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# CXCR3 expression on peripheral CD4+ T cells as a predictive marker of response to treatment in chronic hepatitis C

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HCV load

**Abstract** We monitored in fifty individuals with chronic hepatitis C (CHC) the expression of CCR5 and CXCR3, two chemokine receptors involved in the intra-hepatic recruitment of T cells, at the surface of circulating CD4+ T cells. The percentage of CD4+ T cells expressing CCR5 and/or CXCR3 was increased in patients. The increased percentage of CD4+ CXCR3+ T lymphocytes was linked to serum level of aspartate aminotransferase (AST) and to fibrosis METAVIR score. CD4+ T cell surface CCR5 and CXCR3 densities increased after 6 months of treatment with pegylated interferon-α and ribavirin. The pre-therapeutic percentage of CD4+ CXCR3+ T cells was correlated with alanine aminotransferase serum level at 12 months, and viral load at 24 months after treatment initiation. Thus, in CHC we observed a high CXCR3 expression on peripheral blood CD4+ T cells which correlates with AST serum level and liver fibrosis, and is predictive of the response to treatment.


## Introduction

The Th1-type CD4+ T "helper" lymphocytes play a crucial role in the immune response against the hepatitis C virus (HCV) by activating cytotoxic CD8+ T cells, thereby stimulating cellular immunity [1]. A sustained, vigorous and virus-specific CD4+ T cell response in peripheral blood is needed for spontaneous HCV clearance. Conversely, a weak, delayed or transient response is associated with persistent infection [2]. On the other hand, histological liver damage in chronic hepatitis C (CHC) depends on increased intra-hepatic expression of Th1-associated cytokines which favour cellular immunity [3]. Therefore, both leukocyte trafficking and the recruitment of T cells to specific liver compartments are strongly implicated in the physiopathology of HCV infection.

Selective T cell recruitment depends on chemokine gradients and interactions between chemokines and chemokine receptors [4]. Th1 cells preferentially express the CC-

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chemokine receptor 5 (CCR5) and the CXC-chemokine receptor 3 (CXCR3) [5]. During CHC, there is an increased synthesis by hepatocytes and hepatic endothelial cells of chemokines able to bind to the CCR5 receptor, particularly of regulated upon activation normals T cell-expressed and secreted (RANTES or CCL5), macrophage inflammatory protein-1α (MIP-1α or CCL3) and macrophage inflammatory protein-1β (MIP-1β or CCL4) [6,7]. Increased intra-hepatic production of chemokines that bind to the CXCR3 receptor is also seen including monokine induced by interferon γ (Mig or CXCL9), interferon-inducible protein 10 (IP-10 or CXCL10), and interferon-inducible T cell alpha chemoattractant (I-TAC or CXCL11) [7,8]. The increased synthesis and expression of these chemokines may explain why many liver-infiltrating T lymphocytes express CXCR3 and CCR5 chemokine receptors [6,7,9,10]. Moreover, animal experiments support this model. Thus, in double-transgenic mice for HCV core and a core-specific T cell receptor, there is a drastic increase in Mig and IP-10 intra-hepatic production together with an influx of $CXCR3^+$ anti-core T lymphocytes that may be inhibited by anti-CXCR3 monoclonal antibodies [11].

Because of the key roles played by CCR5 and CXCR3 in CHC, it is of major interest to study their expression at the surface of peripheral blood $CD4^+$ T cells. Previous studies have given conflicting results. Whereas a decrease in the percentage of circulating $CD4^+$ T cells expressing CCR5 in patients with CHC has been reported by a group [12], the opposite result has been reported by another one for CXCR3 [13], and a third group did not find any change in the proportion of circulating T cells positive for CXCR3 [14]. In addition to measure the proportion of $CD4^+$ T cells positive for these chemokine receptors it is also important to quantify the mean number of each receptor present at the lymphocyte membrane as we have previously shown that this cell surface density determines the intensity of the chemotaxis mediated by the receptor [15]. Finally, the therapy may modify chemokine receptor expression. For instance, Larrubia et al. have reported that treatment with pegylated interferon-α (PEG-IFNα) plus ribavirin increased $CCR5^+CXCR3^+CD8^+$ T cells frequency in peripheral blood [16]. Moreover, in the same study, the increase in CXCR3 expressing $CD8^+$ T cells after 6 months of treatment was correlated with the virological response.

For these reasons we decided to monitor CCR5 and CXCR3 expression (percentage and cell surface density) in peripheral blood of HCV-infected subjects before, during and after treatment by PEG-IFNα and ribavirin, looking for correlations with virological, histological and biochemical markers of the disease and with the response to treatment.

## Patients and methods

### Patients

Fifty patients were prospectively included at the Internal Medicine E Department, St Eloi Hospital, Montpellier. Their main characteristics are summarized in Table 1. They ranged in age from 20 to 65 years. CHC was defined as an increase in alanine aminotransferase (ALT) serum levels over a minimum of 6 months and positive results for HCV antibodies and PCR. All included patients had undergone histologic evaluation within the previous year obtained by liver biopsy ($n=37$) or by Fibrotest$^R$ ($n=13$). Histological abnormalities were expressed according to the METAVIR score: necrotico-inflammatory lesions (A) and fibrosis (F). Five patients had a fibrosis score of F0, 19 a score of F1, 13 a score of F2, 8 a score of F3, and 5 a score of F4. Patients were excluded from the study if they presented with decompensated cirrhosis or hepatocellular carcinoma, had been undergoing interferon therapy less than 6 months ago, were co-infected with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), had chronic inflammatory disease, cancer, diabetes mellitus, fever or signs of infection, if they were taking any antibiotic or immunosuppressive drug, or had an alcohol consumption ≥20 g/day.

**Table 1** Patients characteristics.

| | Patients |
|---|---|
| Number | 50 |
| Age: mean ± SD | 51 ± 13 years |
| Sex: male/female | 25/25 |
| Intravenous drug injection | 14 (28%) |
| Blood transfusion | 11 (22%) |
| Surgery | 10 (20%) |
| Other or unknown | 15 (30%) |
| AST: mean (range) | 44 (22–171) IU/mL |
| ALT: mean (range) | 70 (24–408) IU/mL |
| Genotype | |
| 1 | 35 (73%) |
| 2 | 4 (8%) |
| 3 | 7 (15%) |
| 4 | 2 (2%) |
| Non determinable | 2 (2%) |
| Viremia: mean (range) | 930,000 (4700–6,100,000) copies/mL |
| Necrotic-inflammatory activity | |
| A0/A1 | 26 (58%) |
| A2/A3 | 19 (42%) |
| Fibrosis | |
| F0–F1 | 24 (48%) |
| ≥F2 | 26 (52%) |

Twenty-five women and eighteen men (mean age of 39 ± 13 years) were recruited as healthy controls.

Clinical data collected included age, weight, source and supposed date of HCV contamination. Individual measurements of serum levels of aspartate aminotransferase (AST) and ALT were performed. Virological assessment had been obtained during the last 3 months defining the viral genotype (INNO-LIPA, Innogenetics, Les Ulis, France) and viremia by RT-PCR (Amplicor Monicor HCV, Roche diagnosis systems, Meylan, France). The rate of fibrosis progression was calculated by dividing the fibrosis score by the number of years of duration of the infection.

Eleven patients received a bitherapy associating 100 to 180 μg of PEG-IFNα a week and 800 to 1200 mg of ribavirin a day for 6 (genotypes 2 and 3) or 12 (other genotypes) months.

Forty-four controls were recruited among volunteers working in the Department of Internal Medicine and in the