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Expression of HLA-E molecules in the placental tissue of women infected with HIV-1 and uninfected women

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ABSTRACT

Introduction: Expression of HLA-E molecule in the placental extravillous trophoblast is associated with immune system cell inhibition, resulting in immune tolerance to fetus during pregnancy. HIV-1 can infect trophoblast cells and modify the expression of HLA-E, which may inhibit the cytotoxic activity of the immune system.
Aim: The aim of this study was to evaluate HLA-E expression in third trimester placental tissue of women infected with HIV-1 and uninfected women.
Methods: We performed an immunohistochemistry assay to evaluate HLA-E staining in the placental tissue of 99 HIV-1 infected and 85 uninfected women. A pathologist analyzed and classified the HLA-E expression in the placental cells.
Results: Irrespective of the HIV status, HLA-E staining was observed in the extravillous trophoblast cells, endothelial cells and Hofbauer cells, but not in the syncytiotrophoblast. HLA-E staining showed no significant difference between the placental tissue of women infected with HIV-1 and uninfected women ($P = 0.76$). Considering HIV-1 infected women, HLA-E staining was not influenced by HIV-1 viral load ($P = 0.48$), $CD4^+$ T-cell count ($P = 0.10$) and antiretroviral therapy used during pregnancy ($P = 0.54$).
Discussion: Despite the presence of HIV-1 infection, the expression of HLA-E molecules in the placental tissue was not modified when the infection was under antiretroviral therapy control.

1. Introduction

The human leukocyte antigen E (HLA-E) is a nonclassical class I molecule encoded by a gene of the major histocompatibility complex (MHC). The molecule may modulate the immune response in several pathological conditions, including tumors and viral infections, and in physiological conditions such as pregnancy [1,2]. The expression of nonclassical HLA class I molecules in the extravillous trophoblast (EVT) cells of the placenta contributes to maternal tolerance against paternal antigens, permitting a successful pregnancy [3–5]. In pregnant women infected with human immunodeficiency virus-1 (HIV-1), studies indicate that the virus can also infect the EVT cells [6], and the presence of the HIV-1 $p24_{14\text{-}22}$ peptide can increase the expression of HLA-E in lymphocytes [7,8], suggesting that the virus may control the expression of the molecule. The interaction of HLA-E with the natural killer (NK) receptors may inhibit NK cell cytotoxic activity, representing an immune response evasion strategy of HIV-1 [7,8].

Considering that HIV-1 can infect EVT cells and that the viral

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infection can modulate HLA-E expression, it is relevant to analyze whether the EVT cells of women infected with HIV-1 exhibit altered expression of HLA-E when compared to those of uninfected women. Therefore, this study aimed to evaluate the expression of HLA-E in the placental tissue of women infected with HIV-1 and uninfected women.

2. Methods

2.1. Ethical aspects

The study was approved by the Ethics Committee of the College of Nursing of Ribeirão Preto, University of São Paulo, Brazil (protocol # 1330/2011), and all participants signed the Free and Informed Consent Form. This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. Population samples

We analyzed 184 fragments of paraffin-embedded placental tissue, of which 99 fragments were from HIV-1-infected women (HIV+) and 85 from uninfected women (HIV-). All placentas were obtained from successful third trimester (37 weeks or older) pregnancies.

2.3. Clinical data

Clinical and laboratory data of the HIV + group, such as viral load, circulating $CD4^+$ T lymphocyte count ($CD4^+$ T-cells), and type of antiretroviral therapy (ART) used during pregnancy were retrieved from patients' medical records available at the electronic files of the University Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil.

2.4. Immunohistochemistry assay

Fragments of paraffin-embedded placental tissue were cut into 3-μm sections, fixed on silanized slides, and subjected to immunohistochemical assay to evaluate the expression of HLA-E molecules.

