Host cellular factors and latency

PE9

Transcriptional profiling identifies RORC and PPARG as two major mechanisms regulating HIV permissiveness in primary Th17 cells

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Background: Th17 cells are major players in mucosal immunity. Th17 cells are highly permissive to HIV infection, while Th1 cells are relatively resistant. As a consequence, Th17 are depleted in HIV-infected subjects and their frequency is partially restored under antiretroviral therapy. Our recent studies demonstrated persistence of HIV reservoirs in CD4+ T-cells expressing the Th17 marker CCR6 in ART-treated subjects. To identify molecular mechanisms of HIV permissiveness in Th17 cells, we performed a genome-wide analysis of gene expression in Th17 vs. Th1 cells.

Methods: Th17 (CCR4+CXCR3-CCR6+) and Th1 (CCR4-CXCR3+CCR6-) subsets were sorted by flow cytometry and stimulated via CD3/CD28 Abs. The expression of 47,000 probe-sets was tested using the Illumina BeadArray technology. Transcripts were classified by biological functions using Gene Set Variation Analysis and Gene Ontology. Real-time RT-PCR and fluorescence microscopy were used to validate differential gene expression. RNA interference was used to evaluate the role of top-modulated genes in regulating HIV permissiveness. Cytokine production and proliferation was measured by flow cytometry. HIV infection-integration was quantified by HIV-p24 ELISA and nested real-time PCR.

Results: HIV permissiveness in Th17 vs. Th1 was regulated by both entry and post-entry mechanisms. Among 2,533 "present calls", 1,335 and 1,198 probe-sets were upregulated and downregulated, respectively, in Th17 vs Th1 cells. Genes associated with T-cell differentiation (RORC, KLF2, ARNTL), TCR signaling (ZAP-70, Lck, MAP3K4), activation/apoptosis (PTPN13), and HIV replication (PPARG) were upregulated in Th17 vs. Th1 cells. Genes down regulated in Th17 vs. Th1 cells and previously linked to HIV resistance included CCR5-binding chemokines and IFN-induced molecules. HIV permissiveness in Th17 vs. Th1 cells was associated with high sensitivity to TCR triggering, increased proliferation potential, and superior NF-κB DNA-binding activity. RORC RNA interference decreased HIV replication, while PPARG silencing induced opposite effects.

Conclusions: Our study reveals a unique molecular signature for HIV-permissive Th17 cells and identifies RORC and PPARG as major positive and negative regulators, respectively, of HIV replication in these cells. Novel therapeutic strategies aimed at interfering with Th17-specific transcripts may limit HIV replication and reservoir persistence, while preserving the beneficial role of Th17 cells in mucosal immunity.