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Abstract

Background: Following viral infection, plasmacytoid DC (pDC) are activated, undergo maturation and express of new surface markers including CCR7, a homing receptor that facilitates their migration to lymph nodes and facilitate the maturation of myeloid DC and these in turn are critical for T cell responses. CCR7 is also a marker for central memory CD8 T cells that home to lymph nodes. We tested the hypothesis that a homing defect occurs in progressive HIV disease in perinatally infected children/adolescents on long term ART.

Methods: pDC (Lin⁻¹, HLA-DR⁺ CD123⁺) were evaluated in whole blood assay by flow cytometry for expression of maturation markers CD83, CD80, homing receptor CCR7, and intracellular cytokines (TNE- α and IFN- α) after short-term stimulation with a TLR7/8 agonist, resiquimod (RSQ) in perinatally HIV infected children (n=20). RNA was extracted from PBMC and relative CCR7 mRNA relative expression levels was quantified by real-time PCR with the ABI/PRISM 7700 sequence detection system using the comparative threshold cycle (CT) method (2- $\Delta\Delta$ CT). CD4 and CD8 CCR7 expression was assessed by flow cytometry in cryopreserved samples. Spearman correlation and Kruskal-Wallis test were used as statistical tools.

Results: pDC CCR7 expression was decreased in patients with poor immunologic response (IR-) and lack of viral control (VR-) in comparison to healthy donors, and good immunologic/virologic responders (IR+VR+). CCR7 expression was independent of IFN- α response. Selective impairment of CCR7 mRNA expression was observed in PBMC of IR- patients (p = 0.03) compared to IR+ patients. The CCR7 mRNA expression levels in PBMC correlated with CD4% (r = 0.6507, p = 0.0047), with inverse correlation to viral load (r = -0.6625, p = 0.0127). CCR7 expression on CD8 T cells was significantly higher in IR+ than in IR- subjects (p = 0.032), there was no difference in the CCR7 expression on CD4 T cells.

Conclusions: A generalized defect in CCR7 could result in defective homing of DC and other cells of immune system to the lymph node. Further investigations to decipher the mechanism underlying the CCR7 mRNA expression, are warranted.

Introduction

Dendritic cells (DC) play major roles in bridging innate and adaptive immunity. They also play a major role in immune regulation. Defects of myeloid (m) and plasmacytoid (p) DC are now well established in HIV infection. Following highly active antiretroviral therapy (HAART), pDC increase but do not reach normal levels and remain functionally impaired in IFN- α production, whereas mDC normalize.^{1,2}

Pathogenic HIV infection of humans and SIV infection in rhesus macaques (RM) is characterized by generalized immune system activation and CD4 T-cell depletion. In SIV infected RM, disruption of chemokine expression is believed to be responsible, in part, for alterations in the networks of antigen-presenting cells in lymphoid tissues, ultimately contributing to systemic immunodeficiency. It has been reported that the expression of chemokines CCL19 and CCL20 and chemokine receptors CCR7 and CCR6 in lymph node and spleen were increased in acutely infected macaques and decreased with progression to AIDS.³

Similar to the findings in SIV infected macaques, we observed, decreased CCR7 expression in pDC following stimulation with the TLR7/8 agonist resiquimod (RSQ) in perinatally acquired HIV-infected children with progressive disease.⁴ We hypothesize that defects in expression of CCR7 may result in impaired homing of cells to lymph nodes and alter adaptive immune responses contributing to further spread of HIV infection and generalized immune inactivation.

Objectives

- > To determine whether the observed defect in CCR7 expression is at the transcriptional level
- > To determine if CCR7 expression in pDC is correlated with immune activation
- > To determine the expression of CCR7 in other cell types besides DC
- > To determine expression of other chemokine receptors (CCR5, CXCR3) in pDC in response to CpG 2395, a TLR 9 agonist and to Resiquimod (RSQ), a TLR7/8 agonist

Methods

Patient population

25 children with perinatal HIV-infection (ages 5.5-18 years) were evaluated. At the time of study, all patients were on antiretroviral therapy (median duration, 9.25 years) 17 patients were on potent HAART (median duration, 5.6 years)