Tissue fragments were incubated at 60 °C for 1 h and subjected to xylol and alcohol washing for complete deparaffinization and hydration. For antigen recovery, citrate solution (pH 6.0) was used for 40 min at 96 °C. Endogenous peroxidase blocking was performed using phosphate-buffered saline (PBS), methanol, and 30% hydrogen peroxide for 10 min, and to avoid nonspecific binding, the specimens were incubated with 1% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO) for 5 min. The anti-HLA-E antibody (clone MEM-E/02, EXBIO Antibodies, Prague, Czech Republic), diluted at 1:250 in a solution containing 1% BSA, was added to the specimens and incubated overnight at room temperature in a moist chamber. The specimens were then incubated with EasyLink One (EasyPath, Indaiatuba, Brazil) polymer for 30 min in dark. The specimens were then stained with diaminobenzidine (DAB) for 5 min and counterstained with Mayer's hematoxylin for 3 min. Dehydration was performed using different concentrations of alcohol and xylol, and Canada balsam was used for slide mounting.

A pathologist blinded to patient identification and clinical and laboratory data analyzed the tissues and classified the magnitude of HLA-E expression in trophoblast cells by adopting the score used by Li et al. [9]. When less than 5% of the cells expressed HLA-E molecules, the expression was considered to be negative. Positive labeling was classified as 1+ (6–25% of the cells expressing HLA-E), 2+ (26–50%), 3+ (51–75%), and 4+ (>75%).

2.5. Statistical analysis

For data analysis, chi-square test was applied with significance set at 5% and a logistic model was applied. For the analysis, the Odds Ratio (OR) was estimated with a 95% confidence interval (95% CI) by using the software SAS® 9 [10].

3. Results

HIV-1-infected women were aged 18–41 years (mean = 28; SD ± 6.25), and most of them were single, exhibited Caucasian ancestry, were unemployed, and had up to 9 years of schooling. Uninfected women were aged 18–38 years (mean = 24, SD ± 5.54), and most of them presented Caucasian ancestry, had 9–12 years of schooling, were unemployed, and were married (see Table 1).

Regarding obstetric history, HIV-1-infected women gave birth to an average of 3 children (range 1–10) and 20% of them had previous miscarriages, 87% had access to prenatal care, and 51% had cesarean deliveries. Uninfected women had an average of 2 children (range 1–7) and 14% had miscarriages, 100% had access to prenatal care, and 74% had vaginal delivery.

Clinical and laboratory data indicated that 83.7% of HIV-1-infected women had a viral load of <10,000 copies/mL, 44.1% had $CD4^+$ T-cell count between 200 and 499 cells/mm^3, and 95.1% of the patients used protease inhibitors (PI) in combination with antiretroviral drugs.

Irrespective of the HIV status, HLA-E was expressed in the human placental tissue mainly in the EVT cells, endothelial cells, and Hofbauer cells, but was not expressed in the syncytiotrophoblast (see Fig. 1).

The stratification of patients according to the HIV status revealed that the HIV + group presented a relatively higher frequency of 3 + staining and a lower frequency of 1 + staining, whereas the HIV- group showed a relatively higher frequency of 4 + staining and a lower frequency of negative staining. Despite this variation, HLA-E staining of the placentas showed no significant difference between the HIV+ and HIV- groups ($P = 0.76$; Table 2).

The stratification of HLA-E staining in the placentas of the HIV + group according to viral load, $CD4^+$ T-cell count, and type of ART used exhibited no significant difference (Table 2). It should be emphasized that the use of ART was analyzed according to the use or no use of PI in combination with antiretroviral drugs.

Logistic regression results also indicated no statistically

Table 1
Participant distributions in this study according to demographic parameters.

Demographic parameters	HIV+ (%)	HIV- (%)
Marital status		
Single	58	14
Married	15	24
Living common law	19	59
Separated	3	0
Divorced	4	3
Widowed	1	0
Ancestry		
Caucasian	68	53
Admixture	20	47
African	12	0
Years of schooling		
Up to 9 years	60	37
From 9 to 12 years	30	63
More than 12 years	6	0
Undeclared	4	0
Employment		
Yes	33	31
No	67	69

