The impact of pregnancy on the HIV-1-specific T cell function in infected pregnant women

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Abstract Evidences indicate that pregnancy can alter the Ag-specific T-cell responses. This work aims to evaluate the impact of pregnancy on the in vitro HIV-1-specific immune response. As compared with non-pregnant patients, lower T-cell proliferation and higher IL-10 production were observed in T-cell cultures from pregnant patients following addition of either mitogens or HIV-1 antigens. In our system, the main T lymphocyte subset involved in producing IL-10 was CD4+FoxP3−. Depletion of CD4+ cells elevated TNF-α and IFN-γ production. Interestingly, the in vitro HIV-1 replication was lower in cell cultures from pregnant patients, and it was inversely related to IL-10 production. In these cultures, the neutralization of IL-10 by anti-IL-10 mAb elevated TNF-α release and HIV-1 replication. In conclusion, our results reveal that pregnancy-related events should favor the expansion of HIV-1-specific IL-10-secreting CD4+ T-cells in HIV-1-infected women, which should, in the scenario of pregnancy, help to reduce the risk of vertical HIV-1 transmission.
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Introduction

During pregnancy the maternal immune system has a very difficult task; it should be modulated in order to not reject the semi-allogeneic fetus, but at the same time, it must protect the mother and her fetus from infections.

Although the mechanisms by which the fetus is protected from the maternal immune system during pregnancy are not fully understood, predominant Th17 and Th1 phenotypes have been related to recurrent spontaneous abortion (RSA) [1,2]. However, Th2-dominant immunity has also been reported in RSA [3,4]. In fact, the Th1/Th2 paradigm is now insufficient to explain the mechanism of tolerance of maternal immune cells to the fetus.

In this scenario, studies have suggested that, during pregnancy, the hormonal changes operate to dampen the cellular immune response against the semi-allogeneic fetus by inducing maternal regulatory T cells [5,6]. In humans, several types of regulatory T cells (Tregs) have been described, including natural Tregs (nTregs) and the adaptive type 1 regulatory (Tr1) and Th3 subsets. The nTregs are produced in the thymus as a functionally mature subpopulation of CD4+ CD25+ T cells that express intracellularly the transcription factor forkhead box P3 (FOXP3) [6]. It has been proposed that nTregs mediate their suppressive activity mainly via cell-cell contact, most probably via membrane proteins such as Lag-3, galectin-1 and CTLA-4 [7,8]. On the other hand, Tr1 and Th3 cells constitute another regulatory populations, being more tightly related to the production of high levels of IL-10 and TFG-β [9,10], respectively. Both cytokines play predominant anti-inflammatory actions.

Although the great majority of experimental evidences have demonstrated an increase in nTregs at the fetal-maternal interface [11,12], some studies have failed to demonstrate a systemic increase in phenotypically well-defined nTregs [13,14]. Despite ambiguities regarding the elevation of systemic nTreg cells in normal pregnancy, abnormalities in nTregs frequency or function have been reported in patients experiencing RSA [2]. Compared with the decidual CD4+ T cells from women with a normal pregnancy undergoing an elective interruption of pregnancy, the decidual CD4+ T cells from women suffering from RSA show a defect in either FOXP3 expression or IL-10 production [15,16]. Since IL-10 is directly involved in down-regulating Th1 function by inhibiting IFN-γ production, this anti-inflammatory cytokine has been proposed to play an important immunoregulatory role in pregnancy [17,18]. On the other hand, by attenuating Th1-mediated immunity, pregnant women are more susceptible to some infections like toxoplasmosis and listeriosis and have increased risk of severity in viral infections such as influenza and varicella [19]. Therefore, the absence of appropriate immune response during pregnancy might bring adverse pregnancy outcomes.

