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Molecular determinants of HIV-1 permissiveness and persistence in gut-homing CD4+ T-cells expressing the Th17 marker CCR6

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Background: HIV-infected CD4+ T-cells are enriched in gut-associated lymphoid tissues (GALT). The integrin a4β7 and CCR9 mediate imprinting for gut-homing, and their expression is induced by retinoic acid (RA), a vitamin A metabolite produced by GALT dendritic cells. We previously demonstrated that CD4+ T-cells expressing the Th17 marker CCR6 are permissive to HIV in vitro, harbor replication-competent HIV reservoirs in ART-treated subjects, and that RA selectively increases HIV replication in these cells. To identify new molecular determinants of HIV permissiveness/persistence, we performed a genome-wide transcriptional analysis in RA-treated CCR6+ versus CCR6- T-cells.

Methods: CD4+ T-cells were sorted from PBMCs by negative selection using magnetic beads (Miltenyi). Memory (CD45RA-) CCR6+ and CCR6- T-cells were sorted by flow cytometry (BDAriaII). Cells were stimulated via CD3/CD28 and cultivated in the presence or absence of RA (10nM) for 4 days. Total RNA was extracted for microarrays analysis (HT 12v4 BeadChip, Illumina; >46,000 probe sets per chip). Validations of microarrays were performed by real-time PCR and/or flow cytometry. HIV-DNA integration was measured by nested real-time PCR. Functional validations were performed using RNA interference (Amaxa).

Results: Among 15,303 "present calls", [1,5]38 and [1,2]85 probe sets were modulated by RA in CCR6- and CCR6+ T-cells, respectively (p-value < 0.05; fold change cut-off 1.3). Gene Set Variation Analysis (GSVA), Ingenuity Pathway Analysis (IPA), and Gene Ontology tools were used to identify pathways/individual transcripts specifically induced by RA in CCR6+ versus CCR6- T-cells. This signature included an increased expression of gut homing markers (a4β7, CCR9), HIV-1 coreceptors (CCR5, CXCR6), and also pathways linked to the regulation of T-cell activation (CD38, Lck, PTPN13, MAP4K4), glucose metabolism (Glut1, Glut8), cell cycle (GADD45G), HIV replication via CCR5 expression (KLF2), and multidrug resistance (MDR1/ABCB1). In addition, the transcriptome of RA-treated CCR6+ T-cells showed decreased expression of known HIV-1 resistance factors (PPAR-g, CCL3, CCL3L1).

Conclusions: Our studies demonstrate that RA-mediated imprinting for gut-homing is associated with HIV permissiveness in CCR6+ but not CCR6- T-cells and reveal molecular mechanisms underlying these differences. These findings will orient the discovery of new therapeutic strategies aimed at limiting HIV permissiveness, and subsequently the size of HIV reservoirs, specifically in gut-homing Th17 cells.

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