-infected individuals in Bahia, Northeast Brazil, were genotyped for HLA class I *locus*. HLA class I genotyping was performed by hybridization with sequence-specific oligonucleotide probes following amplification of the corresponding HLA-A, HLA-B and HLA-C genes. Statistical analysis was performed using Fisher's exact and ANOVA tests for categorical and continuous variables, respectively. **Results:** We detected a significant association ( $\chi^2 = 4.856$ ;  $p = 0.018$ ) between the presence of HLA-Bw4 and low levels of viremia. Eighteen out of the 147 HIV-infected individuals presented viremia  $\leq 1,800$  copies/mL and 129 presented viremia  $> 2,000$  copies/mL. Ninety and four percent (17/18) of all individuals with viremia  $\leq 1,800$  copies/mL carried HLA-Bw4, compared to 67.4% (87/129) of individuals with viremia  $> 2,000$  copies/mL. Additionally, we found a significantly higher frequency of B\*57 (OR = 13.94; 95% CI = 4.19-46.38;  $p < 0.0001$ ) and Cw\*18 (OR = 16.15; 95% CI = 3.46-75.43;  $p \leq 0.0001$ ) alleles, favoring the group with lower viremia levels, in comparison with those with higher viral load. **Conclusion:** HLA-Bw4-B\*57 and Cw\*18 alleles are associated with lower level of viral load in HIV-infected Brazilian patients. These findings may help us in understanding the determinants of HIV evolution in Brazilian patients, as well as in providing important information on immune response correlates of protection for such population.

**Keywords:** HIV, AIDS, MHC, HLA, polymorphism.

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## INTRODUCTION

The dynamics of HIV-1 replication is a very important factor in determining the clinical course of HIV-1 disease. Although an enhanced production of virus particles results in progressive deterioration of the immune system in most HIV-1 infected individuals, the control of viremia and the rate of progression of HIV-1 disease have been demonstrated to be substantially influenced by host genetic characteristics.

The genetic profile of human leukocyte antigen (HLA) class I molecules plays a very important role in both, the innate and adaptive immune responses during the course of HIV-1 infection. Cytotoxic T lymphocyte (CTL) response depends on the recognition of viral peptides presented in association with major histocompatibility (MHC)

class I molecules on the surface of infected cells.<sup>1</sup> Certain types of *HLA-B* alleles, especially B\*27 and B\*57, seems to strongly influence AIDS progression.<sup>2-6</sup> HIV-specific CTL play a central role in the initial control of virus replication during primary HIV infection, when the immunologic response limits the viral replication and reduces the viremia and the number of infected cells in lymph nodes,<sup>7-10</sup> as well as during chronic HIV infection, since the high number of HIV-specific CTL often persists in late infection in peripheral blood of HIV-infected patients.<sup>11,12</sup> Several studies reported that in HIV-infected long-term survivors the viral load and the number of CD4<sup>+</sup> T cells infected with HIV-1 are very low, and a strong HIV-specific CTL response is detected during the course of infection.<sup>13,14</sup>

The HLA repertoire can be implicated in the innate immunity responses. HLA class I molecules have been identified as killer immunoglobulin receptor (KIR) ligands.<sup>15,16</sup> In particular, all *HLA-B* specificities are grouped in two antigenic families: HLA-Bw4 and HLA-Bw6.<sup>17</sup> HLA-Bw4 and HLA-Bw6 epitopes consist of a single epitope in each *HLA-B* molecule and they are different from the epitopes that establish the *HLA-B* specificity; therefore each *HLA-B* molecule expresses only one of the serological epitopes HLA-Bw4 or HLA-Bw6. HLA-Bw4 is one of the inhibitory or activating ligands for KIR<sup>18,19</sup> that control the natural killer (NK) cell cytotoxic activity. As a component of the innate immune system NK cells might have a very important role in the defense of the HIV-1 infected host,<sup>20</sup> possibly controlling the viremia through lysis of HIV-infected cells.<sup>21,22</sup> Genetic association studies and functional assays report the importance of HLA-Bw4 in the pathogenesis of AIDS. Two studies have shown the protective role of HLA-Bw4 against HIV-disease progression through association with viremia control and CD4<sup>+</sup> T cell count maintenance.<sup>21,23</sup> Qi *et al*<sup>24</sup> reported the synergistic role of *KIR3DS1/Bw4-80Ile* against CD4<sup>+</sup> T cell depletion and development of opportunistic infections. A more recent study has demonstrated that the presence of *HLA-Bw4* is associated with a decreased risk in male-to-female HIV-1 transmission.<sup>25</sup> The objective of this study was to evaluate the association between serological epitopes HLA-Bw4 and HLA-Bw6 and the modulation of viral load and CD4<sup>+</sup> T cell count in the Brazilian HIV-infected patients.