With regard to the human immunodeficiency virus (HIV), the number of infected young women has been significantly increasing [20], mainly in developing countries, where the frequency of pregnancy among them is also elevated. Considering that HIV is an intracellular pathogen, HIV-specific CD4+ Th1-mediated immune response plays a pivotal role in protecting HIV-infected patients from disease progression [21]. These T lymphocytes, by secreting high levels of IFN-γ and IL-2, mobilize and coordinate a variety of other cell types against HIV, such as HIV-specific CD8+ T cells and natural killer (NK) cells [21,22]. These cells may exert a direct anti-viral effect by killing the HIV-infected CD4+ T lymphocyte but also promote an efficient Th1 response by secreting IFN-γ [21]. Interestingly, the risk of disease progression to acquired immunodeficiency syndrome (AIDS) in HIV-infected women does not increase during pregnancy, and until now, no study was performed to evaluate the impact of HIV-1-infection on maternal immune profile and the effect of pregnancy on HIV-1-specific T cell responses, which are the key objectives of this study.

Materials and methods

Patients

For our study, HIV-1-infected pregnant (n=25) and non-pregnant (n=25) women who controlled the plasma viral load (PVL) were recruited from the Obstetrics Service of three Public Hospitals in Rio de Janeiro State. As control for pregnancy- and HIV-1-infection-related immune events, healthy HIV-(1 and 2) negative pregnant (n=10) and non-pregnant (n=10) women, were also recruited.

Clinical data from the HIV-infected pregnant patients were obtained from medical records including antiretroviral therapy, plasma viral load (PVL) and CD4+ T cell count (Table 1). As it is accepted that the majority of HIV-1 vertical transmissions occurs in late gestational times, peripheral blood samples were collected in the last trimester of pregnancy.

All pregnant patients were antiretroviral therapy naïve before pregnancy. Although the antiretroviral drugs were available at the site, some pregnant women were still without treatment at the time of blood sampling, because they only looked for medical assistance just before delivery, and most of them were not aware of their seropositivity. Nevertheless, all pregnant patients received continuous intravenous zidovudine (AZT) infusion during the elective cesarian section. To avoid differences concerning systemic viral load, all HIV-1-infected patients (n=50) had undetectable PVL (<80 copies of RNA/mL) at the moment of the study. Furthermore, patients with any other infection besides HIV-1, who smoked or used illegal substances, were not included in the study.

The infected status of the neonates was confirmed or eliminated by nucleic acid technique (PCR) for env, nef, and vif HIV-1 provirus genes two weeks after the end of the prophylaxis with AZT, that is, 6 weeks after birth.

A written informed consent was obtained from all mothers. This study was approved by the Ethical Committee for Research on Human Subjects of the Federal University of State of Rio de Janeiro (UNIRIO).

T cell cultures and stimulation

The peripheral blood from all women was drawn into tubes containing EDTA and the peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on Ficoll–Hypaque density gradient. In some experiments, PBMC
were submitted to either B cells or CD4\(^+\) cells negative selection by using magnetic beads coated with anti-CD19 mAb or anti-CD4 mAb, respectively. The efficacy of this procedure was about 1–2\% (for CD19\(^+\) cells) and 3\% (for CD4\(^+\) cells) as evaluated by flow cytometry (data not shown). The number of viable cells, measured by trypan blue exclusion in a hemocytometer, was adjusted to 1 \times 10^5/mL and cultured in a 96-well microtiter plate with 200 \(\mu\)L of RPMI 1640 added with 2 \(\mu\)M of L-glutamine ( GibCO, Carlsbad, CA, USA), 10% of FCS, 20 \(\mu\)U/mL of penicillin, 20 \(\mu\)g/mL of streptomycin and 20 \(\mu\)M of HEPES buffer. To induce polyclonal activation, these cultures were maintained with plate bound anti-CD3 mAb (1 \(\mu\)g/mL; OKT3, mouse IgG1; Ortho Biotech, Bridgewater, NJ) plus anti-CD28 (1 \(\mu\)g/mL; clone CLB, Research Diagnostic, Flanders, NJ) for 3 days or with Phorbol-12-myristate-13-acetate (PMA, 20 ng/mL) plus ionomycin (IO, 600 ng/mL) for 18 h. In order to measure HIV-1 specific response, CD19-depleted or CD4-depleted PBMC (1 \times 10^6/mL) were kept for 6 days in the presence of p24 protein (p24HIV-1\(_{\text{Gag}}\), Sigma Co) at 1 \(\mu\)g/mL or a cocktail of immuno-dominant synthetic envelope peptides (ppHIV-1\(_{env}\) T1, T2, TH4, P18, MN, P18 IIIB) at 1 \(\mu\)M each peptide, with or without recombinant human IL-2 (rhl-2) at 20 U/mL (BD Systems, Minneapolis, MN). The concentrations of p24 protein (p24HIV-1\(_{\text{Gag}}\)) and ENV-derived peptides were established in our laboratory as the optimum dose that induced CD8\(^+\) T cell proliferation in acutely HIV-1 infected patients. The cells were cultured at 37 °C in a humidified 5\% CO\(_2\) incubator.

