

ORIGINAL ARTICLE

Iran J Allergy Asthma Immunol
September 2007; 6(3): 115-121

Expression of IP-10 Chemokine is Regulated by Pro-inflammatory Cytokines in Cultured Hepatocytes

Gholamhossein Hassanshahi¹, Abdollah Jafarzadeh¹, Zohreh Ghorashi¹, Nazanin Zia Sheikholeslami, and Alan James Dickson²

¹ Hematology and Immunology Department, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

² Biochemistry Department, Faculty of Life Sciences, The University of Manchester, UK

Received: 22 July 2006; Received in revised form: 2 February 2007; Accepted: 26 June 2007

ABSTRACT

Chemokines are classified in four distinct groups as CXC, CC, CX3C and C, depending on the presence or absence of a motif called ELR (Arg-Leu-Glu) before the first cysteine residue in their structure. CXC chemokines are also subdivided into ELR⁺ and ELR⁻. Increasing evidence has indicated the existence of a chemokine network in the liver which is involved in both physiological responses and, under certain circumstances, pathological and repair processes following hepatic injury. The CXC chemokines play a major role in both these processes, and much attention has been focused on their therapeutic applications to liver disease.

The aim of this study was to examine the response of cultured hepatocytes to exogenous inflammatory cytokines (TNF- α and IFN- γ) regarding expression of IP-10 and growth regulatory oncogen (Gro) chemokines. In this study we employed western and northern analysis to measure chemokines at the level of protein and mRNA by hepatocytes in response to pro-inflammatory cytokines.

We found that, the pro-inflammatory cytokines, TNF- α and IFN- γ , selectively stimulated expression of IP-10 but were without effect on Gro. This confirms a potential direct involvement of these cytokines in chemokine production by hepatocytes. Thus, IFN- γ and TNF- α may play a role in hepatic injury and inflammation and produce some of their biological effects by localized induction of chemokines by hepatocytes.

Given the similarity to an acute phase response, we were able to show that IFN- γ and TNF- α mimicked the effects of cell isolation and culture on induction of IP-10 expression. Further, evidence for linkages between IFN- γ and TNF- α and liver injuries is seen in hepatitis C and hepatitis B in which increased levels of TNF- α and its soluble receptor were reported.

Key words: Chemokine; Hepatocytes; IP-10; IFN- γ ; TNF- α

INTRODUCTION

Corresponding Author: Gholamhossein Hassanshahiraviz, PhD;
Department of Hematology and Immunology School of Medicine,
Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
Tel: (+98 391) 5224 001-3, Fax: (+98 391)5225 209,
E-mail: ghassanshahi@gmail.com

Stimulatory effects of lipopolysaccharide (LPS),¹
TNF- α , IFN- γ ³⁻⁶ and IL-1 β on transcription of

interferon- γ introducing protein-10 (IP-10) has been shown.² It has also been shown that stimulation of keratinocytes with TNF- α and IFN- γ results in a concentration and time-dependent elevation of IP-10 via activation of protein kinase C (PKC).^{4, 7-9} Stimulation of primary human kidney mesangial cells by TNF- α and IFN- γ also increased IP-10 expression through a NF- κ B-dependent pathway, that required co-operation between the interferon- γ signaling response element (ISRE) and nuclear factor-kappa B (NF- κ B) sites on the IP-10 promoter.¹⁰ TNF- α and IFN- γ utilize NF- κ B to regulate IP-10 expression in human fibroblasts cell lines^{4,10} and LPS-stimulated Kupffer cells.⁶ Hepatocytes in patients suffering from chronic hepatitis produce IP-10⁷ and the concentration of IP-10 is increased in serum of patients with cirrhosis, chronic B, C and autoimmune hepatitis.^{7,8} In hepatitis C infection and other liver abnormalities, sinusoidal endothelial cells also produce IP-10⁸ and expression of IP-10 by sinusoidal endothelial cells *in vitro* in response to IFN- γ , TNF- α and IL-1 β suggests that release of these Th₁ cytokines promote expression of IP-10 in hepatitis and patients with alcoholic liver damage.⁹ In experimental models of liver ischaemia/reperfusion pre-treatment with IFN- γ led to expression of several CXC chemokines including ELR⁺ variants such as growth regulation oncogen (Gro) and ELR⁻ variants, specifically IP-10.¹⁰ Expression of Gro in H4 rat hepatoma cell line¹¹ and in hepatectomized mouse liver has been shown and it may play important role in hepatocyte proliferation and liver regeneration.¹² Increased Gro expression is associated with some hepatic injuries and diseases, including, liver ischaemia/reperfusion in mouse and rat.¹⁰ Given the above introductory documents, this study was designed to examine the response of cultured hepatocytes to exogenous inflammatory cytokines (TNF- α and IFN- γ) to characterise the extent to which expression of CXC chemokines IP-10 and Gro due to hepatocyte isolation may mimic the effects of cytokines generated in inflammation or injuries.

