Analysis of placental inflammatory markers according to the class of antiretroviral therapy used during pregnancy in women living with HIV

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Abstract

Antiretroviral therapy (ART) drastically reduces vertical transmission of HIV. However, recent studies have demonstrated associations between ART use during pregnancy and placental dysfunction and inflammation, particularly within protease inhibitor (PI)-based regimens. We sought to analyze the relationship between the class of ART used during pregnancy and associated placental inflammation.

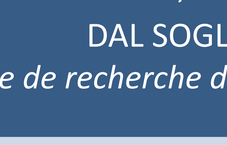
The placentas of 81 women living with HIV (WLWH) treated with ART since conception and 30 uninfected women were collected. All pregnancies were full term. WLWH were stratified into three groups based on class of ART: 22 women treated with nucleoside reverse transcriptase inhibitors (NRTI) + non-nucleoside reverse transcriptase inhibitors (NNRTI), 26 were on NRTI + integrase inhibitors (II), and 33 were on NNRTI+PI. Four randomly selected areas within the villi of each placenta were used to measure cell surface expression of CD45, CD68 (M1; proinflammatory), and CD163 (M2; anti-inflammatory).

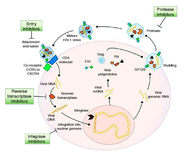
Placentas from WLWH contained significantly more CD45+ cells than those from the uninfected controls. Significantly higher numbers of total and M2 macrophages were observed in placentas from the NNRTI+II and NNRTI+PI groups compared with uninfected controls, while significantly higher M2/M1 ratios were found in placentas from the NRTI+NNRTI group. There were no significant differences between placentas from WLWH and uninfected controls in terms of M1 macrophages.

Placentas from WLWH who were treated on any class of ART during their entire pregnancy exhibited higher levels of anti-inflammatory macrophages compared to uninfected women despite controlling for viral load. Further investigations into the role of M2 cells in the context of macrophage-mediated compensatory mechanisms are required to suggest a protective effect with regards to ART-associated placental dysfunction.

Background & Objectives

ART is a daily treatment regimen which reduces the viral load of HIV but is Not curative, by reducing the risk of transmission, including vertical transmission.





Three main combinations of ART used in this study
- NRTI + NNRTI
- NRTI + PI
- NRTI + II

Previous studies show an association between PI-based regimens and placental dysfunction as well as adverse birth outcomes such as preterm birth. In addition, an increase in DAMPs and other inflammatory markers have been shown to be elevated at the placental level in HIV+ women treated with ART.

We sought to establish a relationship between the class of ART used during pregnancy and corresponding placental inflammation.
We expected to see an association between a pro-inflammatory profile and the use of PI-based ART.

Methods

All pregnancies were full term.
Four randomly selected areas within the villi of each placenta were analyzed via immunohistochemistry to measure expression of:
CD45+ cells
CD68+ cells (M1-like; pro-inflammatory)
CD163+ cells (M2-like; anti-inflammatory)

Multivariate analyses which included factors such as ethnicity, BMI, HIV viral load, CD4 levels and CMV co-infection were performed.
The model produced and the individual factors were found to be non-significant.

Results

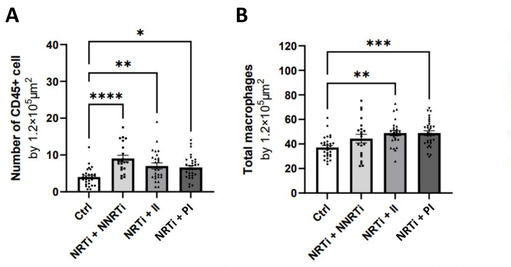


Figure 1: HIV-positive status and ART treatment are associated with an increase in CD45+ cells and total macrophages at the placental level.
Cell-surface expression of (A) CD45+ and (B) total macrophages were measured using immunohistochemistry. (A) The number of CD45+ positive cells is significantly higher in placentas of women infected with HIV on any ART treatment when compared to HIV-uninfected controls. (B) The number of total macrophages is significantly higher in placentas of women on NRTI+II and NRTI+PI based regimens compared to HIV-uninfected controls.