### Proliferation assay

Approximately 1 \times 10^5/well of CD19\(^+\) cell-depleted PBMC were activated with anti-CD3 (1 \(\mu\)g/mL) plus anti-CD28 (1 \(g\)g/mL) for 3 days or with HIV-1-antigens for 5 days. T cell proliferation was measured following addition of 0.5 \(\mu\)Ci/well of [\(^{3}H\)] thymidine during the last 8 h of incubation. After this period, the cells were harvested in glass fiber filters in an automatic cell harvester and radioactive incorporation was measured using a liquid-scintillation counter. The results were shown as stimulation results were shown as mean ± sd of counts per minute (cpm).

### Flow cytometry analysis

Mouse anti-human monoclonal antibodies (mAbs) to CD3-PE, CD8-FITC, CD4-FITC, CD45RO-PE, CD25-APC, CD127-AlexaFluor647, FoxP3-PE, IL-10-APC, IFN-\(\gamma\), AlexaFluor488, and all isotype-control antibodies were purchased from BD Bioscience (San Diego, CA, USA), and were used to characterize the phenotypes of T cells: regulatory (FoxP3\(^+\)CD25\(^-\)CD127\(^{low}\)) and effector IL-10- or IFN-\(\gamma\)-producing T cells. Briefly, freshly purified PBMC (2 \times 10^5/tube) were incubated with various combinations of the aforementioned mAbs for 30 min at room temperature in the dark according to the manufacturer’s instructions. After washing with phosphate-buffered saline (PBS), permeabilization was performed by incubating cells with Cytofix/Cytoperm (BD Pharmingen, San Diego, CA) at 4 °C for 20 min. After washing, the antibodies for intracellular staining, including anti-FoxP3 or the corresponding isotype control anti-IgG1, were added in various combinations and incubated for 30 min at 4 °C.

For analysis of the frequency effector IL-10-producing or IFN-\(\gamma\)-producing T cells, freshly purified PBMC were initially kept for 18 h in the presence PMA and Ionomycin, then submitted to both surface (anti-CD4-FITC, anti-CD45RO-PE, anti-CD8-FITC) and intracellular (anti-FoxP3, anti-IL-10 and anti-IFN-\(\gamma\)) staining. To optimize cytokine staining, brefeldin A was added 10 h (10 \(\mu\)g/mL; Sigma-Aldrich) before the end of incubation time. After washing with PBS, the analysis was performed using Accuri C6 (Accuri™, Ann Arbor, MI, USA) and Flow software (Accuri™, Ann Arbor, MI, USA). Isotype control antibodies and single-stained samples were used to periodically check the settings and gates on the flow cytometer. After acquisition of 20,000 or 30,000 events, lymphocytes were gated based on forward and side scatter properties after the exclusion of dead cells and doublets.

### Cytokine determination

The supernatants collected from CD19\(^+\) cells-depleted or CD4\(^+\) cells-depleted PBMC cultures, stimulated either polyclonal activation or HIV antigens, were submitted to cytokine measurement by OptEIA ELISA kits (BD, Pharmingen, San Diego), according to manufacturer’s protocol. Briefly, each ELISA was performed using pairs of mAbs directed to human IL-1\(\beta\), TGF-\(\beta\), IL-10, IL-4, TNF-\(\alpha\), and IFN-\(\gamma\). The reaction was revealed with streptavidin–horseradish peroxidase, using 3,3’,5,5’-tetramethylbenzidine (TMB) as substrate. Recombinant human IL-1\(\beta\), TGF-\(\beta\), IL-4, IFN-\(\gamma\), TNF-\(\alpha\), and IL-10 ranging from 10–500 \(\mu\)g/mL were used to construct standard curves.