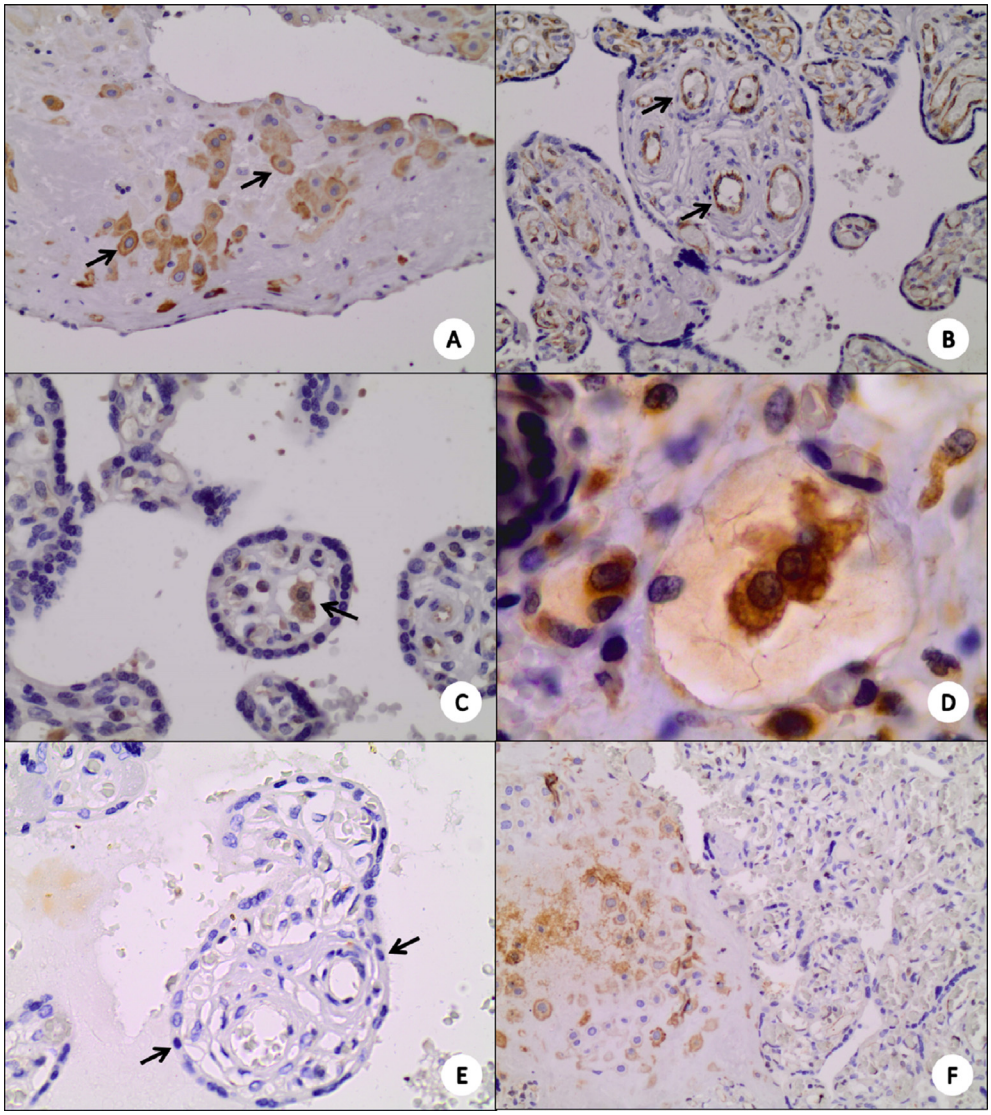

Fig. 1. Expression of HLA-E molecules in the human placental tissue. The anti-HLA-E antibody (clone MEM-E/02, EXBIO) was used for immunohistochemistry analysis. In HIV+ and HIV- placentas, the expression of HLA-E can be observed in the extravillous trophoblast cells (A, F), endothelial cells (B), and Hofbauer cells (C, D), but not in the syncytiotrophoblast (E, F).

Table 2
HLA-E staining frequency (0–4+) stratified according to the following variables: HIV-1 status, HIV-1 viral load, $CD4^+$ T-cell count, and type of antiretroviral regimen used by the patients.