## MATERIAL AND METHODS

### Subjects

Were evaluated 147 HIV-infected individuals, selected from an AIDS cohort established at the Hospital Universitário Professor Edgard Santos (HUPES) and Centro de Referência Estadual de AIDS (CREAIDS) in 1997 with HIV positive patients from the state of Bahia, Northeast Brazil. All the patients had a Western Blot-confirmed HIV-1 infection. The cohort was monitored for CD4<sup>+</sup> and CD8<sup>+</sup> T cell count (cells/mm<sup>3</sup>) and plasma viral load (copies/mL) every six months. The demographic and clinical parameters of all patients are summarized in Table 1. In Bahia state, there is a great genetic mixture of Portuguese and African descendents.<sup>26-28</sup> Informed consent for blood donation and for genetic polymorphism investigation was obtained from each patient. This study was approved by the Comitê de Ética em Pesquisa do Centro de Pesquisa Gonçalo Moniz, Process # 29/2003.

A subset of the patients (18 individuals) was classified as viremia controllers, in accordance with the HIV Controller Consortium.<sup>29</sup> All controllers had been infected for more than two years, remained asymptomatic during the follow up without antiretroviral therapy and maintained the viral load ≤ 1,800 copies/mL. The patients that did not attained to all these criteria (129 individuals) were classified as viremia non-controllers.

**Table 1. Demographic and clinical characteristics of HIV-infected individuals in Bahia**

Features	All patients (n = 147)
Age, mean	45 (24-68)
Sex	
Male (%)	60.1
Female (%)	39.9
Risk of HIV infection	
Homosexual (%)	19.6
Heterosexual (%)	65.2
Blood transfusion(%)	3.1
IDV (%)	3.9
Bisexual (%)	7.6
Unknown (%)	7.6
CD4 <sup>+</sup> T cell count (cells/m <sup>3</sup> ), mean ± SD	303 ± 228
Viral load (copies/mL), mean±SD	107,841 ± 198,925

### HLA and KIR genotyping

Genomic DNA was extracted from whole blood using extraction method GFX™ Kits Genomic Blood DNA Purification (Amersham Pharmacia Biotech, UK). HLA genotyping was performed by using SSO LABType<sup>(R)</sup> method (ONE LAMBDA, Inc., Canoga Park, USA) according to the manufacturer's instructions. Briefly, the polymerase chain reaction (PCR) conditions were: 96°C for 3 minutes, followed by 5 cycles of 96°C for 20 seconds, 60°C for 20 seconds and 72°C for 20 seconds, and 30 cycles of 96°C for 10 seconds, 60°C for 15 seconds and 72°C for 20 seconds, and extend at 72°C for 10 minutes. After amplification of genomic DNA using locus-specific primers that flanked exon two and three, PCR products were denatured and annealed to sequence-specific oligonucleotide (SSO) probes of the corresponding *HLA-A*, *-B* and *-C* genes. The fluorescent intensity of binding probes was identified by LABScan™100 flow analyzer and the results were interpreted using HLA-visual software that established the HLA genotyping. *HLA-A* and *HLA-B* alleles were pooled *HLA-Bw4* and *HLA-Bw6* according to HLA class I sequence alignments<sup>30</sup> (Table 2). KIR3DS1 and KIR3DL1 genotyping was performed by using gene-specific primer pairs according to Martin *et al.*<sup>21</sup> Each reaction contained 15 ng of DNA, 0.1 mM DNTPs, 1.5 mM MgCl<sub>2</sub>, 67 mM Tris-HCl (pH 8.8), 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.25 mM of each primer, and 1U of Taq polymerase in a 10 µL final volume. To improve the reliability of the results, besides the utilization of an internal control, all negative reactions were repeated to confirm non-amplification in the PCR. In addition, positive results were randomly replicated. Phenotype frequencies (f) for the presence of each gene were estimated by direct counting.