MATERIALS AND METHODS

Perfusion, Isolation and Maintenance of Hepatocytes in Culture

Hepatocytes were obtained from fed male Sprague-Dawley rats (BSU, University of Manchester) weighing approximately 200 g. Hepatocytes were isolated from

rats by perfusion of the liver with Krebs–Henseleit bicarbonate (128 mM NaCl, 5 mM KCl, 1 mM KH₂PO₄, 1 mM MgSO₄, 25 mM NaHCO₃, 2.5 mM CaCl₂)/collagenase (Sigma, Poole, UK) under aseptic conditions.¹³ After 10 min the liver was removed and under sterile conditions the liver was gently broken down and filtered through sterile gauze with Krebs–Henseleit bicarbonate. The cells were washed three times by differential centrifugation to harvest a population of large parenchymal cells and each time gently resuspending the pellet in Krebs–Henseleit bicarbonate. The final pellet was resuspended in inoculation medium (serum-free Waymouths MB/721 media; Invitrogen Ltd, Paisley, Scotland, UK) and the viability of the cells was assessed using trypan blue. The hepatocytes were used only if they were greater than 85% viable and were generally 90–95% viable. The hepatocytes were of high purity and, under the light microscope, endothelial cells were rare, (never more than 1% of the population). The hepatocytes were seeded (2×10^6 cell/ml) on to collagen type 1-coated plates (3-cm plates for RNA and 6-cm plates for protein) and cultured in inoculation medium at 37 °C under an atmosphere of 5% CO₂ in O₂. After 3 h in culture, the medium on the cells was replaced with maintenance medium [Waymouths MB/721 media supplemented with BSA (0.2% w/v) and sodium oleate (0.0005% w/v)]. The cells were treated as described in the figure legends.

Northern Blot Analysis

Hepatocytes were isolated from male Sprague Dawley rat liver and these cells (2.5×10^6) were added to culture plates that were pre-coated with collagen type-I. Cells were firstly suspended in inoculation medium and added to culture plates that already were primed with inoculation medium, after 3h inoculation medium was replaced by fresh pre-warmed maintenance medium. Hepatocytes were cultured for 24h and then culture was continued in freshly made maintenance medium (control) or supplemented with IFN- γ (15ng/ml) or TNF- α (30ng/ml). RNA (20 μ g) was electrophoresed on a 1% agarose/17% formaldehyde gel before being transferred and fixed onto Hybond-NTM nitrocellulose membrane (Amersham International, Aylesbury, UK). Part-length cDNA of *mob-1* was random labelled with [α -³²P] dATP (50 μ Ci) (ICN Biomedicals Ltd). To standardize the RNA loading, the filters were reprobred with 18S rRNA which was labelled with [α -³²P] dATP (20 μ Ci) using