### Quantification of the in vitro HIV-1 replication

To evaluate the in vitro HIV-1 replication, B cell-depleted PBMC cultures were stimulated with anti-CD3 plus anti-CD28 mAbs (1 \(\mu\)g/mL) and the supernatants were collected 7 days later. To evaluate the impact of endogenous production of IL-10 in vitro HIV-1 replication, saturating doses of anti-IL-10 mAb (22 \(\mu\)g/mL; BD\&D System, Minneapolis, MN) were added in some wells at the time of cell cultures stimulation. As controls, some wells were kept in the presence of the isotype-matching control antibody (IgG2a). This time was chosen because in previous experiments performed in our laboratory the peak of in vitro HIV-1 replication occurred at this point (data not shown). Supernatants were stored at –70 °C for posterior HIV RNA quantification by a commercial HIV-1 RNA quantitative reverse transcriptase polymerase chain reaction (AmpliCor HIV Monitor Test, Roche Molecular System, Branchburg, New Jersey, USA), with an average detection threshold of 50 copies/mL.

### Statistical analysis

Statistical analysis was performed using Prism (GraphPad software). Statistical significance within groups was analyzed with Kruskal-Wallis Test. The nonparametric Mann–Whitney U test was applied to determine whether the two groups were statistically different for each given variable. Spearman’s correlation was used to analyze the correlation...
between the levels of in vitro cytokine production and HIV-1 replication. The significance in all experiments was defined as p < 0.05.

Results

Characteristics of the HIV-infected patients

To evaluate the dual impact of HIV-1 infection and pregnancy on the women’s immunological profile in response to HIV-specific and non-specific stimuli, samples of peripheral blood were drawn from HIV-1-infected non-pregnant (HIV-1 pos.nPW, n = 25) and pregnant (HIV-1 pos.PW, n = 25) women who control (< 80 copies of RNA/mL) their plasma viral load (PVL) (Table 1). As control for each virus-infected group, we also worked with healthy HIV-1-seronegative non-pregnant (HIV-1 neg.nPW, n = 10) and pregnant (HIV-1 neg.PW, n = 10) women.

As we evaluated the HIV-1-specific T cell response, we worked with patients immunologically preserved, that is, with detectable CD4+ and CD8+ T cells. The diagnostic of HIV-1 infection among the children was evaluated by proviral DNA-PCR in the second month of baby’s life.

Figure 1 Impact of pregnancy on in vitro T-cell proliferation of HIV-1-infected women. Cell cultures from CD19+ cell-depleted PBMC (1 x 10^6/mL), obtained from non-pregnant (nPW) and pregnant (PW) women infected or not with HIV-1, were maintained for 3 days in the presence of polyclonal activators anti-CD3 (1 μg/mL) plus anti-CD28 (1 μg/mL) or for 6 days in the presence of HIV-1 antigens, P24 antigen or a cocktail of peptides from HIV envelope protein (ppHIV-1ENV). In some experiments human recombinant IL-2 (20 U/mL) was added at the beginning of cultures. The values are presented as the mean ± sd of cpm for each group and the p values are indicated at the figure.
asymptomatic, with undetectable plasma viral load and a satisfactory CD4+ T cell counts (\(>400\) cells/mm\(^3\)) at the time of the experiments. Concerning the quantification of peripheral CD8+ T cells, as expected, the mean values were significantly higher in HIV-1-infected patients, in comparison with each control group (Table 1). With regard to HIV-1 treatment, 19 out of 25 (80%) of the pregnant women were under antiretroviral therapy (ART) introduced between 20 and 32 weeks of gestation, as soon as the HIV infection was confirmed. Among them, 04/19 were treated with AZT monotherapy during pregnancy. All non-pregnant and 15/19 pregnant patients were treated with a three-drug combination, consisting of two nucleosidic reverse transcriptase inhibitors and a protease inhibitor.

Finally, although all infants were HIV-1-negative and born at term, babies born from HIV-1-infected mothers had a tendency to a lower birth weight, as compared with control group (\(p=0.056\)) (Table 1).