**Table 2.** Bw4 and Bw6 epitopes and their corresponding alleles observed in the HIV-infected individuals in Bahia

Serological epitope	Amino-acid at position					Corresponding alleles
	77	80	81	82	83	
Bw4	Asn	Ile	Ala	Leu	Arg	B*1516 B*1517 B*1524 B*3801 B*4901 B*51
						B*5201 B*53B* 57 B*58 A*23 A*24
	Asn	Thr	Ala	Leu	Arg	B*13 B*44
	Asp	Thr	Leu	Leu	Arg	B*2703 B*2713 B*3701
Bw6	Ser	Ile	Ala	Leu	Arg	A*32
	Ser	Asn	Leu	Arg	Gly	B*07 B*0801 B*1401 B*1402 B*1501 B*1502 B*1503 B*1504 B*1507 B*1510 B*1520 B*1554 B*1801 B*1820 B*35 B*39 B*4001 B*4001 B*41 B*42 B*4501 B*5001 B*55 B*8101

### Statistical analysis

Mean viral load and CD4<sup>+</sup> T cells count for each patient (before starting antiretroviral therapy) was estimated and used as representative value in the statistical analysis. ANOVA and Mann-Whitney tests were used to compare continuous variables. Fisher's exact test was used to investigate associations between categorical variables and correction for multiple tests was applied when necessary. Hardy-Weinberg Equilibrium analysis was performed for each population (controllers and non-controllers of viremia) using Genepop software.<sup>31</sup> Additionally, Odds Ratio (OR), and their 95% confidence interval was used to determine the strength of the allele-specific associations that were statistically significant.

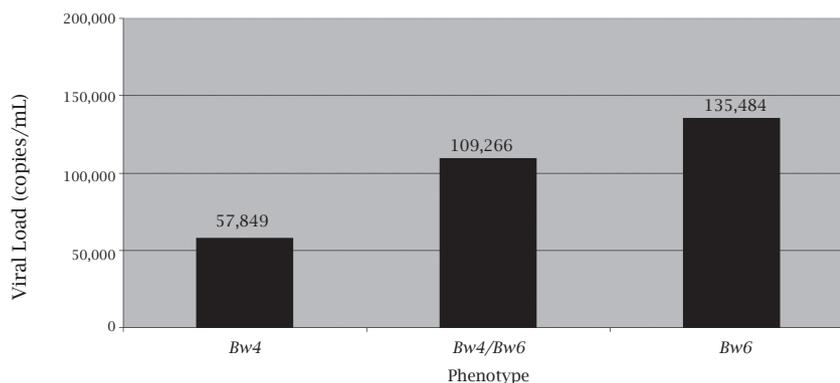
### RESULTS

We detected a direct correlation between viral load and CD4<sup>+</sup> T cell count, fundamental markers for HIV-infected patient monitoring. It was statistically significant ( $R = -0.307$ ;  $p < 0.001$ , by Pearson's correlation) and the regression analysis suggested there was a logarithmic relationship between both variables.