Regulation of IP-10 Chemokine Expression by Pro-inflammatory Cytokines

Nick translation. The membranes were prehybridized at 42 °C in 50% (v/v) formamide containing 5× SSPE, 2× Denhardt's, 0.1% (w/v) sodium dodecyl sulphate (SDS) and 0.1 mg/ml heat-denatured salmon sperm DNA. The membranes were then hybridized with one of the labelled cDNA oligonucleotide probes in fresh hybridization mixture at 42 °C overnight. The membranes were washed twice for 15 min at room temperature with 2× SSC/0.1% (w/v) SDS, before a final 20 min wash at 55 °C with 0.1× SSC/0.1% (w/v) SDS. Then the membranes were autoradiographed using intensifying screens at -70 °C. Quantitation of the hybridization intensity was by phosphorimage analysis.

Western Blot Analysis

Hepatocytes were isolated from Sprague Dawley rat liver. Isolated hepatocytes ($2-5 \times 10^6$) were cultured on collagen type-I matrix pre-coated culture plates. Cells were initially suspended in inoculation medium and added to the pre-wetted plates. Cells were cultured up to 24h and then culture was continued in fresh maintenance medium in the absence (control) or presence of either TNF- α (30 ng/ml) or IFN- γ (15ng/ml). Medium was removed at indicated time points, centrifuged and subjected to SDS-PAGE and immunoblotting. The immunoblotting data were quantified by densitometry analysis. At indicated time points, medium was removed from hepatocyte cultures and centrifuged. Clarified supernatants from 0h and incubated samples were used for SDS-PAGE. Immunoblotting and densitometry were performed to quantify the expression of IP-10 and Gro. Equal amounts of protein (35 μ g) were loaded and resolved on a 10% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. After blocking with 3% (w/v) milk in Phosphate Buffer Saline- Tween-20 (PBST) (10 mM Tris, pH 7.4 containing 140 mM NaCl, 0.1% (v/v) Tween-20) the nitrocellulose membrane was incubated overnight at 4 °C in TBST containing 3% (w/v) milk including anti-rat IP-10 and Gro (Chemokine.com, Houston, USA supplied by AMS Biotechnology, Abingdon, UK). Subsequently, anti-rabbit or anti-mouse horseradish peroxidase-conjugated antibodies (diluted, 1:1000) were used accordingly and the ECL detection system (Amersham International) was used to define protein localization and amount.

Statistical Analysis

All data are expressed as mean \pm SEM (Standard errors of mean). Comparisons between two groups

were performed using an unpaired Student's *t* test. Differences were considered significant when $P < 0.05$.

RESULTS

Analysis of Regulation of IP-10 and Gro at Protein Level by Pro-inflammatory Cytokines

As is clear from Figure 1 and Figure 3, the inclusion of the pro-inflammatory cytokines, TNF- α or IFN- γ , produced a profound and significant stimulation of expression of IP-10, with effects observed at the earliest time point studied (1h after cytokine addition v relevant time control; $P < 0.05$). The effect was transient, returning towards control expression by 8h after cytokine addition. By 24h after cytokine addition, IP-10 expression had returned to control values. In contrast, analysis of Gro showed there was no significant difference in expression of Gro in response to either IFN- γ or TNF- α (except after 24h stimulation with TNF- α) (Figure 2 and 4).

Analysis of Regulation of IP-10 and Gro at mRNA Level by Pro-inflammatory Cytokines

Analysis of IP-10 mRNA level showed that both TNF- α and IFN- γ could produce a profound stimulation of expression.

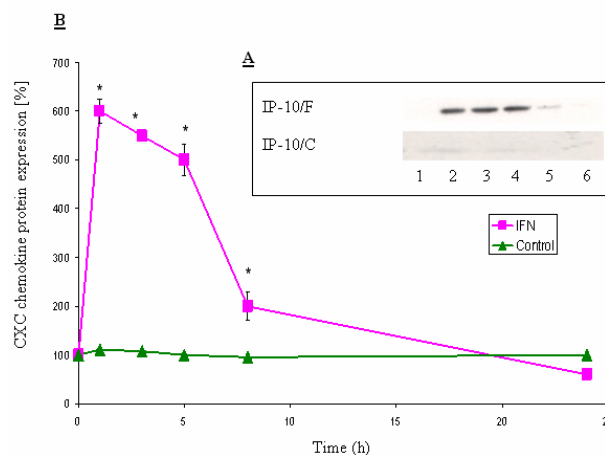


Figure 1. Expression of the CXC chemokine IP-10 protein in the presence and absence of IFN- γ .