	HLA-E expression			*P*-value
	0 (≤5%)	1,2,3 (6–75%)	4 (>75%)	
HIV-1 Status				
Uninfected	14.1% (12)	60.0% (51)	25.9% (22)	0.76
Infected	15.2% (15)	63.6% (63)	21.2% (21)	
Viral Load				
<10,000 copies/mL	17.3% (14)	61.7% (50)	21.0% (17)	0.48
>10,000 copies/mL	6.3% (1)	75.0% (12)	18.8% (3)	
$CD4^+$ T-cells				
≥500 cells/mm^3	22.0% (9)	63.4% (26)	14.6% (6)	0.10
200–499 cells/mm^3	12.8% (5)	56.4% (22)	30.8% (12)	
<200 cells/mm^3	6.7% (1)	86.7% (13)	6.7% (1)	
ART				
Without PI	25.0% (1)	75.0% (3)	0% (0)	0.54
With PI	14.1% (13)	64.1% (59)	21.7% (20)	

ART: antiretroviral therapy.
PI: protease inhibitors.

significant association, emphasizing that HLA-E staining in the placentas of the infected and uninfected women was not affected by HIV-1 infection.

4. Discussion

HLA-E is expressed in the placental tissues and has immunomodulatory properties. The effect of HIV-1 infection on HLA-E expression, however, has not yet been studied. Therefore, in this study, we evaluated HLA-E expression in the placental tissues of HIV-1-infected and uninfected women.

The expression of HLA nonclassical class I molecules in the placental tissues has been associated with one of the many factors that collaborate with the immunological tolerance during pregnancy, indicating that these molecules may inhibit the cytotoxic activity of uterine NK cells, guaranteeing a successful pregnancy [11]. Our results showed that HLA-E molecules were expressed in the EVT, endothelial, and Hofbauer cells, but not in the syncytiotrophoblast. Menier et al. [12] analyzed the expression of HLA-E in the first trimester placentas of uninfected women and showed that

HLA-E was expressed in the EVT, endothelial, and Hofbauer cells, but not in the perivillous trophoblast and syncytiotrophoblast.

It has been reported that HIV-1 induces the expression of HLA-E in lymphocytes to inhibit the host's immune system. The expression can be regulated by the HIV-1 $p24_{14-22}$ peptide, and its interaction is mediated by the CD94/NKG2A inhibitory receptor of NK cells. This interaction results in the inhibition of NK cytotoxic activity, representing an evasion strategy used by HIV-1 [8]. In addition, Nattermann et al. [7] demonstrated that lymphocytes infected with HIV-1 *in vitro* induced the expression of HLA-E and decreased the cytotoxic activity of NK cells, potentially contributing to the establishment of a chronic infection. At the placenta level, our results show that the presence of HIV-1 infection did not interfere with the expression of HLA-E. Possibly, the control of viral load during pregnancy may have influenced this result once all patients were using ART.

The role of HLA-E and HLA-G in mother-to-child transmission of HIV-1 has been analyzed in women not treated with ART during pregnancy [13–16]. Despite the lack of studies regarding the modulation of HLA-E by ART, the interference of antiretroviral drugs on the modulation of HLA-G expression has been analyzed. Cabello et al. [17] reported an increased number of monocytes expressing HLA-G in patients using antiretroviral drugs when compared to that in untreated patients. Rivero et al. [18] evaluated the expression of HLA-G in different HIV therapeutic regimens and revealed that nucleoside analog reverse-transcriptase inhibitors increased HLA-G expression in circulating $CD4^+$ monocytes and lymphocytes, whereas protease inhibitors did not affect lymphomononuclear cell HLA-G expression. These data are very important because they offer new immunological perspectives on ART, combining the drug pharmacological properties with the modulation of immunomodulatory molecules.

Although there are no studies regarding HLA-E expression in the placental tissue, stratified according to HIV-1 viral load and $CD4^+$ T-cell count, the evaluation of another immunomodulatory molecule showed that the placental expression of HLA-G1 in HIV-infected women exhibited a significant correlation between maternal viral load and the expression of HLA-G1 [19].

Our results indicated that in the placental tissue of HIV-1-infected women with infection under control, the expression of HLA-E in the EVT cells was not modified when compared to that in the placentas of uninfected women. The expression level of HLA-E was not influenced by factors such as viral load, $CD4^+$ T-cell count, and use of PI with ART. Although further research studies are needed to understand the influence of HIV-1 on the expression of HLA-E, our results indicate that ART reduces HIV-1 viral load and the peptides that modulate HLA-E expression. Thus, the maintenance of HLA-E expression in HIV-1-infected women may propitiate a pregnancy similar to uninfected women.

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