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A rapid chemokine response of MIP-1α, MIP-1β and RANTES is associated with a sustained virological response in the treatment of chronic hepatitis C

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Abbreviations: ALT, alanine aminotransferase; HCV, hepatitis C virus; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, Regulated on Activation, Normal T Expressed and Secreted; TNF, tumor necrosis factor.

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ABSTRACT

The role of chemokines in chronic hepatitis C virus (HCV) infection is not fully understood. The aim of this study was to characterize the baseline serum concentrations and the initial β-chemokine response to treatment of interferon-α and ribavirin with respect to the final clinical outcome of virological response to treatment. Serum concentrations of alanine aminotransferase (ALT) and of the CC subfamily chemokines (macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1 and Regulated on Activation, Normal T Expressed and Secreted (RANTES)) were measured in patients with chronic HCV infection and in healthy individuals. Necroinflammation and fibrosis were scored in liver biopsies. Treatment outcomes were classified as with or without a sustained virological response after a full course treatment according to the genotypes. The main treatment group consisted of 72 patients with chronic hepatitis C whereas 24 hrs blood samples were available for 41 patients. Increased baseline levels of all CC-chemokines were found in the two responder groups compared to the healthy controls, but reached significant levels only for MIP-1α and MCP-1. No correlation was observed between chemokines levels and serum ALT levels, any histological necroinflammatory parameters, or to the fibrosis grade. After 24 hrs of treatment increases of MIP-1α, MIP-1β and RANTES were exclusively observed in the group of sustained virological response. MCP-1 was also significantly increased after 24 hrs in both responder groups, but no differences were observed between the two responder groups. In conclusion, an early response of MIP-1α, MIP-1β, and RANTES may predict a sustained virological treatment response.

Introduction

The understanding of a successful virus elimination mediated by the immune response to the hepatitis C virus (HCV) infection is far from settled. Both viral and host factors are determinants for the spontaneous elimination or persistence of the virus. In HCV infection some 50-90 % of the patients develop chronic disease [1]. There is evidence that unspecific CD8 cells and a subset of antigen-specific CD4 cells deviating T-helper cells in a TH1 profile (interferon (IFN) and tumor necrosis factor (TNF)-α) play a pivotal role of the immune response to the HCV [2]. A rapid and strong host T cell response plays a major role in an effective clearance of the HCV virus. Most likely the tendency towards chronic infection represents a lack of a strong and specific immune response to viral antigens as well as an ineffective rapid clearance of the HCV [for review, see 3, 4].

The recruitment of effector cells from the blood stream to the liver is dependent of local chemokines and their receptors. Chemokines constitute a large family of small proteins consisting of four main subfamilies (CXC, CC, C, CX3C) mediating their effects on a family of 7-transmembrane domain G-protein coupled receptors [5]. The chemokines are responsible for the leukocyte migration by creating a chemical gradient from the vascular endothelium to the infected cells,[6] and by selective expressions of differential chemokine receptors on the leukocytes [7].There is increasing evidence for a role of chemokines as inflammatory mediators in hepatitis C infection [8].

The CC chemokines macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4) and RANTES (CCL5) are expressed by the portal vessel endothelium and recruit macrophages and lymphocytes into the liver [6,9]. These chemokines bind to their corresponding receptors CCR5 on lymphocytes with a type 1 cytokine secretion [9,10]. Theoretically, both the chemokine response to HCV and the availability of the corresponding

receptors on the lymphocytes are obligate for a strong immunological response to the virus. Genetically determined loss of CCR5 expression has been linked to chronic hepatitis [11]. Most studies although not all, have shown increased intrahepatic and/or blood levels, as well as increased expression, of the CXC and CC families of chemokines in chronic HCV infection (for review, see [8]). However, the exact role of chemokines both in spontaneous remission, and in response to immunomodulatory agents such as interferon-α, is so far poorly understood. Among the few studies reported, high baseline serum levels of MIP-3α [12] are associated with a positive prognostic response, whereas high levels of interferon gamma-inducible protein 10 are associated with negative prognostic response to treatment [13,14]. Moreover, increase of CXCR3 expressing CD8+ cells during treatment has been associated with achievement of viral control [15]. However, the dynamics of the apparent critical initial rapid CC-chemokine response with emphasis on clearance of virus is so far not well investigated.