A) Representative profile of IP-10 protein in control and IFN- γ stimulated cultures by immunoblotting. Lane 1= 0h; lane 2= 1h; lane 3= 3h; lane 4= 5h; lane 5= 8h; lane 6= 24h after stimulation. [C: Control; F: IFN- γ].

B) The expression of IP-10 in 0h time point in control (not stimulated) was assigned as 100% of expression and other time points and conditions are related to those. Values are expressed as mean \pm SEM for 4 different experiments. [$*P < 0.05$ v relevant time control].

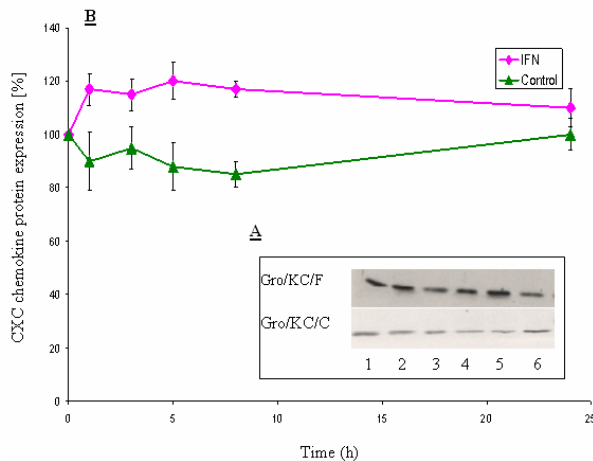


Figure 2. Expression of the CXC chemokine Gro protein in the presence and absence of IFN- γ

A) Representative profile of Gro/KC protein in control and IFN- γ stimulated cultures by immunoblotting. Lane 1= 0h; lane 2= 1h; lane 3= 3h; lane 4= 5h; lane 5= 8h; lane 6= 24h after stimulation. [C: Control; F: IFN- γ].

B) The expression of Gro/KC in 0h time point in control (not stimulated) was assigned as 100% of expression and other time points and conditions are related to those. Values are expressed as mean \pm SEM for 4 different experiments.

Significant expression was seen 1h after addition of either cytokine and the subsequent patterns of expression were similar for each (1h after cytokine addition v relevant time control; $P < 0.05$) (Figure 5). A slight variation in the pattern of transient response was seen at 8h but there was a return to control levels for both by 24h. The pattern of change contrasted to the relatively constant and high level of expression of Gro mRNA. There were no significant differences in expression of Gro mRNA in response to either IFN- γ or TNF- α (Figure 6). Overall, this study showed that there was no significant change in expression of Gro by hepatocytes at either protein or mRNA level in response to IFN- γ or TNF- α . Herein, we also showed that addition of IFN- γ and TNF- α to the basal culture of hepatocytes mimicked the transient stimulation of IP-10 protein and mRNA expression that hepatocytes experienced during isolation and culture.