The patients were designated as **Immunologic responders (IR+)** if CD4 counts >25% **Virologic responders (VR+)** if HIV RNA < 400 copies/mL. Collectively they were classified as IR+VR+, IR+VR- or IR-VR-

Healthy controls

10 HIV negative controls were evaluated (ages 6-25 years)

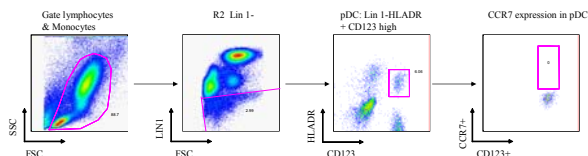
Immunology laboratory assays

Phenotypic and functional analyses of dendritic cells & T cell subsets by cell culture and flow cytometry. Quantitative real time PCR for CCR7 mRNA

Whole blood dendritic cell assay⁵

Peripheral pDC were stimulated in whole blood (WB) using TLR7 agonist RSQ (10 μ M) and TLR9 agonist CpG 2395 (50 μ g/mL). 180 μ L WB was cultured with 20 μ L of 10X ligand for 5 hours at 37°C. Cells were lysed and fixed for flow cytometric analysis. Chemokine receptors (CCR7, CXCR3, CCR5) expression was assessed in pDC defined as Lin⁻¹HLA-DR⁺CD123⁺ high after 5 hours.

Gating strategy: To study chemokine receptor expression on pDC in whole blood DC assay



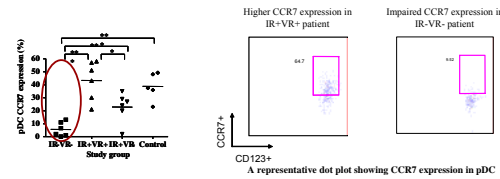
CCR7 mRNA expression in pediatric samples. Total RNA was isolated from stored PBMC and CCR7 mRNA was analyzed by quantitative real time PCR. The relative expression of CCR7 mRNA was normalized using housekeeping gene HPRT amplification levels by the 2- $\Delta\Delta$ CT comparative method described in *Applied Biosystems User Bulletin*.

Whole blood stimulation, preservation and determination of CD4 and CD8 T cell subsets.

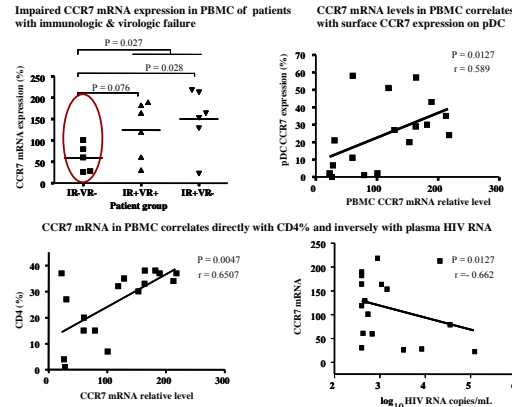
The 1mL WB samples were cryopreserved following red cell lysis. Batch analysis for T cell phenotype was performed using flow cytometry. Cells were stained for CD3/CD4/CD8/CCR7 and CD3/CD8/CD38/HLA-DR. Isotype controls were used for nonspecific staining.

Results

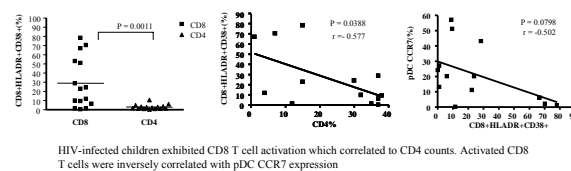
Reduced surface CCR7 expression in pDC of HIV-infected children with immunologic and virologic failure (IR-VR-)



Reduced CCR7 mRNA expression in HIV-infected children

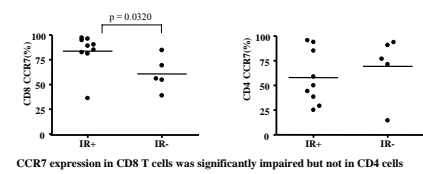


pDC CCR7 expression is inversely associated with immune activation in HIV-infected children