Therefore, in this study we have described the blood concentrations of MIP-α, MIP-β, MCP-1 and RANTES and their relationship to liver enzyme levels, microbiological and histological parameters at baseline, and then described the association between the initial (24 hrs) chemokine response and the final clinical outcome of the treatment of chronic hepatitis C.

Materials and methods

Patients

Seventy-two patients with chronic hepatitis C virus infection were included. The patients were admitted to the University Hospital of North Norway, Tromsø and Nordland Hospital, Bodø,

Norway, for diagnosis and treatment between 1998 and 2005. Blood tests were taken for measurement of alanine aminotransferase (ALT), chemokines and HCV tests before (all 72 patients) and after 24 hrs (46 patients). The inclusion criteria for treatment were elevated ALT, positive HCV serology, and detectable viremia by PCR. Patients with concomitant active hepatitis B infection and patients with severe diseases were excluded.

Patients included before 2001 received interferon-α-2b (Introna®, Schering Plough AS, Oslo, Norway) 3 MIU trice weekly (mono therapy). After 2001, treatment was given as a combination of pegylated interferon-α-2b (PegIntron®, Schering Plough AS, Oslo, Norway) 1.5 μg/kg once weekly and ribavirin (Rebetol®, Schering Plough AS, Oslo, Norway) 600-1200 mgs per day (duo therapy), for either 24 weeks (genotype 2 or 3) or 48 weeks (genotype 1 or 4). The effect of treatment was classified as sustained virological response (SVR; negative HCV PCR 24 weeks after cessation of treatment) and no SVR (comprising two subgroups: relapse (negative/positive HCV PCR at end of treatment and a positive test after additional 24 weeks, respectively) and non-response (positive HCV PCR at 12 weeks of treatment)). The treatment responses of the 72 patients were 39 with SVR and 33 patients with no SVR (17 patients with relapse, and 16 patients with non-response). Liver biopsies were performed at regular basis before start of treatment. Modified hepatic activity index (HAI) grading necroinflammatory scores (0-18) and fibrosis grading (0-6) were performed according to Ishak (16).

In Table 1 the baseline characteristics and clinical profiles are presented for the 72 patients (mono therapy: 40, duo therapy: 32) included in the study. In 12 patients (all in mono therapy group), genotype determination was not performed due to the combination of lack of available method at the time of inclusion and lack of blood to test at later time point. Histological examinations of liver biopsies were performed in 65 patients. In 7 patients biopsy specimens

were not available, either due to patient refusal to participate or a regular missed biopsy. Of the 24 hrs analyses, only blood samples from 42 patients were available (SVR: 23; no SVR 19 (relapse: 12; non-response:7)).

Analysis of chemokines

Serum from blood samples was obtained after centrifugation at +4 ºC, and subsequently kept at –27 ºC. Measurement of the CC-chemokines MIP-1α, MIP-1β, MCP-1 and RANTES was performed with commercial ELISA kits (Quantkine®, human chemokines, R&D, system Europe Ltd., Oxon, UK). Blood samples from 5 healthy volunteers were used as controls.

Approvals

All participants were informed and signed written consent. The Regional Committee for Medical and Health Research Ethics approved of the study, and permissions for storage of biological material and the research database were granted from the Norwegian Ministry of Health and the Norwegian Social Science Data Services (NSD), respectively.