DISCUSSION

In this study we have assessed the effects of pro-inflammatory cytokines, such as IFN- γ and TNF- α , on expression of specific CXC chemokines, Gro and IP-10 in cultured hepatocytes. We have demonstrated that CXC chemokine IP-10 but not Gro, could be induced at the level of protein and mRNA in primary rat

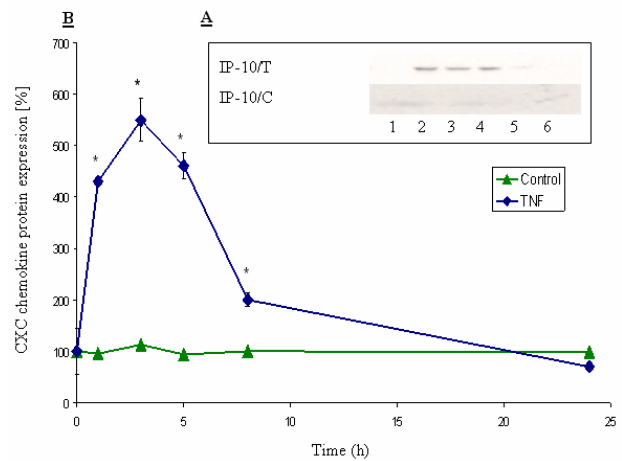


Figure 3. Expression of the CXC chemokine IP-10 protein in the presence and absence of TNF- α

A) Representative profile of IP-10 protein in control and TNF- α stimulated cultures by immunoblotting. Lane 1= 0h; lane 2= 1h; lane 3= 3h; lane 4= 5h; lane 5= 8h; lane 6= 24h after stimulation. [C: Control; T: TNF- α].

B) The expression of IP-10 in 0h time point in control (not stimulated) was assigned as 100% of expression and other time points and conditions are related to those. Values are expressed as mean \pm SEM for 4 different experiments. [$*P < 0.05$ v relevant time control].

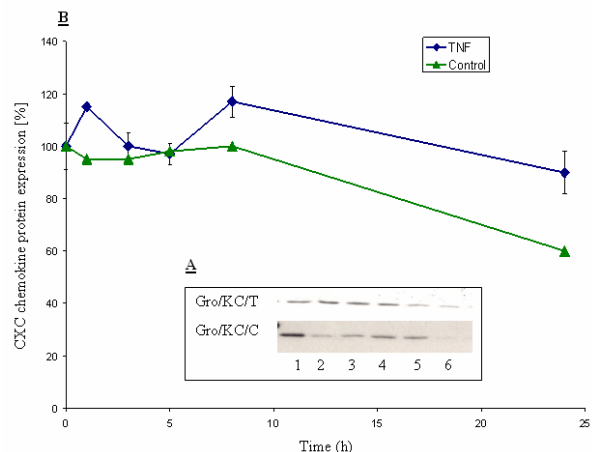


Figure 4. Expression of the CXC chemokine Gro protein in the presence and absence of TNF- α

A) Representative profile of Gro/KC protein in control and TNF- α stimulated cultures by immunoblotting. Lane 1= 0h; lane 2= 1h; lane 3= 3h; lane 4= 5h; lane 5= 8h; lane 6= 24h after stimulation. [C: Control; T: TNF- α].

B) The expression of Gro/KC in 0h time point in control (not stimulated) was assigned as 100% of expression and other time points and conditions are related to those. Values are expressed as mean \pm SEM for 4 different experiments. [$*P < 0.05$ v relevant time control].

hepatocytes by pro-inflammatory cytokines IFN- γ and TNF- α . In several cases of liver damage, pro-inflammatory cytokines (IFN- γ and TNF- α) have been identified as key mediators of activation of signalling cascades and induction of immediate early genes.¹⁴

Regulation of IP-10 Chemokine Expression by Pro-inflammatory Cytokines

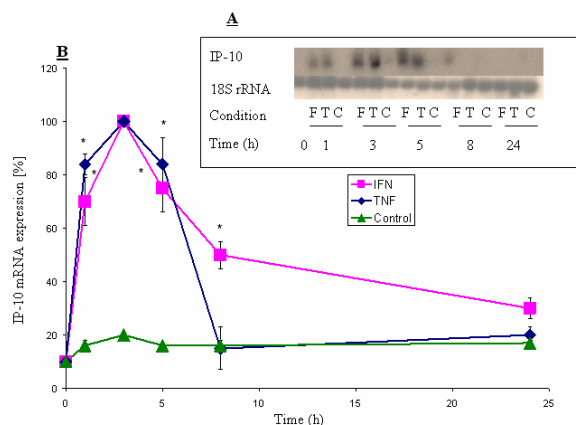


Figure 5. Expression of the CXC chemokine IP-10 mRNA in the presence and absence of IFN- γ and TNF- α