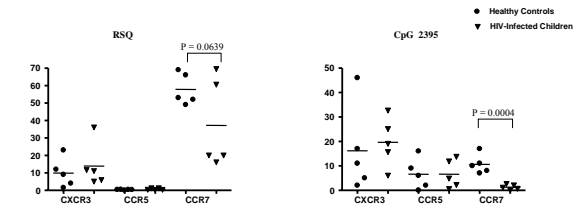


HIV-infected children exhibited CD8 T cell activation which correlated to CD4 counts. Activated CD8 T cells were inversely correlated with pDC CCR7 expression

CCR7 expression is reduced in CD8 T cells



Reduced CCR7 expression in pDC stimulated through TLR 7 and TLR 9



Surface chemokine receptor expression in plasmacytoid dendritic cells stimulated by TLR 7 ligand RSQ and TLR9 ligand CpG 2395 in healthy controls (n=5) and HIV-infected children (n=5)

Summary

- ❖ We analyzed CCR7 expression in pDC of children with perinatally acquired HIV-infection using TLR7/8 agonist RSQ and TLR9 agonist CpG 2395 and real time PCR respectively. Defect in pDC CCR7 upregulation was noted in children with progressive disease.
- ❖ The defect in CCR7 was at the transcription level as suggested by significantly lower CCR7 mRNA expression in PBMC of HIV-infected children with ongoing viral replication and poor immune reconstitution (p = 0.027 in IR-VR- vs. IR+VR+ & IR-VR- groups of patients).
- ❖ The CCR7 mRNA expression levels in PBMC correlated with the CCR7 protein levels on pDC (r = 0.5898, p = 0.0027). CCR7 mRNA levels correlated positively with CD4% (r = 0.6507, p = 0.0047) and inversely with plasma viral load (r = -0.6625, p = 0.0127).
- ❖ HIV-infected children exhibited CD8 T cell activation which correlated to CD4 counts but not with plasma HIV RNA (not shown). pDC CCR7 expression was inversely correlated with activated CD8 T cells.
- ❖ Significantly lower levels of CCR7 expression was also observed in CD8 T cells (p = 0.032) but not in CD4 T cells in HIV-infected children with poor immune reconstitution.
- ❖ Decreased CCR7 upregulation was observed in pDC activated through TLR 9 (p = 0.0004) or TLR7 (p = 0.063) in perinatally acquired HIV-infected children compared to healthy controls. No defects were noted in other chemokine receptors CXCR3 and CCR5.

Conclusion

Defects in lymph node homing marker CCR7, may result in impaired homing of pDC & CD8 T cells and this defect may contribute to impaired immune response against HIV resulting in persistent HIV replication in the lymph node and disease progression.

References

1. Azzoni L, Rutstein RM, Chehimi J, Farabaugh MA, Nowmoss A and Montaner LJ. Dendritic and natural killer cell subsets associated with stable or declining CD4+ cell counts in treated HIV-1-infected children. *J Infect Dis* 2005; 191:145-59
2. Finke JS, Shodeji M, Shah K, Siegal FP and Steinman RM. Dendritic cell numbers in the blood of HIV-1 infected patients before and after changes in antiretroviral therapy. *J Clin Immunol* 2004; 24:647-52
3. Choi YK, Fallert BA, Murphey-Corb MA, Reinhart TA. Simian immunodeficiency virus dramatically alters expression of homeostatic chemokines and dendritic cell markers during infection in vivo. *Blood*. Mar 1 2003; 101(5):1684-1691
4. Desai S, Chaparro A, Liu H, Scott G, Haslett P, Pahwa R, and Pahwa S. "Dendritic Cell Function in Surviving Children and Adolescents with Long-Term HIV Infection Acquired via Perinatal Route". 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO # R-165, 2006
5. Iida JA, Shrestha N, Desai S, Pahwa S, Hancock WA and Haslett PA. A whole blood assay to assess peripheral blood dendritic cell function in response to Toll-like receptor stimulation. *J Immunol Methods* 2006. 310:86-99

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