Data analysis

If not otherwise stated, data are presented as mean (95% CI for mean). For log-linear variables (chemokine and ALT measurements) geometric mean was used. For further analyses logarithmic transformation was used when appropriate. Ordinal variables (HAI, fibrosis score) were analysed by non-parametric statistics (Wilcoxon). Correlation statistics were performed by Pearson correlation. Categorical data were analysed by Chi-square. Analysis of basal chemokine levels were performed by one-way ANOVA followed by a simple contrast with healthy controls as reference. The 0-24 hrs differences showed normal distribution, and non-transformed data could be entered into t-test analysis. Comparisons of

chemokine levels between groups were analysed by student's t-test for independent observations, and within groups one-sample t-tests were used. None of the analyses violated the assumption of normal distribution or the assumption of equality of variances.

Linear and logistic regression analyses were performed to test for confounding effects of sex, age, type of treatment, ALT, genotype and histological data.

Receiver-operator characteristics (ROC) analysis was performed with 0-24hr differences in chemokine levels as continuous variable and SVR/non-SVR as state variable.

All analyses were performed in SPSS 16.0.

Results

Clinical characteristics at baseline

In the whole group of 72 patients no significant differences were detected in ALT, HAI score nor fibrosis score across the treatment groups with and without SVR (Table 1) or when further subgrouping according to SVR and the two no SVR subgroups relapsers and non-responders (data not shown). As expected, there were more SVR in the genotype 2+3 group than in the genotype 1+4 group (p=0.008, Chi-square). Moreover, logistic regression analysis showed that genotype 1+4 (P = 0.006), but not age, sex nor ALT were independent predictors of treatment response (SVR versus no SVR)

Baseline chemokines in HCV patients and in healthy controls

There were increases in all chemokines in the SVR group and the no SVR group (relapsers and non-responders) when compared to healthy controls, but only MIP-1α and MCP-1 levels were at significant levels (Table 2). No significant differences were observed between the various chemokines in the various responder groups (data not shown). When comparing the levels of each of the 4 chemokines, linear regression analyses showed no significant effects of

factors such as ALT, sex, age, type of treatment, and treatment response on the chemokine levels. Moreover, no significant correlations could be detected between histological and microbiological parameters versus chemokine levels (data not shown).

Early chemokine changes during initiation of hepatitis C treatment

In the group of 42 patients (16 with mono therapy and 26 with duo therapy) with 24 hrs blood samples available, significant increases from baseline (within groups) were exclusively observed in the SVR group for MIP-1α, MIP-1β, and at non-significant level for RANTES (Fig. 1). MCP-1 levels were significantly increased in both responder groups (Fig. 1), whereas in the no SVR group the significant increase was only observed in the subgroup of relapsers (data not shown). Moreover, the mean differences between SVR and no SVR groups at 24 hrs were significant (between groups) for MIP-1α , MIP-1β and RANTES but not for MCP-1 (Fig. 1).

These observed differences in response were also observed after adjustment for sex, age, genotype, and treatment protocol in a linear regression analysis.

ROC analysis

To evaluate if the early chemokine response could predict SVR, an ROC analysis was performed. Only MIP-1α and MIP-1β had significant areas (Fig. 2). A cut-off of 0 pg/mL 0-24 hr increase in serum MIP-1α would yield a sensitivity of approximately 0.8 and a specificity of 0.5 for virological response. A cut-off of 0 pg/mL 0-24 hr increase in serum MIP-1β would yield a sensitivity of approximately 0.8 and a specificity of 0.4 for SVR.

Discussion

In this study we have shown that baseline serum levels of the CC chemokines MIP-1α, MIP-1β, MCP-1 and RANTES were increased compared to healthy controls. There were no significant relationships between the serum levels of the chemokines and liver function tests, microbiological and histological parameters. Of the various chemokines studied a rapid (24 hrs) response exclusively associated to a sustained virological response (SVR) were observed only for MIP-1α, MIP-1β and RANTES. MCP-1 was increased in both the SVR and no SVR group, but no differences between the two responder groups were observed.

