DIMINISHED CD4+ T CELL SURFACE CCR5 EXPRESSION IN ALCOHOLIC PATIENTS

PASCAL PERNEY,1,2* PIERRE PORTALÈS,2 JACQUES CLOT,2 FRANÇOIS BLANC1 and PIERRE CORBEAU2

1Service de Médecine Interne E and 2Laboratoire d’Immunologie, Hôpital Saint Eloi, 80 avenue Augustin Fliche, 34293 Montpellier cedex 5, France

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Abstract — Aims: The C–C chemokine receptors, particularly the CCR5, appeared to play an important role in T cell-mediated inflammatory reactions. The aim of our study was to assess the impact of chronic alcohol consumption on the in vivo CCR5 expression.

Methods: Fourteen alcoholic men hospitalized for a detoxification programme were prospectively included and compared with 49 age-matched controls.

Results: The CD4+ T cell surface CCR5 densities were drastically lower in alcoholic patients [mean, 5319 molecules/cell; 95% confidence interval (CI) 4477–6162] as compared with CCR5 densities of the controls (10 944 molecules/cell [CI 9929–11 959]; P < 10−4). Conclusions: Chronic alcohol consumption is associated with a significant decrease of CCR5 expression, which could favour Th1/Th2 imbalance.

INTRODUCTION

One of the immune abnormalities seen in alcoholics is a decreased Th1 (cellular immunity)/Th2 (noncellular immunity) balance (Cook, 1998). Th1 cells express the C–C chemokine receptor CCR5 and produce CCR5-binding chemokines that act in synergy with Th1 cytokines (Dorner et al., 2002). Therefore, we tested the hypothesis that alcohol consumption could alter CCR5 expression in vivo and thereby alter Th1 immunity.

METHODS

We prospectively recruited 14 men, HIV and HCV infection free and without liver disease, but with a consumption of >80 g of alcohol per day for at least a year. They were compared to 49 age-matched men whose mean alcohol consumption was ≈30 g of EtOH per day. CCR5 and CXCR4 density at the surface of CD4+ T cells was measured by a commonly used quantitative flow cytometry assay, as previously described (Reynes et al., 2001). Briefly, cells were indirectly labelled with the anti-CCR5 or anti-CXCR4 monoclonal antibody (mAb) (Pharmingen, San José, CA) and fluoroscein isothiocyanate (FITC)-conjugated anti-Ig. After washing, the cells were directly labelled with phycoerythrin (PE)-conjugated anti-CD4 mAb (Beckman-Coulter, Margency, France). Three-color flow-cytometric analysis was then performed on a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). CCR5 and CXCR4 expression by CD4+ T cells was measured by converting FITC fluorescence intensity into antibody-binding capacity, which corresponded, at the saturating concentrations we used, to the number of CCR5 and CXCR4 molecules present on the cell surface. Fluorescence intensity was converted into CCR5 and CXCR4 densities by means of a calibration curve obtained with standard micro-beads (DAKO QIFIKIT, Glostrup, Denmark) pre-coated by the manufacturer with various densities of mAb and subsequently labelled with the FITC-conjugated anti-Ig probe.

RESULTS

There was no difference between control and patient group, either in the percentage of CD4+ T cells (44 ± 11 and 48 ± 12, respectively), nor in CD4+ T cell surface CD4 density (Mean Fluorescence Intensity, 131 ± 43 and 138 ± 41, respectively). The CD4+ T cell surface CCR5 densities were lower in the alcoholic patients [mean 5319 molecules/cell; 95% confidence interval (CI) 4477–6162] compared with those of the non-alcoholic men (10 944 molecules/cell; CI 9929–11 959; P < 10−4) (Fig. 1).

*Author to whom correspondence should be addressed at: Service de Médecine Interne E, Hôpital Saint Eloi, 80 avenue Augustin Fliche, 34295 Montpellier cedex 5, France. Tel.: +33 467 337026; Fax: +33 467 337869; E-mail: p-perney@chu-montpellier.fr

Fig. 1. Mean number of CCR5 molecules per peripheral blood CD4+ T cell expressing CCR5 in alcoholic and nonalcoholic subjects.
CI 17–27) as against 28% in the controls (CI 24–31; \( P = 0.05 \)). Conversely, CXCR4 densities were not different. The patients’ mean was 1531 molecules/cell (95% CI 1243–1820) while that of the controls was 1415 molecules/cell (95% CI 1237–1594; \( P = 0.73 \)).

DISCUSSION

Our data have shown for the first time in alcoholics a significantly decreased CCR5 density on CD4\(^+\) T cells, together with a decreased percentage of CD4\(^+\) T cells expressing CCR5. CCR5 is preferentially expressed on human Th1 cells, and correlates well with the efficient attraction of Th1 cells by the appropriate C–C chemokine ligands (Ward et al., 1998). The major ligands of CCR5 are the macrophage inflammatory proteins (MIP)-1\(\alpha\), MIP-1\(\beta\) and RANTES (regulated and normal T-cell expressed and secreted) which function together with IFN-\(\gamma\) as type 1 cytokines (Ward et al., 1998; Dorner et al., 2002). The expression of CXCR4, which belongs to another subfamily of chemokine receptor and was used as control index in our study, was unchanged in our alcoholic patients. CXCR4 is expressed on B cells (Mackay, 2001) and humoral immunity is not usually affected in alcoholics (Cook, 1998). As CD4 and CXCR4 expressions were not affected, the decrease in CCR5 expression is unlikely to be due to a direct cytotoxic effect of alcohol on CD4\(^+\) T cells.

Contrary to our findings, Wang et al. (2002) have shown that alcohol increases CCR5 expression in monocyte-derived macrophages. This discrepancy could be explained by the different methods used. Thus, Wang et al. (2002) studied the effect of acute alcohol exposure (24 h) on a different type of cell (macrophages). Moreover, their study was performed in vitro on isolated macrophages. In vivo, chemokine receptor expression and its association with Th1 and Th2 phenotypes are affected by other cells and cytokines, such as interleukine-2, interferon-\(\alpha\) or tumor growth factor-\(\beta\) (Sallusto et al., 1998; Ward et al., 1998).

CONCLUSIONS

Chronic alcohol consumption was associated with a significantly decreased CCR5 expression on CD4\(^+\) T cells. This could favour the Th1/Th2 imbalance previously described in alcoholics, and might represent a pathophysiological mechanism for several alcohol-associated diseases.

REFERENCES