A) For northern blotting analysis, cultures were continued up to the stated time point. At each time point, supernatant was removed, Trizol™ reagent was added, RNA was extracted and purified. RNA was separated on a RNA gel and transferred onto nylon membranes. A northern analysis was performed and probed with [α -32p] dCTP-labelled IP-10 cDNA. 18S rRNA was used to standardize for loading efficiency. [C: Control; F: IFN- γ ; T: TNF- α].

B) The densitometry data presented are the means \pm SEM of 4 separate experiments. The data was calculated as a ratio of IP-10 to 18S RNA. Samples treated with either IFN- γ or TNF- α at 3h after stimulation assigned as 100% and other samples are related to those. [*P<0.05 v 3h stimulation].

Given the similarity to an acute phase response, we were able to show that IFN- γ and TNF- α mimicked the effects of cell isolation and culture on induction of IP-10 expression. In contrast, Gro expression was not affected. IFN- γ has previously been reported to increase expression of IP-10 in histiocytic lymphoma cell lines (U937 cells), RAW 264.7 mouse macrophage cells,¹ Kupffer cells, hepatocytes¹⁵ and cultured liver sinusoidal endothelial cells.⁸ Although IP-10 can be induced by IFN- γ or TNF- α in different cell types, the exact signalling pathways involved have yet to be defined, but the linkage to the presence of NF- κ B, ISRE, AP-1 binding sites in the promoter of IP-10 is again important.¹⁶ Both the p55 and p75 components of the TNF- α receptor and IFN- γ receptor are constitutively expressed by hepatocytes.¹⁷ The involvement of p38 in the ISRE-binding complex and, also, the translocation of the interferon regulatory factor-1 (IRF-1) to the nucleus and ability of this transcription factor to bind ISRE in hepatocytes have been demonstrated.¹⁸ A pathway involving p38, IRF-1 and ISRE can explain the effect of TNF- α on induction of IP-10 in this pathway. Studies have revealed that TNF- α is released by hepatocytes in response to ischemia/reperfusion and partial hepatectomy.^{19,20}

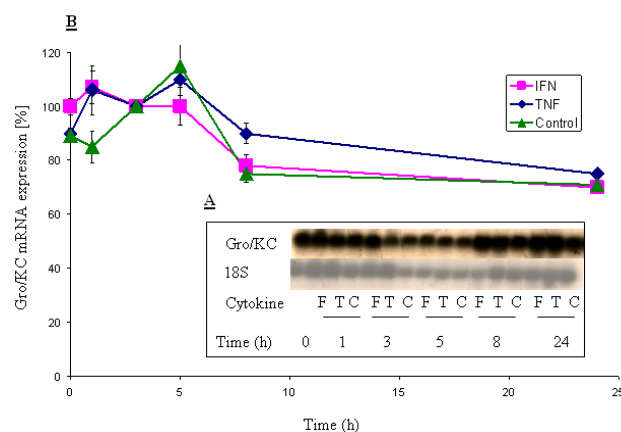


Figure 6. Expression of the CXC chemokine Gro mRNA in the presence and absence of IFN- γ and TNF- α

A) For northern blotting analysis, cultures were continued up to the stated time point. At each time point, supernatant was removed, Trizol™ reagent was added to the plate, RNA was extracted and purified. RNA was separated on a RNA gel and transferred onto nylon membrane. A northern analysis was performed and probed with [α -32p] dCTP-labelled Gro/KC cDNA. 18S rRNA was used to standardize for loading efficiency. [C: Control; F: IFN- γ ; T: TNF- α].

