Research Letter

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Heroin use in Indonesia is associated with higher expression of CCR5 on $CD4^+$ cells and lower ex-vivo production of CCR5 ligands

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Opioid use may affect HIV infection through altered expression of HIV co-receptors. This was examined in Indonesia among antiretroviral therapy-naive HIV patients, many of whom use drugs. C-C chemokine receptor type 5 (CCR5) expression on $CD4^+$ cells was higher in heroin ($P = 0.007$), methadone ($P = 0.024$) and former opioid users ($P = 0.003$) compared to nonusers, whereas production of RANTES and other CCR5 ligands was similar or lower. This suggests that opioids can affect HIV susceptibility through up-regulation of CCR5 or down-regulation of its ligands.

Higher expression of the chemokine receptors C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4), which act as co-receptors for HIV, increases susceptibility of cells to HIV infection [1] and viral replication [2,3], whereas chemokines as natural ligands for these receptors can antagonise these effects [4–6]. Injecting drug use (IDU) is a risk factor for HIV transmission, but it may also change the natural course of HIV infection [7–9]. We previously showed that HIV-infected individuals with a history of IDU have a more rapid $CD4^+$ cell decline in the absence of antiretroviral therapy (ART) [9]. One possible explanation is the use of heroin and other opioids, as in-vitro studies have shown that opioids can up-regulate the expression of HIV co-receptors [10–12] and affect the expression of CCR5-binding chemokines RANTES [13–16], MIP-1α [15] and MIP-1β [17]. It is unknown if this also takes place *in vivo*. We therefore studied the relationship of opioid use with CCR5 and CXCR4 expression, and ex-vivo chemokine production in HIV-infected individuals in Indonesia, which has a concentrated HIV epidemic strongly driven by IDU [18].

We included 84 ART-naïve HIV-infected adults with no signs of opportunistic infections and $CD4^+$ cell counts above 100 cells/μl in West Java, Indonesia. Four groups of study participants were included: current heroin injectors; current users of methadone maintenance therapy (MMT); previous users of heroin or methadone (>1 year previously); or non-users. After consent was obtained, study participants underwent interviews and blood taking. This study was approved by the Health Research Ethics Committee at the Faculty of Medicine of Padjadjaran University/Dr Hasan Sadikin General Hospital in Bandung, Indonesia.

Using three-colour flow cytometry (BD FACSCalibur, CellQuest Pro Software), CCR5 and CXCR4 expression was measured as a proportion (%) of positive cells and mean fluorescence intensity (MFI). For whole blood stimulation, blood was diluted 1 : 5 with culture medium only or in combination with either live *Candida albicans* (10^6 cells/ml), *Mycobacterium tuberculosis* lysate (10 μg/ml) or *Escherichia coli* lipopolysaccharide (LPS) (10 ng/ml). After 24 or 48 h incubation, the expression of chemokines stromal cell-derived factor 1 (SDF-1) (which binds CXCR4), and monocyte chemotactic protein (MPC)-2, macrophage inflammatory protein (MIP)-1α, MIP-1β and regulated upon activation, normal T cell expressed and secreted (RANTES) (which bind CCR5) was measured using a multiplex beads assay (Merck Millipore, Billerica, Massachusetts, USA), and corrected for $CD4^+$ cell counts (picogram per 10^5 $CD4^+$ cells).

We compared differences between groups using chi-square for categorical variables, analysis of variance for normally distributed variables, and Kruskal–Wallis and Mann–Whitney analyses for non-normally distributed variables. If characteristics were different between groups, we also examined the association between these variables and our outcomes. Results were found statistically significant (two-sided) at a level of 0.05, resulting in 0.016 after Bonferroni adjustment for multiple tests. All statistical analyses were performed using the SPSS, version 18.0 (SPSS Inc., Chicago, Illinois, USA) and graphs were created using GraphPad Prism, version 5.0 (GraphPad Software Inc., San Diego, California, USA).

In total, 84 ART-naïve HIV-infected individuals were included: 17 active heroin users, 18 individuals receiving MMT, 19 individuals who had stopped using opioids and 30 individuals who never used heroin. Participants were mostly men (85.7%, $n = 72$), with a median $CD4^+$ cell count of 336 cells/μl [interquartile range 214–505 cells/μl]. The median $CD4^+$ cell count was 387 cells/μl for heroin users, 237 cells/μl for MMT clients, 385 cells/μl for former users and 290 cells/μl for controls ($P = 0.518$). Compared to the other groups, individuals who had never used any opioid were slightly younger ($P = 0.001$), but no differences were found between groups in sex ($P = 0.479$) or total lymphocyte count ($P = 0.592$).

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