#### Effects of HLA-Bw4 on viral load and CD4<sup>+</sup> T cell count modulation

Viral load averages for Bw4, Bw4/Bw6 and Bw6 phenotypes are shown in Figure 1, which emphasizes the increasing

**Figure 1:** HLA-Bw4 presence associated with low levels of plasmatic viral load. Viral load average was calculated for 147 HIV-infected individuals and is shown on the top of the respective bars.



gradient from absence of *HLA-Bw6* to presence of *HLA-Bw4*. ANOVA test showed a borderline significant difference between and within the groups that was lost after correction ( $p = 0.059$ ;  $pc = 0.076$ ) (Table 3). The categorization into *Bw4*<sup>+</sup> (*Bw4/Bw4* and *Bw4/Bw6*) and *Bw4*<sup>-</sup> (*Bw6/Bw6*) patients groups yielded a more significant association. Mann-Whitney test revealed a statistically significant difference between *Bw4*<sup>+</sup> and *Bw4*<sup>-</sup> groups, suggesting an association of the presence of *HLA-Bw4* with viral load reduction ( $Z = -2.052$ ;  $p = 0.040$ ) (Table 3). Additionally, we observed that 18 out of the 147 HIV-infected individuals presented viremia  $\leq 1,800$  copies/mL and 129 presented viremia  $> 2,000$  copies/mL. Ninety four percent (17/18) of all individuals with viremia  $\leq 1,800$  copies/mL carried *HLA-Bw4*, compared to 67.4% (87/129) of individuals with viremia

$> 2,000$  copies/mL (Table 4). We detected a statistically significant association (OR = 8.21; 95% CI = 1.08-63.76;  $p = 0.018$ ; Fisher's exact test) between the presence of *HLA-Bw4* and low levels of viremia.

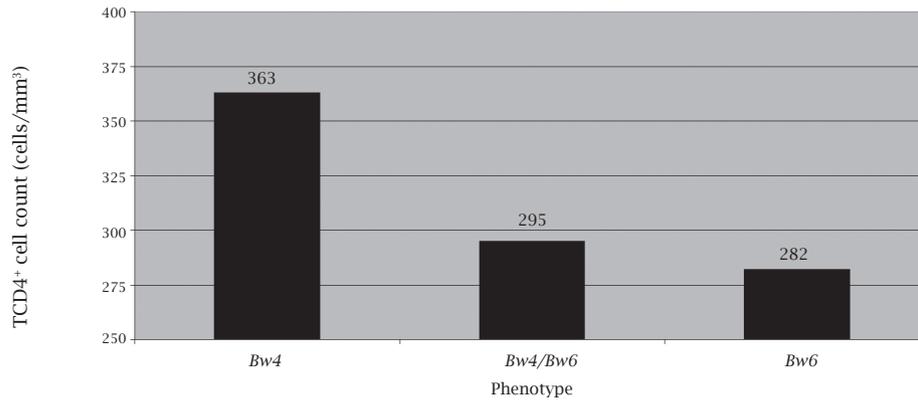
The same comparative analysis used for viral load was used for CD4<sup>+</sup> T cell count average (Figure 2). Although individuals presenting *Bw4*<sup>+</sup> had a higher mean CD4<sup>+</sup> T cell count than individuals not presenting this allele, the comparison of the groups revealed no significant association between the presence of *HLA-Bw4* and higher CD4<sup>+</sup> T cell count ( $p = 0.282$ ), even after stratifying patients into *Bw4*<sup>+</sup> (*Bw4/Bw4* and *Bw4/Bw6*) and *Bw4*<sup>-</sup> (*Bw6/Bw6*) groups ( $Z = 1.220$ ;  $p = 0.222$ ) (Table 3), suggesting no influence of *HLA-Bw4* on CD4<sup>+</sup> T cell count modulation.