*p = 0.05
**p = 0.01
***p = 0.005
****p = 0.001

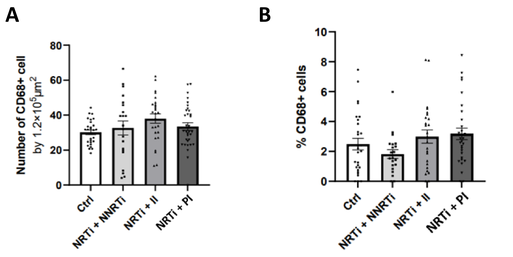


Figure 2: There is no association between M1-like pro-inflammatory profile and HIV status and ART use.
Cell surface expression of CD68 was measured using immunohistochemistry. (A) The absolute number of CD68+ cells and (B) the percentage of CD68+ cells are not significantly associated with HIV status and ART use.

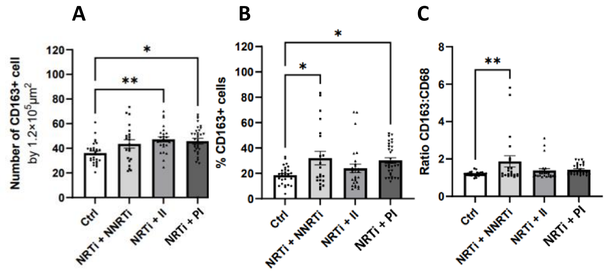


Figure 3: HIV-positive status and ART treatment are associated with an M2-like anti-inflammatory profile at the placental level.
Cell surface expression of CD163 and CD68 was measured using immunohistochemistry. (A) The absolute number of CD163+ cells was significantly higher in placentas of women infected with HIV on NRTI+II and NRTI+PI based regimens compared to HIV-uninfected controls. (B) The ratio of anti-inflammatory (CD163) to pro-inflammatory (CD68) was significantly higher in placentas of women infected with HIV on NRTI+NNRTI based regimens compared to HIV-uninfected controls. (C) The percentage of CD163+ cells was significantly higher in placentas of women infected with HIV on NRTI+NNRTI and NRTI+PI based regimens compared to HIV-uninfected controls.

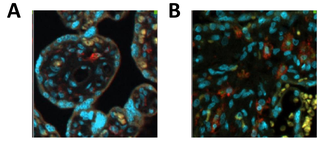


Figure 4: Immunofluorescent imaging of CD68 and CD163 cells, with blue corresponding to Dapi staining, green to CD68+ cells, and red to CD163+ cells. (A) Placental sample of an HIV-uninfected control patient. (B) Placental sample of HIV-infected patient on NRTI + PI regimen.

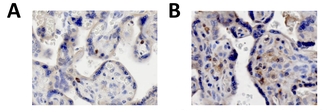


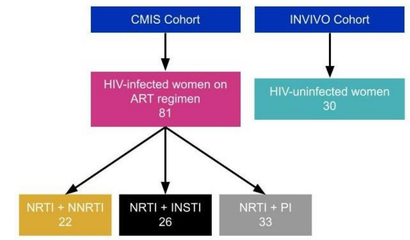
Figure 5: Immunohistochemical staining of placental samples of (A) uninfected control patient and (B) HIV-infected patient on NRTI+NNRTI regimen.

Study Population

Table 1: Demographic data of HIV-infected groups

	Classification		
Characteristic	NRTI + NNRTI (n=21)	NRTI + PI (n=32)	NRTI + II (n=26)
Mode of Delivery			
Elective C-section	4 (19.0)	10 (31.2)	6 (23.1)
Non-elective C-section	1 (4.8)	8 (25.0)	1 (3.8)
Vaginal	16 (76.2)	14 (43.7)	19 (73.1)
Ethnicity			
African	16 (76.2)	22 (68.7)	14 (53.8)
Asian	0	1 (3.1)	1 (3.8)
Caribbean	3 (14.3)	4 (12.5)	7 (26.9)
Caucasian	2 (9.5)	3 (9.4)	3 (11.5)
Haitian	0	0	1 (3.8)
Hispanic	0	1 (3.1)	0
Middle Eastern	0	1 (3.1	0
BMI			
18.5-24.9	5 (23.8)	11 (34.4)	8 (30.8)
25-30	9 (42.8)	9 (28.1)	7 (26.9)
>30	2 (9.5)	10 (31.2)	8 (30.8)
No data	5 (23.8)	2 (6.2)	3 (11.5)