In agreement with several reports the serum levels of CC-chemokines were increased in patients with chronic hepatitis C when compared to healthy controls (for review, see [8]). Increased intrahepatic expressions have also been reported for CC chemokines [17,18]. Furthermore, in our study, serum levels of the CC-chemokines were not associated with the grade of necroinflammatory activity, or the fibrosis grade. In literature there are conflicting results on this topic: in one study serum MIP-3α and ALT correlated significantly [12], in another study intrahepatic RANTES expressions correlated well with the histological activity [17], whereas in yet another study genetic expressions of neither RANTES nor MIP-1β in the liver correlated to necroinflammation activity scores [18]. Most likely, during a chronic hepatitis C virus infection there is an inflammatory circle: an activated chemokine response is responsible for recruitment of inflammatory cells which in turn activate further production of cytokines and chemokines, This inflammatory circle is in turn responsible for the chronic necroinflammation and the development of cirrhosis [13]. However, the exact contribution of chemokines and especially the CC-chemokines to this inflammatory circle is unsettled and needs further studies.

The conventional treatment of chronic hepatitis C consists of a combination of the immuno-modulatory and antiviral agents interferon-α and ribavirin. Several studies have shown that a rapid viral clearance from the general circulation is associated with a high rate of sustained virological response [19]. It has been proposed that the natural immune response to the HCV has to be rapid, strong and complete to the various epitopes to prevent virus escape with a subsequent chronic inflammation [20]. Therefore, this study was designed to study early (24 hrs) responses of the CC-chemokines during initiation of antiviral treatment. Significant increases exclusively associated to a sustained virological response were observed for MIP-1α and MIP-1β. For RANTES the response at 24 hrs were less pronounced but were at significant level between the two groups. Of interest to note was that MCP-1 was also increased at 24 hrs treatment in the sustained response group and in the relapser group, but not in the non-responder group. Our data indicate that of the various chemokines mediating the immune response during the treatment, MIP-1α, MIP-1β and RANTES, in contrast to MCP-1, apparently play an important role inducing an effective clearance of the hepatitis C virus.

Interestingly, the present data indicate a possible role of early chemokine response as a predictor of SVR response. As can be seen form the ROC curves in figure 2 the assays cannot yield both high sensitivity and specificity at the same time, as this would require a steep sigmoid curve shape with an area approaching 1. The clinical use of such a test would be to decide if a patient should discontinue treatment assuming the patient will not respond to treatment. In consequence, the cut-off values were set for high sensitivity of response to give the patient the benefit of doubt.

Our study indicates that various chemokines play a role in mediating an effective immunomodulatory role during treatment of hepatitis C. However, our results must be

interpretated with great caution. Firstly, the number of patients studied was low and two types of treatment with interferon-α were used in this clinical material. Secondly, the study was not primarily designed to test independent 24 hr chemokine responses to the final clinical outcome. A future study should be prospective and properly stratified for potential confounding factors such as sex, genotypes, and grade of liver cirrhosis. In this setting a possible role of early chemokine response as a clinical predictor of sustained virological response could be better evaluated.

In conclusion, serum levels of CC-chemokines are elevated in chronic hepatitis C but not associated to the grade of necroinflammation. An early response of MIP-1α, MIP-1β and RANTES to treatment may predict an effective HCV clearance.

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Transparency Declaration

The authors declare no conflict of interest in relation to this study.

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Table 1: Clinical characteristics at baseline in patients with chronic hepatitis C grouped according to response to treatment.

	All	Sustained response	Non-sustained response
N	72	39 (54%)	33 (46%)
Male/female	42/30	22/17	15/18
Age (range 23-59)	38 (36 to 40)	36 (34 to 38)	41 (36 to 45)
ALT (U/L)(n=72)	99 (86 to 114)	111 (91 to 135)	87 (72 to 106)
HAI index (n=65)	9.0 (8.3 to 9.8)	8.4 (7.4 to 9.3)	10.0 (8.9 to 11.0)
Fibrosis score (n=65)	1.5 (1.1 to 1.8)	1.4 (1.0 to1.8)	1.6 (1.0 to 2.2)
Genotype 1:2:3:4 (n=60)	26:4:27:3	6:4:16:1	20:0:11:2

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Genotype 1:2:3:4 (n=60)	26:4:27:3	6:4:16:1	20:0:11:2

Data are presented as mean (95 % CI) (for ALT geometric mean) or n (%).

