

IFN-beta stimulates the production of beta-chemokines in human peripheral blood monocytes. Importance of macrophage differentiation

Abstract

We investigated the effect of IFN-beta on beta-chemokine expression in differentiating human peripheral blood monocytes. MCP-1, MIP-1alpha and MIP-1beta were constitutively expressed in 1 day-cultured monocytes, and their secretion increased with time in culture despite any change in mRNA accumulation. IFN-beta treatment of differentiating monocytes resulted in a

marked and dose-dependent increase of beta-chemokine secretion, which was regulated differently with respect to the differentiation stage. In particular, IFN-beta upregulated MCP-1 secretion in monocytes at all stages of differentiation although its effect was significantly higher in 1-day cultured monocytes as compared to monocyte-derived macrophages (MDM). In contrast, MIP-1alpha and MIP-1beta secretion was up-regulated by IFN-beta only in MDM. Although MCP-1, MIP-1alpha and MIP-1beta mRNA expression was up-regulated by IFN-beta in both 1 day-cultured monocytes and MDM, no correlation was found between mRNA level and protein secretion. These results suggest that the regulation of beta-chemokine secretion in monocytes/macrophages by IFN-beta occurred through different mechanisms, involving both a direct effect of this cytokine on chemokine gene expression and translational/post-translational steps of regulation more likely linked to the differentiation process. This finding reveals a novel role for this cytokine in the recruitment of specific cell types during the immune response, which may be relevant in the control of viral infections in vivo.

Full text

INTRODUCTION

Monocytes and macrophages are involved differently in a variety of immunoregulatory, phagocytic and secretory functions. During maintenance of immune homeostasis, monocytes circulate and transmigrate through vascular endothelium and localize in peripheral tissues [1, 2]. Human peripheral blood monocytes mature into different types of tissue histiocytes when they migrate from the bloodstream to various tissues. This differentiation is essential for their functional competence and is probably triggered by environmental signals. *In vitro* cultivation of blood monocytes results in their adherence to the plastic surface and in the initiation of a series of morphological, biochemical and functional changes closely resembling their *in vivo* maturation to macrophages [3, 4]. This process is characterized by an increased expression of some macrophage-specific antigens [4-6], an enhanced production of some cytokines in response to LPS stimulation [6], and an increased sensitivity to the antiviral activity of IFN as the result of an increased expression of IFN receptors [7, 8]. Macrophages are generally considered to be important elements in the natural resistance against infections [9]. These cells are intimately linked to the

IFN system and the existence of a so-called IFN-macrophage alliance has been proposed [9].

IFN are cytokines which are spontaneously produced at low levels under normal physiological conditions [10], and whose expression is highly enhanced soon after cell exposure to viruses or other stimuli [11]. Because of their direct antiviral activity, which is combined with stimulatory effects on NK cells, type I IFN are considered important mediators of innate immunity. Originally considered as simple antiviral substances, type I IFN have recently been reconsidered as important cytokines for the generation of a protective, T cell-mediated immune response to viral infections and tumors [12].

Recruitment of monocytes from the blood compartment into tissues is an important process in the inflammatory response and immunity [13]. Directional migration is regulated by locally produced, cell-secreted proteins, bacterial peptides and phospholipid metabolism products [14, 15]. In the past few years, a new superfamily of small, structurally related chemiotactic molecules has been described. Chemokines are key regulators of leukocyte trafficking and play a pivotal role in host defense mechanisms [13, 16] as well as in the development and homeostasis of the immune system [17, 18]. Macrophages represent a major source of soluble factors, such as cytokines and chemokines and, at the same time, their functions are strongly regulated through the activity of these mediators [19]. Some studies have revealed complex network of interactions between cytokines and chemokines in the development of both local and systemic immune responses [20]. In particular, it has been shown that cytokines are important modulators of the biological activity of chemokines by virtue of their capacity of regulating both chemokine receptor expression and ligand secretion [21-26]. In turn, chemokines have been reported to regulate the activity of some cytokines and to utilize signal transducers previously identified as factors specific to cytokine receptors signal transduction [27-29].

In this study, we investigated the effect of IFN-beta on the expression of beta-chemokines in human peripheral blood monocytes, and the role of differentiation in the IFN response. We report that IFN-beta is a potent inducer of MCP-1, MIP-1alpha and MIP-1beta in monocytes/macrophages. Interestingly, MCP-1 is preferentially released by monocytes, while MIP-1alpha and MIP-1beta are only produced in differentiated macrophages, after IFN-beta stimulation. The differential expression of these related polypeptides during monocyte

differentiation in response to IFN-beta may be related to the differential activities and functions of blood monocytes with respect to tissue macrophages.