**Table 3. Genetic factors associated with clinical markers in HIV-infected patients without antiretroviral therapy**

Genotype	Patients (n = 147)	Clinical markers		Clinical markers		
		<sup>a</sup> CD4 <sup>+</sup> count, cells/mm <sup>3</sup>	<sup>b</sup> p value	<sup>a</sup> Viral Load, RNA copies/mL	<sup>b</sup> p value	<sup>c</sup> pc value
<i>HLA Bw4/Bw4</i>	26 (17.7)	363 (233)	0.282	57,849 (81,414)	0.059	0.076
<i>HLA Bw4/Bw6</i>	78 (53.1)	295 (226)		109,266 (221,885)		
<i>HLA Bw6/Bw6</i>	43 (29.2)	282 (227)		135,484 (202,527)		
<i>Bw4</i> <sup>+</sup> ( <i>Bw4/Bw4</i> ; <i>Bw4/Bw6</i> )	104 (70.7)	315 (228)	0.222	96,411 (197,268)	0.040	
<i>Bw4</i> <sup>-</sup> ( <i>Bw6/Bw6</i> )	43 (29.2)	282 (227)		135,484 (202,527)		
<i>Bw4</i> <sup>+</sup> non- <i>B*27</i>	102 (69.4)	317 (230)	0.698	84,115 (147,066)	0.044	
<i>Bw4</i> <sup>+</sup> non- <i>B*57</i>	89 (60.5)	312 (230)	0.781	93,523 (153,732)	0.201	
<i>Bw4</i> <sup>+</sup> non- <i>B*27</i> non- <i>B*57</i>	87 (59.2)	315 (231)	0.721	93,990 (154,644)	0.194	
<i>Bw4</i> <sup>+</sup> - <i>Ile80</i> <sup>+</sup>	80 (76.9)	319 (240)	0.792	105,455 (219,144)	0.966	
<i>Bw4</i> <sup>+</sup> - <i>Ile80</i> <sup>-</sup>	24 (23.1)	288 (184)		66,297 (89,930)		
<i>KIR3DL1</i>	107 (72.8)	303 (233)	0.875	104,035 (163,404)	0.360	
<i>KIR3DS1</i>	32 (21.8)	297 (199)		141,678 (303,066)		
<sup>d</sup> Non <i>KIR3DL1-3DS1</i>	08 (5.4)	376 (279)		16,054 (21,926)		
<i>KIR3DL1</i> + <i>Bw4</i> <sup>+</sup> <i>Ile80</i> <sup>+</sup>	62 (59.6)	320 (243)	0.923	92,632 (171,015)	0.354	0.934
<i>KIR3DL1</i> + <i>Bw4</i> <sup>+</sup> <i>Ile80</i> <sup>-</sup>	15 (14.4)	303 (193)		86,802 (108,495)		
<i>KIR3DS1</i> + <i>Bw4</i> <sup>+</sup> <i>Ile80</i> <sup>+</sup>	19 (18.3)	302 (215)		183,544 (362,791)		
<i>KIR3DS1</i> + <i>Bw4</i> <sup>+</sup> <i>Ile80</i> <sup>-</sup>	08 (7.7)	265 (176)		44,174 (51,328)		

<sup>a</sup>Mean  $\pm$  SD; <sup>b</sup>p values were computed using Mann-Whitney, ANOVA and the  $\chi^2$  Tests. <sup>c</sup>pc values were computed using Kruskal-Wallis Test. <sup>d</sup>Non considered in the analysis.

**Figure 2:** HLA-Bw4 presence associated with high levels of CD4<sup>+</sup> T cell count. CD4<sup>+</sup> T cell count average was calculated for 147 HIV-infected individuals and is shown on top of the respective bars.