B) The densitometry data (Section 2.4.6) presented are the means \pm SEM of 4 separate experiments. The data was calculated as a ratio of Gro/KC to 18S RNA. Sample treated with either IFN- γ or TNF- α at 3h after stimulation was assigned as 100% and other samples are related to those.

Thus, TNF- α may play a role in hepatic injury and inflammation and produce some of its biological effects by localised induction of chemokines by hepatocytes.²⁰ Further, evidence for linkages between TNF- α and liver injuries is seen in hepatitis C²¹ and hepatitis B⁸ in which increased levels of TNF- α and its soluble receptor were reported.²² However, in studies *in vivo* it is not possible to specify which type of liver cells (parenchymal or non-parenchymal) are the source of chemokines. Studies *in vivo* have shown clear increased levels of IFN- γ and TNF- α in the liver and serum in hepatitis.²² Our studies *in vitro* show that parenchymal cells can express IP-10 in response to IFN- γ and TNF- α . This confirms a potential direct involvement of these cytokines in chemokine production by hepatocytes. Consistent with these findings, the expression of I-TAC (another IFN- γ -inducible CXC chemokine, reported to be induced in hepatitis) has been shown to be stimulated by IFN- γ and TNF- α in cultured Huh-7 hepatoma cells.²³ It has also been shown that LPS-induced expression of IP-10 was increased by IFN- γ in hepatocytes.²² Romagnani *et al.*, (2002) showed that primary kidney mesangial cells and human visceral epithelial cells express both IP-10 and monokine

induced by gamma interferon (MIG) in response to IFN- γ and TNF- α or a combination of both cytokines. They also showed that NF- κ B was essential for stimulation of chemokine expression.⁵ We have shown in our study that the expression of the CXC chemokine Gro was not affected by IFN- γ and TNF- α and similar results have been obtained by several investigators in liver cells. IFN- γ selectively decreased LPS-induced expression of Gro in mouse peritoneal macrophages and human neutrophils.²² Hassanshahi and colleagues in another study showed that Gro is constitutively expressed by H4 hepatoma cells and this was not affected by different environmental stimuli such as hyper smolarity, hydrogen peroxide and heat shock, which in a way is in agreement with these results.¹¹

In conclusion the results of this study showed that, pro-inflammatory cytokines, TNF- α and IFN- γ , selectively stimulated expression of IP-10 but were without effect on Gro in cultured hepatocytes.