Criteria of Inclusion:
- Term delivery
- Singleton pregnancy
- Initiation of ART at or before conception
- No change in ART during pregnancy



Conclusions

HIV seropositivity and treatment with any class of ART during entire pregnancy is associated with increased placental inflammatory infiltrate.
This corresponds to an anti-inflammatory profile in HIV+ women treated with ART delivering at term.
This suggests a potential protective effect of anti-inflammatory M2-like cells with regards to ART-associated placental dysfunction.

Further investigations into the role of M2 cells in the context of macrophage-mediated compensatory mechanisms are required to suggest this effect.

Further investigations into the association between PI-based ART regimens and preterm birth are required to explain the prevalence of preterm birth among HIV-positive women, particularly on PI-based treatment.

Acknowledgements & References

Fonds de recherche Santé Québec
Réseau SIDA-MI

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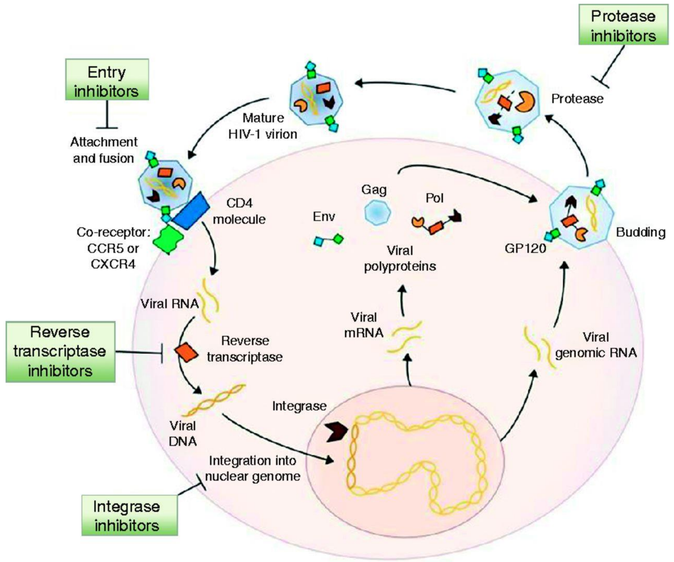