Figure 2  Cytokine profile in polyclonally-activated T cells obtained from HIV-1-infected women. B cell-depleted PBMC cultures (\(1 \times 10^6/mL\)), obtained from non-pregnant [HIV-1-positive (\(n=15\)) and HIV-1-negative (\(n=10\))] and pregnant [HIV-1-positive (\(n=15\)) and HIV-1-negative (\(n=10\))] women were stimulated with anti-CD3 (1 \(\mu g/mL\)) plus anti-CD28 (1 \(\mu g/mL\)) mAbs. After 3 days, the supernatants were collected and the dosage of IL-1\(\beta\), TNF-\(\alpha\), IFN-\(\gamma\), IL-4, IL-10 and TGF-\(\beta\) was quantified by ELISA. The data are presented as mean±sd and \(p\) values are indicated at the figure.

Figure 3  Frequency of systemic nTreg in HIV-1-infected women. Freshly isolated PBMC (\(0.2 \times 10^5/tube\)), obtained from HIV-1-infected non-pregnant (nPW, \(n=12\)) and pregnant (PW, \(n=14\)) women were stained with mAbs to CD4 and CD25, followed by permeabilization and staining with mAb to FoxP3. In (A), representative dot plots showing CD4+ T cell subsets expressing CD25 and FoxP3 in the two different groups. In (B), the mean (± sd) frequency of CD4+ T cell subsets expressing CD25 and FoxP3 in HIV-1-infected nPW and PW. The data are presented as mean±sd and \(p\) values are indicated at the figure.
Lower lymphoproliferative response and higher IL-10 production were observed in HIV-1-infected pregnant women

The first immune event analyzed in our study was T cell proliferation. As shown in the Fig. 1, among HIV-1-infected non-pregnant (nPW, n = 12) and pregnant (PW, n = 14) women, were stimulated with PMA (20 ng/mL) plus ionomicin (600 ng/mL). After 18 h, PBMCs were stained with mAbs to CD4 (or CD8) followed by permeabilization and staining with mAb to FoxP3 and IL-10. In (A) and (B), representative dot plots showing, respectively, CD4⁺ (A) and CD8⁺ (B) T cell subsets expressing IL-10 and FoxP3 after activation of PBMC cultures with PMA/ionomicin for 18 h. In (C), the mean frequency of IL-10-producing T cell subsets (CD4 and CD8) co-expressing FoxP3 in the groups of HIV-1-infected women. Furthermore, as expected, cell cultures from healthy HIV-1-negative women did not respond to HIV-1 antigens.

During pregnancy, the hormonal changes operate to down-regulate the cellular immune response by favoring the induction of regulatory T cells [5,6]. In this context, as demonstrated in Fig. 2, the production of anti-inflammatory cytokine IL-10 was significantly higher in pregnant women, infected or not by HIV-1. Concerning TGF-β, their levels were very low in all polyclonally-activated cell cultures and no statistical difference was observed between the groups. In contrast, the production of the pro-inflammatory cytokines TNF-α and IFN-γ was significantly higher in HIV-1-infected non-pregnant women as compared with all other groups. With regard to IL-4 production, no detectable difference was observed. Of note detectable spontaneous release of cytokines was not observed in our system (data not shown).
Higher production of IL-10 in HIV-1-infected pregnant women was mainly derived from CD4+ FoxP3− T cells

Among the HIV-1-infected patients, higher levels of IL-10 production were detected in polyclonally-activated cell cultures from pregnant women. Therefore, we sought to evaluate if this event would be related to the frequency of systemic nTreg cells (CD4+CD25+FoxP3+). As demonstrated in the Figs. 3A and B, no statistical difference in the frequency of this CD4+ T cells subset was observed between the two groups of HIV-1-infected women (p=0.107). As expected, these nTregs were CD127 negatives (data not shown).

Indeed, IL-10-producing T cells were negative for FOXP3 protein, and were significantly expanded in pregnant patients (Figs. 4A–C). Furthermore, CD4+ T cell subset was the main source of IL-10. On the other hand, higher frequency of IFN-γ-producing T cells was identified in the non-pregnant patients, mainly into the CD8+CD45RO− T cell subset (Figs. 5A–D).