**Table 4.** HLA and KIR genotype in 147 HIV-1 infected patients according to control of viremia

Genotype	Controller of viremia (n/%) (n = 18)	Non-controller of viremia (n/%) (n = 129)	p value <sup>a</sup>	OR (95% CI)
HLA Bw4/Bw4	04 (22.2)	22 (17.0)	0.020	
HLA Bw4/Bw6	13 (72.2)	65 (50.4)		
HLA Bw6/Bw6	01 (5.5)	42 (32.5)		
Bw4 <sup>+</sup> (Bw4/Bw4;Bw4/Bw6)	17 (94.4)	87 (67.4)	0.018	8.21 (1.08-63.76)
Bw4 <sup>+</sup> (Bw6/Bw6)	01 (5.5)	42 (32.5)		
Bw4 <sup>+</sup> -Ile80 <sup>+</sup>	13 (76.5)	67 (77.0)	0.790	0.97 (0.28-3.31)
Bw4 <sup>+</sup> -Ile80 <sup>-</sup>	04 (23.5)	20 (23.0)		
KIR3DL1	14 (77.8)	93 (72.1)	0.287	2.26 (0.49-10.51)
KIR3DS1	02 (11.1)	30 (23.2)		
<sup>b</sup> Non 3DL1 <sup>+</sup> -3DS1 <sup>+</sup>	02 (11.1)	06 (4.6)		
KIR3DL1+Bw4 <sup>+</sup> Ile80 <sup>+</sup>	11 (64.7)	48 (55.2)	0.313	
KIR3DL1+Bw4 <sup>+</sup> Ile80 <sup>-</sup>	02 (11.7)	12 (13.8)		
KIR3DS1+Bw4 <sup>+</sup> Ile80 <sup>+</sup>	01 (5.9)	15 (17.2)		
KIR3DS1+Bw4 <sup>+</sup> Ile80 <sup>-</sup>	01 (5.9)	06 (6.9)		
<sup>b</sup> Non 3DL1 <sup>+</sup> -3DS1 <sup>+</sup> +Bw4 <sup>+</sup>	02 (11.7)	06 (6.9)		
<b>Loci/allele</b>				
HLA-A	-	-	0.828	
HLA-B				
B*27	1 (5.5)	1 (0.8)	0.100	7.53 (0.45-126.02)
B*57	8 (44.4)	7 (5.4)	< 0.0001	13.94 (4.19-46.38)
HLA-C				
Cw*18	05 (27.8)	03 (2.3)	< 0.0001	16.15 (3.46-75.43)

Note: OR, Odds ratio; CI, confidence interval.<sup>a</sup>p values were computed using Fisher's exact test; <sup>b</sup>Non considered in the analysis.

### Protective *B\*57* and *Cw\*18* alleles in the viremia controller

*HLA* class I alleles play a role in the biology of both NK and CTL responses, in the control of viral load levels. To evaluate the influence of *HLA* class I alleles in viremia control we compared the frequencies of *HLA-A*, *HLA-B*, and *HLA-C* alleles for viremia controller and non-controller groups. No significant deviations from Hardy-Weinberg equilibrium were found in all loci analyzed: *HLA-A* ( $p = 0.33$ ), *HLA-B* ( $p = 0.34$ ), and *HLA-C* ( $p = 0.30$ ). No statistically significant difference for *HLA-A* allele frequencies was found between the groups ( $p = 0.828$ ) (Table 4). However, we detected a significant difference in the *B\*57* (OR = 13.94; 95% CI = 4.19-46.38;  $p < 0.0001$ ) and *Cw\*18* (OR = 16.15; 95% CI = 3.46-75.43;  $p < 0.0001$ ) allele frequencies, which were relatively more frequent in the viremia controller than in non-controller group (Table 4). These data suggest a protective role of the *B\*57* and *Cw\*18* alleles in the control of viral load. Curiously, 100% of *Cw\*18* carriers (5/5) that control the viremia are also *B\*57* carriers, while this last allele was found in 66.7% (2/3) of the non-controller group.

### Influence of *HLA-B\*57* allele in the protection conferred by *HLA-Bw4*

Genetic and functional studies have shown a protective effect of *HLA* class I alleles, in particular *B\*27* and *B\*57*, in the HIV disease progression. These alleles belong to the *HLA-B* *Bw4* group. In order to evaluate the effects on NK and CTL responses to that regard, we analyzed the role of *HLA-Bw4* on HIV disease progression markers in the presence and absence of *B\*27* and *B\*57* alleles. The protective effect of *HLA-Bw4* remained significantly upon removal of the individual positive for *B\*27* ( $p = 0.044$ ), but was entirely lost in the absence of *B\*57* ( $p = 0.201$ ) (Table 3), indicating that the protection conferred by *HLA-Bw4* is completely dependent on the presence of *B\*57*.