REFERENCES

1. Ohmori Y, Wyner L, Narumi S, Armstrong D, Stoler M, Hamilton TA. Tumor necrosis factor-alpha induces cell type and tissue-specific expression of chemoattractant cytokines in vivo. *Am J Pathol* 1993; 142(3):861-70.
2. Vanguri P, Farber JM. Identification of CRG-2. An interferon-inducible mRNA predicted to encode a murine monokine. *J Biol Chem* 1990; 265(25):15049-57.
3. Majumder S, Zhou LZ, Chaturvedi P, Babcock G, Aras S, Ransohoff RM. p 48/STAT-1alpha-containing complexes play a predominant role in induction of IFN-gamma-inducible protein, 10 kDa (IP-10) by IFN-gamma alone or in synergy with TNF-alpha. *J Immunol* 1998; 161(9):4736-44.
4. Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). *J Exp Med* 1987; 166(4):1084-97.
5. Romagnani P, Lazzeri E, Lasagni L, Mavilia C, Beltrame C, Francalanci M, et al. IP-10 and Mig production by glomerular cells in human proliferative glomerulonephritis and regulation by nitric oxide. *J Am Soc Nephrol* 2002; 13(1):53-64.
6. Kopydlowski KM, Salkowski CA, Cody MS, Rooijen NV, Major J, Hamilton TA, et al. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. *J Immunol* 1999; 163(3):1537-44.
7. Tamaru M, Nishioji K, Kobayashi Y, Watanabe Y, Itoh Y, Okanoue T, et al. Liver-infiltrating T lymphocytes are attracted selectively by IFN-inducible protein-10. *Cytokine* 2000; 12(4):299-308.
8. Shields PL, Morland CM, Salmon M, Qin S, Hubscher S, Adams DH. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. *J Immunol* 1999; 163(11):6236-43.
9. Nagayama K, Enomoto N, Miyasaka Y, Kurosaki M, Chen CH, Sakamoto N, et al. Overexpression of interferon gamma-inducible protein 10 in the liver of patients with type I autoimmune hepatitis identified by suppression subtractive hybridization. *Am J Gastroenterol* 2001; 96(7):2211-7.
10. Kobayashi H, Narumi S, Tamatani T, Lane GJ, Miyano T. Serum IFN-inducible protein-10: a new clinical prognostic predictor of hepatocyte death in biliary atresia. *J Pediatr Surg* 1999; 34(2):308-11.
11. Hassanshahiraviz G, Kazemi Arababadi M, Dickson AJ. Expression of CXC Chemokines Gro and SDF-1 alfa in Rat H4 Hepatoma Cells in Response to Different Stimuli. *Iran J Immunol* 2006; 3(2):1-4.
12. Su AI, Guidotti LG, Pezacki JP, Chisari FV, Schultz PG. Gene expression during the priming phase of liver regeneration after partial hepatectomy. *Proc Natl Acad Sci U S A* 2002; 99(17):11181-6.
13. Seglen PO. Preparation of isolated rat liver cells. *Methods Cell Biol* 1976; 13:29-83.
14. Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ, et al. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. *Am J Physiol* 1992; 263(4 Pt 1):G579-85.
15. Narumi S, Tominaga Y, Tamaru M, Shimai S, Okumura H, Nishioji K, et al. Expression of IFN-inducible protein-10 in chronic hepatitis. *J Immunol* 1997; 158(11):5536-44.
16. Park JW, Gruys ME, McCormick K, Lee JK, Subleski J, Wigginton JM, et al. Primary hepatocytes from mice treated with IL-2/IL-12 produce T cell chemoattractant activity that is dependent on monokine induced by IFN-gamma (Mig) and chemokine responsive to gamma-2 (Crg-2). *J Immunol* 2001; 166(6):3763-70.
17. Varley CL, Dickson AJ. Hepatocyte isolation stimulates formation of interferon stimulatory response element DNA-protein complexes. *Biochem Biophys Res Commun* 1999; 263(3):627-31.
18. Satoh S, Nussler AK, Liu ZZ, Thomson AW. Proinflammatory cytokines and endotoxin stimulate ICAM-1 gene expression and secretion by normal human hepatocytes. *Immunology* 1994; 82(4):571-6.

Regulation of IP-10 Chemokine Expression by Pro-inflammatory Cytokines

19. Zylbeberg H, Rimaniol AC, Pol S, Masson A, Groote DD, Berthelot P, et al. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. *J Hepatology* 1999; 30(2):185-191.
20. Colletti LM, Green M, Burdick MD, Kunkel SL, Strieter RM. Proliferative effects of CXC chemokines in rat hepatocytes in vitro and in vivo. *Shock* 1998; 10(4):248-57.
21. Itoh Y, Okanoue T, Ohnishi N, Sakamoto M, Nishioji K, Nakagawa Y, et al. Serum levels of soluble tumor necrosis factor receptors and effects of interferon therapy in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1999; 94(5):1332-40.
22. Wang H, Gao X, Fukumoto S, Tademoto S, Sato K, Hirai K. Differential expression and regulation of chemokines JE, KC, and IP-10 gene in primary cultured murine hepatocytes. *J Cell Physiol* 1999; 181(2):361-70.
23. Helbig KJ, Ruszkiewicz A, Semendric L, Harley HAJ, McColl SR, Beard MR. Expression of the CXCR3 ligand I-TAC by hepatocytes in chronic hepatitis C and its correlation with hepatic inflammation. *Hepatology* 2004; 39(5):1220-9.