Table 2: Serum chemokine concentrations at baseline in patients with chronic hepatitis C grouped according clinical outcome.

	n	MIP-1α pg/ml	MIP-1β pg/ml	MCP-1 pg/ml	RANTES ng/ml
Sustained response	39	7 (3 to 14)*	77 (56 to 107)	239 (200 to 286)*	33 (27 to 41)
Relapse	17	11 (6 to 22)*	79 (54 to115)	225 (179 to 284)*	31 (22 to 43)
Non-response	16	5 (1 to 20)	87 (63 to 120)	181 (83 to 398)*	31 (21 to 47)
Healthy controls	5	1 (0.8 to 2.0)	64 (33 to126)	61 (51 to 73)	21 (17 to 26)
P (ANOVA)		0.003	n.s	<0.0005	n.s

Data are presented as geometric mean (95 % CI). Asterisk denotes significant difference from healthy controls by simple contrast.

Legends to figure

Figure 1.

Early serum chemokine responses in treatment of chronic hepatitis C infection. Values are geometric mean (95% CI for geometric mean). P values are generated by t-test on differences 0-24 hrs for within groups (long underline) and between groups (short underline).

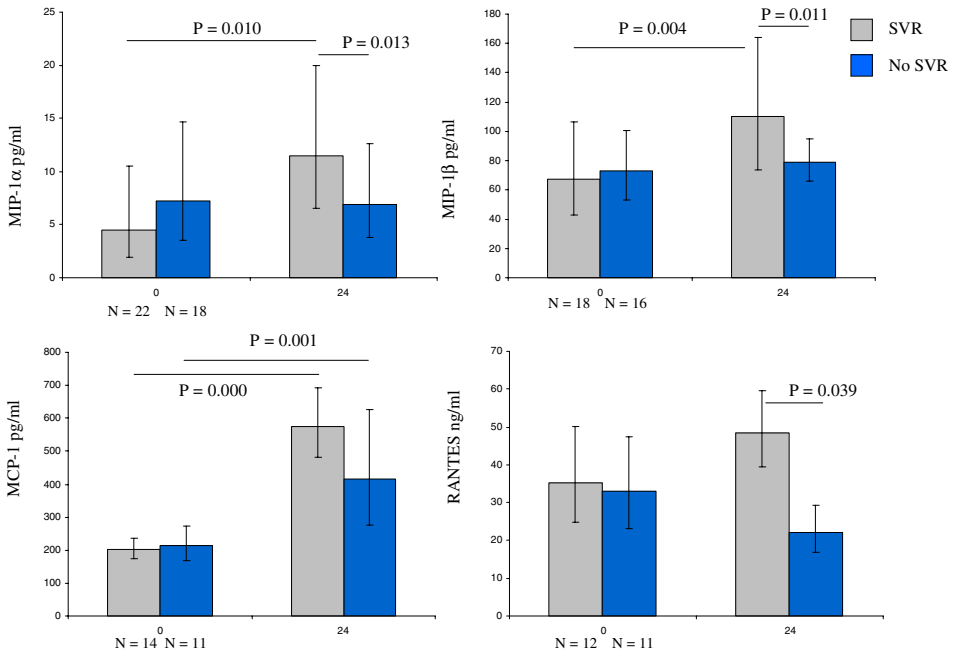

Figure 2.

Receiver-operator characteristics for MIP-1α and MIP-1β increase at 24 hrs after initiation of interferon-α therapy. The basis of this analysis is a continuous variable (chemokine value) and an actual state variable (SVR/no SVR). A choice of cut-off will defines values as positive or negative, and when comparing this value to the actual state gives the estimate of sensitivity and specificity. Different cut-off values then defines the blue curve in the plot. Both observed areas are significant and cut-off values can be deduced by inspecting the plot coordinates and their corresponding chemokine values (not shown).