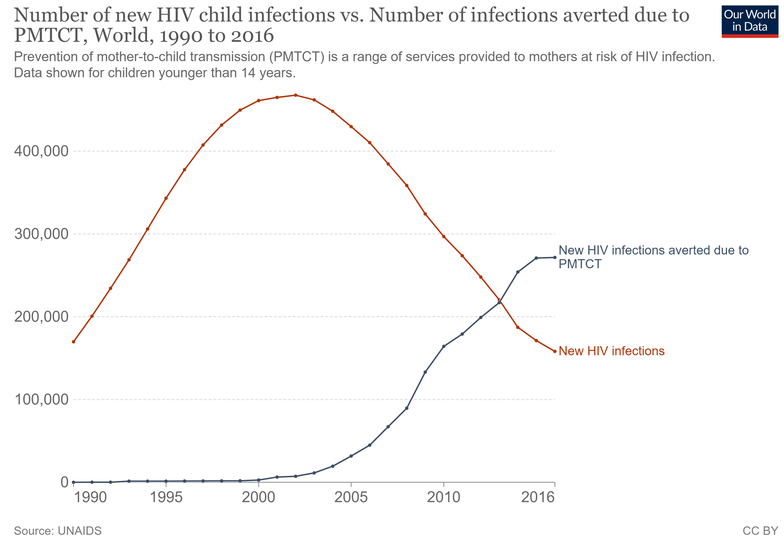

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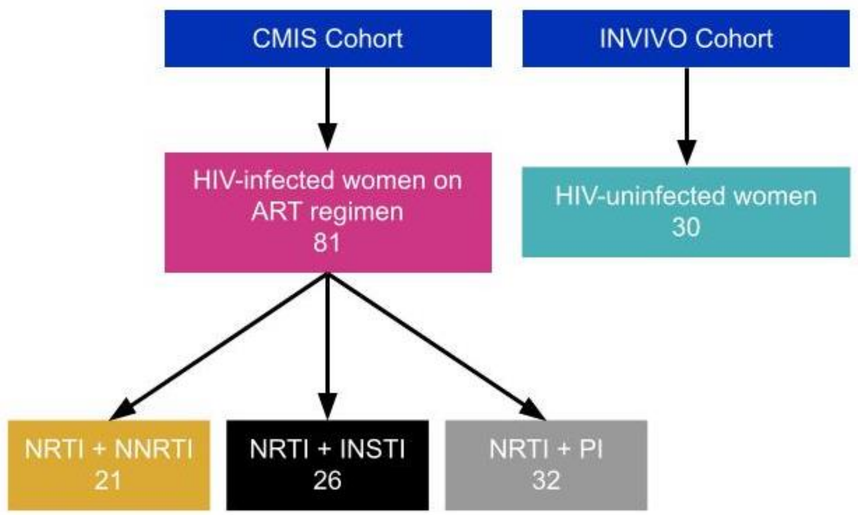

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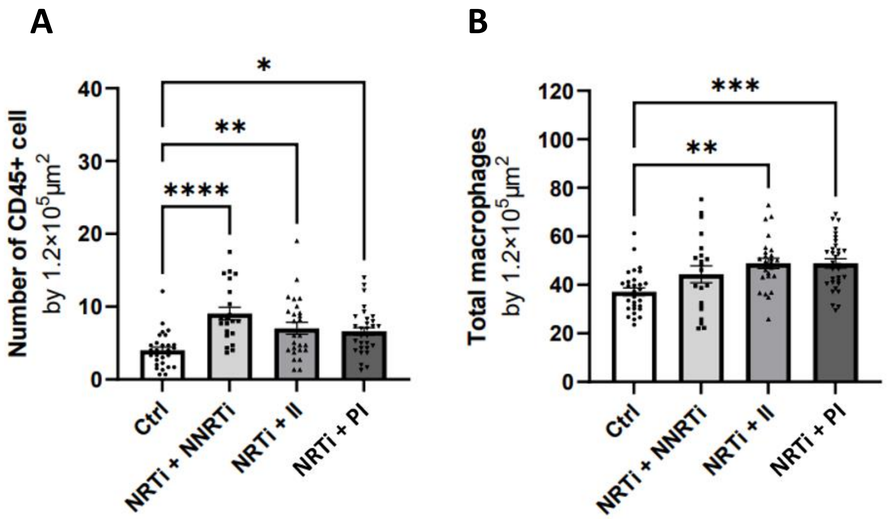

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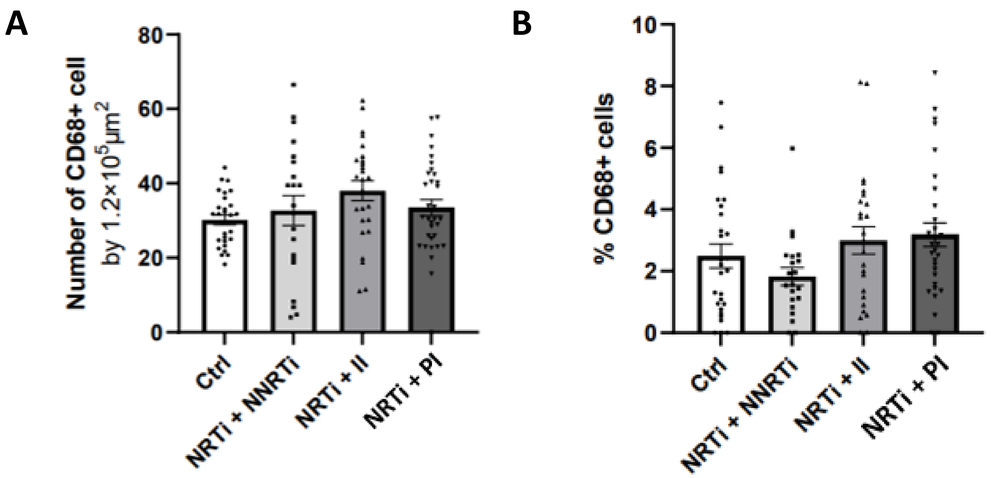

