Intracellular HIV-1 p24 Expression in Cerebrospinal Fluid T-Cells by Flow Cytometry

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Objective

High cerebrospinal fluid (CSF) viral load correlates with increased CSF white blood count (WBC). The aim of our research is to determine if CSF WBCs produce HIV-1, and which cell phenotypes correlate with viral production. We developed a flow cytometric assay to allow identification of cells that produce viral antigen.

Methods

We used intracellular anti-p24 monoclonal antibody staining after cell permeabilization. Analysis of blood and CSF cells from HIV-1 seronegative donors was used as negative controls. We found that non-specific anti-p24 staining occurred in monocytes from seronegative donors, in a fashion that could not be altered by alterations in staining conditions, including permeabilization techniques, blocking, and staining duration and concentration. In contrast, we found no evidence of non-specific anti-p24 staining in CD4+ lymphocytes, which became the focus of our research. We used CD11b bead depletion to remove the majority of monocytes and remaining granulocytes from blood samples, then used CD4 antibody staining to gate remaining monocytes out of the analysis. CSF cells were not CD11b depleted because of the percentage of monocytes and granulocytes in CSF is low.

Results (continued)

Figure 2: HIV infected cells in culture do not necessarily express CD4 receptor on their surface. Therefore, we decided to adjust our staining protocol to allow detection of p24 antigen expressing cells regardless of CD4 expression.

Figure 3: Surface and intracellular staining of PBMCs and CSF cells which indicates identification of HIV-1 p24 expressing cells in both compartments. The presence of HIV-1 gag DNA in p24 antigen expressing cells was confirmed using a Real-time PCR based assay.

Figure 4: In the subjects studied here, there is a wide range of viral load and white cell counts, which are correlated.

Figure 5: We evaluated 37 paired CSF blood samples from 13 different subjects. HIV-1 p24 expression was detected in CSF cells at a median of 0.66% (range 0.23 to 2.39%). The percentage of T cells expressing HIV-1 in the blood was lower than in CSF (p=0.001), with a median of 0.26% (range 0.23 to 1.1%). Analysis of CSF and blood of 39 HIV-1 uninfected subjects indicated that false positive p24 staining of CD3+ CD14- cells did not exceed 0.2% (mean blood 0.19%, CSF 0.21%).

Figure 6: CD4 in blood correlates negatively (p=0.013) to p24% in CSF.

Figure 7: Eight subjects were followed longitudinally, of which 2 were undergoing structured treatment interruption and 2 were initiating therapy. Longitudinal data of cell-free viral load and p24 antigen expression in cells from 1 patient starting therapy is shown.

Conclusions

p24+ cells can be detected in blood and CSF by flow cytometry in the specific range of viral load and white cell counts, which are correlated. The higher percentage of virus producing T-cells inside the CSF compartment may reflect higher levels of T-cell activation outside of the brain. Future Questions

The higher percentage of virus producing T-cells inside the CSF compartment may reflect higher levels of T-cell activation observed among CSF cells (data not shown). HIV-1 viral load in the cell free fraction of CSF does not correlate with the percentage of CSF cells expressing p24, even though the CSF white cell counts correlate with the CSF viral load. The source of CSF viral load may be in cells other than CSF cells, including systemic lymph nodes, meninges and perivascular space in brain tissue.

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