CD8⁺ T Cells in PML-IRIS Lesions Express CCR5: Rationale for the Use of Maraviroc

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ABSTRACT

Background: Therapeutic strategies that modulate the deleterious immune response underlying progressive multifocal leukoencephalopathy-associated immune reconstitution inflammatory syndrome (PML-IRIS) are warranted. Maraviroc, an antagonist of the CCR5 chemokine receptor, has been suggested to be beneficial in preventing or treating PML-IRIS. Since chemokine receptors play a major role in inflammatory cell migration, we investigated whether the molecular target of maraviroc is expressed on pathogenic T cells infiltrating PML-IRIS lesions.

Methods: We analyzed brain lesions of seven patients with inflammatory forms of PML, five HIV-infected patients and two with iatrogenic immunosuppression.

Results: In all cases, histological analysis revealed demyelinating lesions and the presence of JC virus-infected cells, confirming PML. CD8⁺ T cells dominate in the inflammatory infiltrates of all patients. From 87 to 100% of the perivascular and parenchymal CD8⁺ T cells express CCR5 in PML-IRIS patients regardless of their HIV status. The two non HIV-infected patients were treated with maraviroc to prevent or treat PML-IRIS, with a favorable outcome.

Conclusion: CD8⁺ T cells that dominate the inflammatory response in patients with PML-IRIS highly express CCR5. By inhibiting migration and/or activation of effector cells expressing CCR5, CCR5 antagonists might be beneficial to prevent and/or treat the deleterious immune responses that occurs during immune recovery, deserving further studies.

BACKGROUND AND AIM

- Progressive multifocal leukoencephalopathy (PML) is a life threatening opportunistic infection associated with HIV infection or immunosuppressive therapies for conditions such as multiple sclerosis (MS) or lymphomas[1]. Recovery of JC virus (JCV)-specific immune responses is pivotal for the prognosis of PML[2], but may cause disproportionate tissue damage to the host in the setting of an immune reconstitution inflammatory syndrome (IRIS)[3].
- Management of PML-IRIS relies on steroids in most severe cases. However, steroids have a profound impact on the JCV-specific T-cell response and might compromise the subsequent control of JCV replication[4]. Therapeutic strategies that modulate the deleterious immune response underlying IRIS are warranted.
- Two case reports have suggested that maraviroc, an antagonist of the chemokine (C-C motif) receptor 5 (CCR5), can be beneficial in preventing or treating PML-IRIS[5,6].
- **In order to assess whether CCR5 could be implicated in the migration of pathogenic immune cells, we analyzed their CCR5 expression in infiltrating lesions from five HIV-infected patients who developed PML-IRIS after initiation of antiretroviral therapy (ART) and in two non HIV-infected patients who developed PML after immunosuppressive therapies, one who developed PML-IRIS, and the other at high risk of PML-IRIS. Maraviroc was introduced in the two latter patients.**

METHODS

- Paraffin-embedded brain specimens of inflammatory PML, obtained through diagnostic stereotactic brain biopsy, were analyzed from five HIV-infected patients and two non HIV-infected patients. Biopsies were performed before any steroid therapy, and before maraviroc was started. As a control, we collected brain autopsies from 4 HIV-infected patients who developed classic PML (without IRIS).
- Immunohistochemical staining for JCV-infected cells, CD3, CD8, CD20, CD138, CD68 and CCR5 were performed on paraffin sections and analyzed by light and confocal microscopy. Quantification of cells was performed on consecutive sections by using an ocular morphometric grid covering an area of 0.25 mm² (in sections with dense infiltration, i.e., for inflammatory PML cases) or 1.0 mm² (in sections with low infiltration, i.e., for classic PML cases). Quantification of CD8⁺ and CCR5⁺ single and double-labeled cells was performed on CD8 and CCR5 fluorescence stained sections. For each case, single and double-labeled cells were counted in 3 confocal microscope recorded images with a total volume of 0.15 mm².

RESULTS

PATIENTS

- The five HIV-infected patients with PML-IRIS were males with a mean age of 43±2 years[7]. Three developed PML and worsened due to delayed PML-IRIS, while two developed simultaneous PML-IRIS. At baseline, the mean blood CD4⁺ T cell count was 103±34/mm³ and the plasma HIV viral load 5.4±0.2 $\log_{10}$ copies/mL. At IRIS onset, the mean increase in circulating CD4⁺ T cells was +106±34/mm³, and the decrease in HIV viral load was -3±0.1 $\log_{10}$ copies/mL.
- The clinical history of the two non HIV-infected patients is briefly summarized below:
  - Patient 1 developed PML-IRIS soon after he was treated for chronic lymphocytic leukemia (CLL) with four cycles of rituximab, fludarabine, and cyclophosphamide (RFC), resulting in a complete response. Maraviroc was started (300 mg bid) in order to alleviate IRIS, and the patient progressively improved (Figure 1A-D).
  - Patient 2 who was received 36 infusions of natalizumab (NTZ) for relapsing-remitting MS (RR-MS) developed PML with inflammatory features. Because she was considered at high risk of developing PML-IRIS, maraviroc was introduced (300 mg bid) before six plasmapheresis were performed. She notably improved and remained so far free of clinically significant IRIS.

