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Mechanism of CNS-Immune Reconstitution Inflammatory Syndrome with HIV Infection

Tory Johnson, Peter Calabresi and Avindra Nath

**Background:** In the post-HAART era, a new syndrome, Immune Reconstitution Inflammatory Syndrome (IRIS) has emerged in HIV-infected patients. In some patients, it involves the brain only, in the absence of opportunistic infections and may rapidly progress to cause severe encephalitis resulting in death. CNS-IRIS is largely mediated via activated-T cells, which differentiates it from HIV encephalitis that is accompanied by an infiltration of macrophages and a paucity of T cells. This paradoxical infiltration of the brain with T cells in patients who were immune suppressed with low peripheral CD4 cell counts due to HIV infection represents a diagnostic challenge and a treatment dilemma.

**Methods:** After isolation of lymphocytes from blood of normal human donors, the cells were seeded into a 96-well plate at 2X10^5 cells/ well. Cells were treated with 200 nM Tat protein in the presence or absence of 10 uM TPCK (NFκB blocker), 100 nM RO-32-0432 (pan-PKC inhibitor), 10 uM chloroquine, 10 uM pyramethamine and CD3/CD28 antibodies. To map the region of Tat important in activating T cells, isolated lymphocytes were exposed to 15-mer Tat peptides derived from HIV clade B (BRU strain) overlapping by 10 amino acids. Culture supernatants were collected and concentration of Granzyme B was measured by ELISA as an indicator of T cell activation. The mean±SEM was obtained from 5 donors and analyzed by ANOVA with Dunnet correction.

**Results:** The HIV-encoded protein Tat, transactivator of transcription, activates T cells in a dose-responsive manner. This activation requires the NF-kB pathway (p=0.032) but is independent of PKC activation. The endocytotic pathway is important in Tat-mediated activation of T cells as treatment with chloroquine, decreased T cell activation by Tat (p=0.021). No effect was seen with pyramethamine. The cysteine rich region of Tat is critical in this activation of CD4+ and CD8+ T cells (p=0.0039; p=0.0134).

**Conclusions:** HIV Tat protein activates T cells to secrete effector molecules as exemplified by Granzyme B release. The mechanism of Tat activation is dependent on endocytosis of Tat and NF-kB signaling which may represent novel targets for development of treatment for IRIS. The cysteine rich region of Tat is critical for mediating these effects. Thus human populations infected with clade B virus are more likely to develop IRIS.

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