Lower pro-inflammatory cytokines production associated with higher IL-10 release was also observed in response to HIV-1 antigens and was inversely related to the in vitro viral replication

Previous results showed dominant anti-inflammatory profile during pregnancy, even in HIV-1-infected women. Interestingly, the same pattern was observed in B cell-depleted PBMC cultures from pregnant patients following addition of P24 antigen (Fig. 6A). The addition of human recombinant IL-2 to these cultures elevated the release of TNF-α and IFN-γ but the IL-10 production remained significantly higher (Fig. 6A). The same pattern was observed after stimulation with ENV peptides plus IL-2 (data not shown).

In polyclonally-activated T cell cultures from pregnant patients, CD4+ T cells are normally the main source of IL-10. Therefore, CD4+ cells were removed from pregnant patients-derived PBMC before a six-day stimulation with P24 plus IL-2. As demonstrated in Fig. 6B, while the
depletion of CD4+ cells enhanced TNF-α and IFN-γ production, the release of IL-10 was dramatically reduced. This high tendency to produce IL-10 by HIV-specific CD4+ T cells could be related to a lower ability to control in vitro HIV-1 replication. Nevertheless, the extent of HIV-1 replication was significantly lower in pregnant-derived cell cultures (Fig. 7A). Finally, an analysis of the in vitro HIV-1 replication and cytokine profile revealed that, while the level of TNF-α correlated directly with viral replication, an inverse relationship was observed with IL-10 production (Fig. 7B).

In order to understand the extent of this inverse correlation, we added saturating doses of anti-IL-10 mAb at the beginning of the incubation periods, and evaluated the viral replication 7 days later. As demonstrated in Fig. 6, the blockade of IL-10 activity clearly enhanced both HIV-1 replication (Fig. 7C) and TNF-α (Fig. 7D) release in these cultures.

**Discussion**

Women now represent the major group of newly HIV-1-infected people, mainly in developing countries where the frequency of pregnancy among them is also elevated. During pregnancy, suppression of cell-mediated immunity has been accepted as a mechanism that promotes maternal tolerance to the fetus. On the other hand, it also contributes to increased morbidity and mortality for both mother and fetus following viral infections [2,19]. Interestingly, in our study, we have described pregnancy-related events that attenuate HIV-1-specific Th1 response without elevating the in vitro HIV-1 replication.

Here, lower in vitro T cell proliferation induced by either mitogens or HIV-1 antigens was identified in HIV-1-infected pregnant patients. Additionally, in these cell cultures, IL-10 was the main cytokine produced, which seems to be a characteristic of pregnancy.

IL-2 is a very important cytokine for T cell proliferation [23] and it is not normally present in appreciable levels during pregnancy [24]. In our system, although IL-2 addition to pregnant patients-derived cell cultures had elevated both T-cell proliferation and TNF-α and IFN-γ production in response to HIV-1 antigens, IL-10 continued to be the main cytokine produced. Interestingly, depletion of CD4+ cells from these cultures enhanced the production of Th1-related cytokines and diminished dramatically the level of IL-10 released in response to HIV-1 antigens. These results suggest that pregnancy-related events tend to favor the expansion of HIV-1-specific IL-10-producing CD4+ T cells which are able to attenuate the HIV-1-specific Th1-mediated immune response. Unfortunately, it was not possible to re-evaluate HIV-1-specific immune response because, after delivery, the clinical follow-up of these women was transferred to other institutions.
In our system, the higher tendency to produce IL-10 was not due to pregnancy-related nTregs expansion, since no difference in the frequency of CD4+CD25+FoxP3+ T cells subset was identified between HIV-1-infected pregnant and non-pregnant patients. This finding is in agreement with other studies that have failed to demonstrate a systemic increase in human nTregs in pregnancy, mainly during the last trimester [13,14], exactly when the blood samples were collected. Two recent studies, however, have demonstrated conflicting findings about the frequency of peripheral nTregs in HIV-1-infected pregnant women. Study by Richardson and Weinberg [25] has identified that, as compared to healthy HIV-1-negative, a higher expansion of nTreg during the second trimester was observed in HIV-1-positive pregnant women followed by a significant reduction during third trimester. On the other hand, Kolte et al. [26] published data showing a significant elevation in the frequency in this regulatory T cells only in HIV-1-negative pregnant women during the second trimester. Unfortunately, we did not measure the frequency of nTregs in the early times of pregnancy. Nevertheless, differently from the two studies above, all pregnant patients here were ARV naïve before pregnancy, had undetectable plasma viral load and had higher preserved CD4+ T cell counts.