HISTOLOGIC ANALYSIS OF INFLAMMATORY PML LESIONS AND CCR5 EXPRESSION ON IMMUNE CELLS INFILTRATING THE CNS

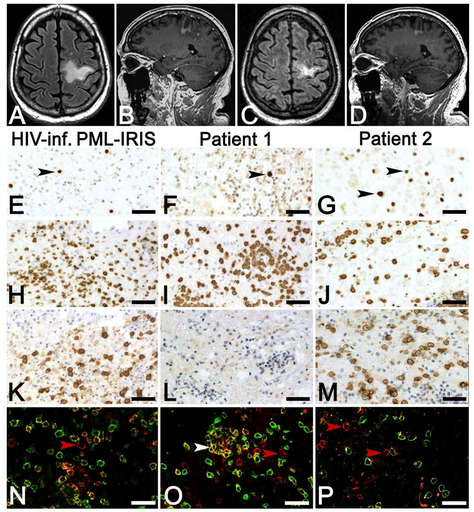
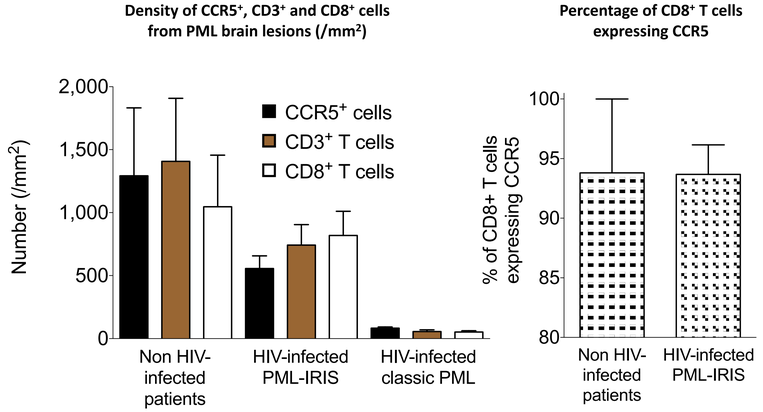


Figure 1: Brain MRI and histopathology

A-D: Brain MRI of patient 1: A-B: At the onset of the PML-IRIS showing white matter hyperintensities of the left perirolandic area (A, axial T2-WI) with multiple foci of gadolinium uptake (B, sagittal post-contrast T1-WI). C-D: Six weeks after the initiation of maraviroc demonstrating a decrease in both lesion volume (C, axial T2-WI) and contrast enhancement (D, sagittal post-contrast T1-WI).
E-P: Histopathology of HIV-infected PML-IRIS patients (left row), Patient 1 (middle row) and Patient 2 (right row). Bars: E-M: 50µm, N-P: 25 µm.
E-G: Staining for SV40 showing JCV-infected cells (arrowheads).
H-J: Staining for CD3 revealing strong T-cell infiltration in all cases.
K-M: Staining for CD138 showing the presence of plasma cells in the HIV-infected PML-IRIS patient and in patient 2 but not in the Rituximab-treated patient 1.
N-P: Double staining for CD8 (green) and CCR5 (red) showing that in all patients most CD8 cells in perivascular space (white arrowhead in panel O) and parenchyma express CCR5. The red arrowheads in N, O and P indicate the presence of CD8⁻ CCR5⁺ cells which, based on morphology and size, are probably CD4⁺ T cells.

Figure 2: Histologic analysis of brain biopsies from PML patients



DISCUSSION AND CONCLUSION

- **We show here that brain lesion-infiltrating CD8⁺ T cells, the likely drivers of tissue damage[7], highly express CCR5 in HIV-infected and non HIV-infected patients developing inflammatory forms of PML.**
- The CCR5/CCL5 axis is implicated in T cell activation and in leukocyte trafficking to the brain in the setting of neurotropic infections and in experimental models of multiple sclerosis (MS) as well as in human MS[8].
- We hypothesize that CCR5 antagonists might prevent and/or treat the deleterious inflammatory reaction that occurs during immune recovery in PML patients, by interfering with the activation and/or migration of CCR5-expressing activated CD8⁺ T cells. We therefore introduced maraviroc in the two non HIV-infected patients with inflammatory PML. Maraviroc was well tolerated and might have participated to the favorable outcome in the first case of PML-IRIS, and prevention of clinical worsening related to IRIS in the second case while this natalizumab-treated MS patient underwent plasmapheresis. Because CD8⁺ T cells in PML-IRIS lesions of HIV-infected patients also express CCR5, we propose that including maraviroc in HIV-infected patients with PML at the initiation of ART might reduce the IRIS burden. In this context, the optimal duration of maraviroc treatment still has to be determined.
- **Building on these observations, our results suggest that CCR5 antagonists can mitigate the deleterious immune response underlying PML-IRIS, deserving further clinical trials.**

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