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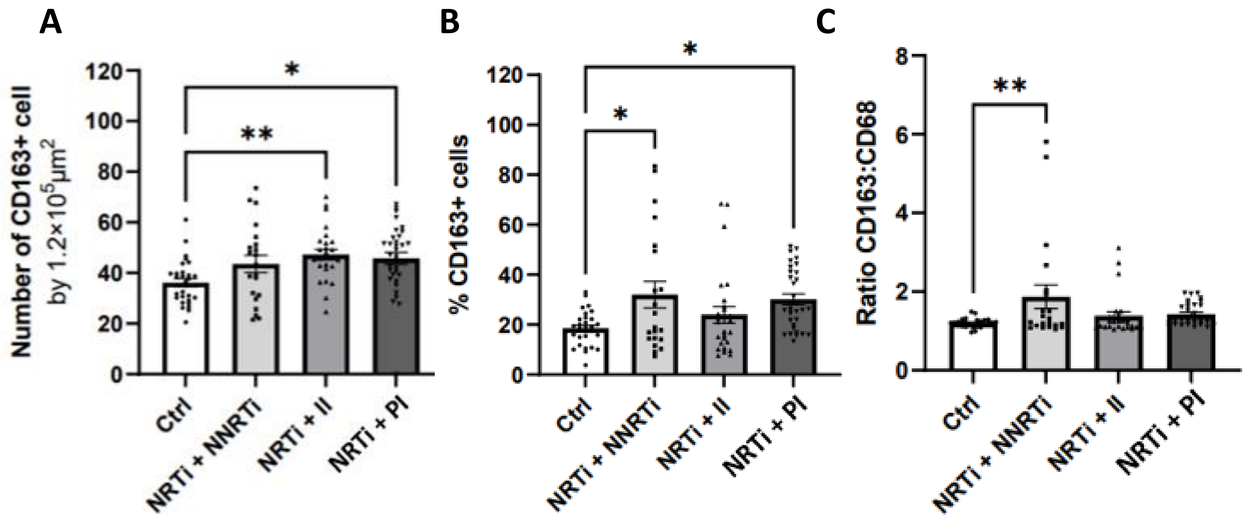

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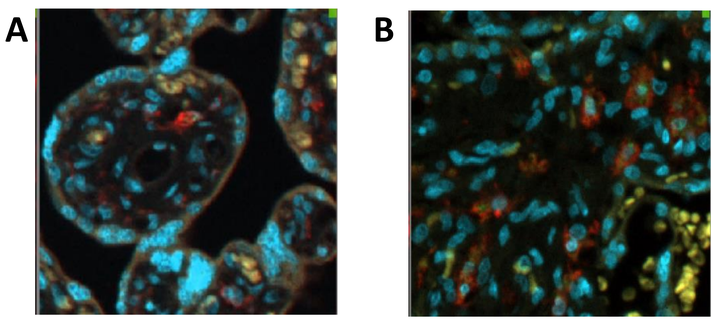

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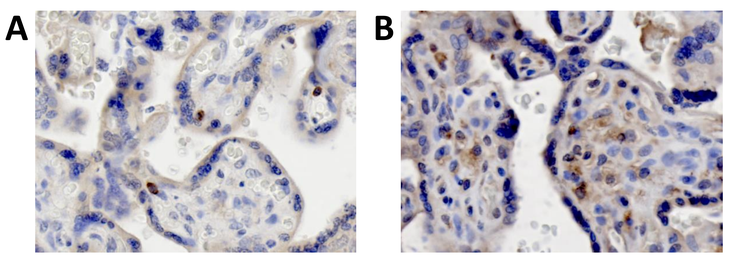

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