In our study, the production of IL-10 was mainly restricted to CD4+ FoxP3+ T cells. Therefore, the data...
presented here suggest that pregnancy elevates the frequency of HIV-1-specific regulatory T cells that produce IL-10, which are phenotypically compatible with type 1 regulatory T (Tr1) cells [27].

IL-10 increases in early pregnancy and remains elevated until the onset of labor [28], possibly regulating maternal immunity and allowing acceptance of the fetal allograft. In this context, polyclonally-stimulated PBMC from women with recurrent miscarriage produced lower levels of IL-10 and higher levels of IL-2, IFN-γ and TNF-α [15,29]. Indeed, IL-10 acts directly on antigen presenting cells [17], reducing their IL-12 production and thus suppressing Th1 cell differentiation [18]. We believe that hormonal changes during pregnancy might be involved in the immune modulations described in HIV-1-infected women. In a study performed in individuals infected with varicella zoster virus (VZV), the in vitro VZV-specific T cell proliferation and Th1-related cytokine production were significantly decreased by pregnancy-related doses of estrogen and progesterone [30]. At the moment, we are dedicating our efforts to evaluate the impact of gestational hormones on in vitro HIV-1-specific immune response.

In contrast to pregnant patients, a dominant pro-inflammatory profile was detected in HIV-specific T cells from HIV-1-infected non-pregnant women. Interestingly, unlike the pregnant patients, the main source of IFN-γ production was CD8+CD45RO T cells, which seems to present as terminally-differentiated effector cells [31]. In the context of pregnancy, strong maternal Th1-dominant response against intracellular pathogens could compromise pregnancy. For example, maternal infection with Human parvovirus B19 or Hepatitis B results in concurrent increase in concentrations of IFN-γ and TNF-α in the placenta [32,33], and consequently might elevate the risk of miscarriage. Due to its ability of activating NK cells or macrophages, TNF-α accelerates placenta damage [34].

Interestingly, despite the lower in vitro IFN-γ production in HIV-1-infected pregnant women, the extent of HIV-1 replication was significantly lower in cell cultures derived from these patients. This phenomenon was directly related to IL-10 release. In contrast, higher HIV-1 replication was observed in activated T cell cultures that produced high levels of TNF-α.

HIV-1 disease is characterized by a gradual decline in circulating CD4+ T cells as infection progresses, leading to death mostly due to opportunistic infections [35]. In this scenario, there is a broad consensus among investigators that, during the pathogenesis of HIV infection, disease progression is closely associated with the level of immune activation [36–38]. Some works have found a significant direct correlation between plasma viral load and systemic TNF-α and IL-1 production [39–41]. These inflammatory cytokines, produced by several immune cells, can favor intense HIV replication, as well as various immune disturbances even in non-infected CD4+ T cell, such as activation-induced cell death [42–46]. In the context of pregnancy, TNF-α is also able to support HIV-1 replication in trophoblast, revealing, thus, a potential impact of placental inflammation in enhancing the risk of vertical transmission [47]. Here, the endogenous IL-10 neutralization enhanced both TNF-α production and HIV-1 replication in pregnant patients-derived cell cultures. In agreement with our results, some works have demonstrated that IL-10 is able to inhibit in vitro HIV-replication [48–52]. Therefore, pregnancy-related immune events could help to diminish the risk of fetal HIV transmission.

Conclusions

In conclusion, our results reveal that pregnancy-related events should favor the expansion of HIV-1-specific IL-10-secreting CD4+ T cells in HIV-1-infected women, which could help to subvert the viral replication by reducing the maternal pro-inflammatory cytokines. These immune events could help to explain, at least in part, why pregnancy is not considered a risk factor for disease progression in immunologically preserved women. It will be important to reanalyze the HIV-1-specific T cell response after delivery.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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