### Effects of *KIR3DS1/3DL1* on viral load and $CD4^+$ T cell count modulation

*HLA-Bw4* molecules with isoleucine at position 80 (*Bw4-80Ile*) are ligands for inhibitory *KIR3DL1* receptors and *Bw4-80Ile* in combination with the activating *KIR3DS1* found to result in delayed progression to AIDS. To assess the effect of the alleles *Bw4-80Ile*, *KIR3DL1* and *KIR3DS1* on viral load and  $CD4^+$  T cell count modulation we analyzed their distribution in this population. Initially, we compared the viral load and  $CD4^+$  T cell average between the patients *Bw4-80Ile*<sup>+</sup> (*80Ile/80Ile* or *80Ile/80Thr*) and *Bw4-80Ile*<sup>-</sup> (*80Thr/80Thr*) (Table 3). We observed a non protective influence of *Bw4-80Ile* on viral load ( $p = 0.687$ ) or  $CD4^+$  T cell count ( $p = 0.687$ ) modulation. Next, we compared the frequencies of *Bw4-80Ile* and *Bw4-80Thr* in the viremia controllers and non-controllers groups (Table 4). We found no statistically

significant difference between the groups ( $p = 0.790$ ). This result may suggest that the association of *HLA-Bw4* with viral load reduction in the HIV-1 infection found here is not specifically attributed to the *Bw4-80Ile*. The influence of *KIR3DL1* and *KIR3DS1* subtypes on clinical markers modulation was also tested: *KIR3DL1* and *KIR3DS1* were carried by 72.8% (107/147) and 21.8% (35/147) of patients, respectively. Eight patients (5.4%) who were negative for *KIR3DL1* and *KIR3DS1* were not included in the analysis (Table 3). We did not find any significant association between the presence or absence of *KIR3DL1/KIR3DS1* genotype and the clinical markers of HIV-1 infection (Tables 3 and 4). We observed that all patients who carried protective *B\*57* and *Cw\*18* (*B\*57* = 15/147; *B\*57-Cw\*18* = 7/15; *Cw\*18* = 8/147) alleles also carried *KIR3DL1*, but carried no *KIR3DS1*. These data suggest no effect of *KIR3DS1/3DL1* genotype on viral load and  $CD4^+$  T cell count modulation.

## DISCUSSION

Previous findings have shown the important role of cellular immunity in controlling HIV-1 replication.<sup>8,10,11,32</sup> HIV-specific CTL, selected according to *HLA* class I repertoire associated to viral peptides, can control the viral replication efficiently through lysis of infected cells before new viral particles arise.<sup>14</sup> In this study *HLA-A* and *HLA-B* alleles were grouped according to serological antigens *HLA-Bw4* and *HLA-Bw6* (Table 2). Some studies have reported the important influence of *HLA-Bw4* in HIV-1 pathogenesis. Martin *et al* (2002) demonstrated epistatic interaction between *KIR3DS1/Bw4-80Ile* and delayed progression to AIDS by genetic analysis.<sup>21</sup> Qi *et al* have demonstrated that *KIR3DS1/Bw4-80Ile* genotype is involved directly in the protection against HIV-1, controlling the viral load through lysis of infected cells, and/or indirectly preventing or delaying the onset of opportunistic infections.<sup>24</sup> This corroborates our findings.

Here, we observed an association between *HLA-Bw4* and viral load reduction. The *HLA-Bw4* phenotype seems to modulate the viral load levels, when considering the whole infected population (Figure 1 and Table 3). Ninety four percent of these patients, who consistently presented viral load levels  $\leq 1,800$  copies/mL, during a period of 2 to 12 years without antiretroviral therapy, carried *HLA-Bw4*, which confirms the protective role of this specific allele for HIV-infected individuals. However, the protective influence of *HLA-Bw4* on the control of viremia is completely dependent on the presence of *B\*57* allele (Table 3), a finding that confirm the already reported favorable effect of *B\*57* on HIV-1 viral load.<sup>6</sup>

Beyond the *B\*27* and *B\*57*, *HLA-C* alleles can play an important role in the HIV/AIDS context. Lazaryan *et al* have demonstrated that *Cw\*18* allele is associated with lower viral load among all subjects with and without *B\*5703* in the Zambian cohort.<sup>33</sup> In our study, 27.8% of the viremia

controllers individuals carried *Cw\*18* allele, which was significantly associated with lower viral load levels, suggesting a protector effect on HIV-1 infection in this population. As 100% (5/5) of controllers of viremia individuals who carried *Cw\*18* also carried *B\*57* and these alleles are in linkage disequilibrium it is possible to attribute the protective effect of *Cw\*18* to the presence of *B\*57*. Theoretically, the role of *Cw\*18* and *HLA-Bw4/B\*57* may be independent, although it can be involved in the same context to produce effective NK and CTL responses.

*HLA-Bw4* epitope and *HLA-C* allotypes are ligands for NK cell receptors *KIR3DL1/3DS1* and *KIR2DL1/2DL2/2DL3*, respectively<sup>34</sup> and stimulate its cytotoxic activity that is complementary to CTL responses. Inhibitory *KIR3DL1* and activating *KIR3DS1* receptors can play a protective effect on AIDS progression in the presence of *HLA Bw4-80Ile* ligand in the infected cell.<sup>21,35</sup> Inhibitory *KIR3DL1* allele highly expressed on the cell enhance protection conferred by *HLA Bw4-80Ile* allele, including *B\*57* specifically.<sup>35</sup> In our work *HLA Bw4-80Ile*, *KIR3DL1* and *KIR3DS1* alleles had no influence in the modulation of clinical markers in HIV-1 infection, even in face of high *KIR3DL1* (72.8%) and moderate *KIR3DS1* (21.8%) expression.

Involvement of *Cw\*18* in NK cell activity will not be discussed here because we did not verify the distribution of *KIR2DL(1-3)* receptors to *HLA-C* allotypes, and therefore it is beyond the scope of this work. We cannot define the exact mechanism by means both populations of T lymphocytes (CTL and NK) might modulate the virus level in favor of the host. However, once the pivotal role of CTL is influenced by the *HLA* profile, we could suggest that the allelic groups of *HLA-B* that compound the *HLA-Bw4* group and *HLA-Cw\*18* allele are specific and more efficient in presenting peptides of circulating viral variants in this population, being able to produce an effective cellular immune responses. In addition, according to our results, it is possible to suggest that *HLA-Bw4* and *HLA-Cw\*18* might function as activators of NK cell cytotoxic activity to destroy HIV-infected cells, which represent one of the main viral reservoirs. As a consequence of the destruction of these reservoirs mediated by combined CTL and NK activity, the plasmatic viral levels would decrease. Obviously, functional assays would be necessary to confirm this hypothesis.

Our data not only confirm the findings of other studies, in different populations, but also reinforce the important influence of the MHC polymorphisms in HIV/AIDS natural history, regardless the ethnic groups. It is very important to emphasize that the Brazilian urban populations are quite heterogeneous in terms of ethnic/genetic structure, and unique (a wide genetic mixture involving Amerindians, Portuguese and Africans),<sup>26-28,36</sup> when compared to other populations around the world. Bahia, in particular, has a bigger genetic contribution of Africans and Portuguese,<sup>26-28</sup>

what becomes genetically different of the populations from other regions of Brazil. Additionally, environmental factors involved in HIV infection, such as social-economic and cultural factors, are also particular to this specific Brazilian region (Bahia), which resembles African countries, not only due to its genetic composition but also to social structure, culture, and health problems.<sup>28,37</sup> Therefore, we could deduce that the profile of the association found here will be able to serve as orientation foretells for politics of public health, with reference to infection/illness prevention, and that the *HLA* profile of the studied patients can be used for therapeutic monitoring in order to improve the effectiveness of the AIDS treatment.

## CONCLUSIONS

*HLA-Bw4-B\*57* and *Cw\*18* alleles are associated with lower level of viral load in HIV-infected patients from Bahia. This is the first study to demonstrate that serological epitopes *HLA-Bw4* and *HLA* class I alleles are associated with disease progression markers in HIV-1 infection involving patients from Bahia, and provides important information about how the host genetic factors influence the AIDS clinical course.

Taken together, these findings may help us in understanding the determinants of HIV evolution in Brazilian patients, as well as in providing important information on immune response correlates of protection for such population. The prevention of the AIDS development in the Bahia population will reduce the costs with medicines that are necessary for who has already developed the disease. However, it is necessary to confirm these data through a study with a larger number of samples.

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