METHODS

Isolation, culture and phenotypic characterization of peripheral blood monocytes. PBMC were obtained from 18 - 40-year-old healthy males as previously described [6]. Monocytes were separated from lymphocytes by Percoll gradient centrifugation [30]. Cells were then cultured in endotoxin-free Iscove's medium containing 15% FCS (0.22 mum filtered), for 24 hours (defined here as 1-day monocytes) or 7 days [7-day monocyte-derived macrophages (MDM)]. To evaluate the expression of differentiation markers, cells were directly stained with specific FITC-labeled monoclonal antibodies against CD71, CD14, HLA-DR and CD11b (Becton Dickinson, Heidelberg, Germany), and analyzed by fluorescence-activated cell sorter (FACS) as previously described [50]. Cytochemical (*i.e.* sodium fluoride-inhibited esterase activity) and surface marker (*i.e.* CD14 Ag) analysis revealed that the adherent cell population consisted of > 95% monocytes.

Reagents. IFN-beta was a gift from the InterPharm Laboratories Ltd (Serono, Italy).

*Measurement of MCP-1, MIP-1*alpha *and MIP-1*beta *content.* The levels of MCP-1, MIP-1alpha and MIP-1beta present in culture supernatants were measured using enzyme-linked immunosorbent assay (ELISA). ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA). The detection limit was 5 pg/ml for MCP-1, 10 pg/ml for MIP-1alpha and 4 pg/ml for MIP-1beta.

Analysis of chemokine mRNA. For the RNase protection assay, total RNA was extracted by the method of Chirgwin [31]. For beta-chemokines mRNA analysis, 5 mug of total RNA was hybridized to a radiolabeled multiprobe produced by an *in vitro* transcription kit (PharMingen, San Diego, CA, USA) as indicated in the manifacturer's instructions. For RNA quantification, the amount of mRNA corresponding to MCP-1, MIP-1alpha and MIP-1beta present in each sample was normalized to the control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using a densitometer (UltroScan XL; Pharmacia LKB, Milwaukee, WI, USA). Values are given as chemokine/GAPDH mRNA ratio.

RESULTS

Peripheral blood human monocytes spontaneously differentiate into macrophages when cultured for a few days. The expression of a panel of surface antigens was routinely monitored as a control for monocyte differentiation. Figure 1 shows the results of a representative phenotypic characterization in which the surface expression of CD71, CD14, HLA-DR and CD11b was evaluated. A significant increase in the expression of these antigens was observed during the *in vitro* culture, indicating a well defined, time-dependent differentiation of monocytes to macrophages [4-6].

In a first set of experiments, the spontaneous production of beta-chemokines was analyzed in monocytes at different stages of differentiation. One day-cultured monocytes released variable amounts of MIP-1alpha, MIP-1beta and, to a larger extent, of MCP-1. As shown in Figure 2, the secretion of MCP-1 (panel A), MIP-1alpha and MIP-1beta (panel B and C) was consistently increased during *in vitro* differentiation.

We then investigated the effect of IFN-beta on the production of beta-chemokines in monocytes at different stages of differentiation. As shown in Figure 3, different patterns of beta-chemokine induction were observed. In particular, IFN-beta consistently induced the secretion of MCP-1 in both 1 day-cultured monocytes and MDM (Figure 3A). However, higher levels of MCP-1 production were found in 1 day-cultured monocytes with respect to MDM. In contrast, an enhanced production of MIP-1alpha and MIP-1beta was induced by IFN-beta-treatment of MDM but not of 1 day-cultured monocytes (Figure 3, B and C). Moreover, the enhancing effect of IFN-beta on the production of MCP-1, MIP-1alpha and MIP-1beta was dose-dependent and required low concentrations of this cytokine. As shown in Figure 3A, B and C, as low as 10 units of IFN-beta was already sufficient to induce a significant increase in MCP-1, MIP-1alpha and MIP-1beta secretion that was markedly enhanced at the concentration of 100 units. No further increase was observed with higher IFN-beta concentrations. Similar results were obtained by stimulating monocytes/macrophages with IFN-alpha (data not shown).

Experiments were then carried out to evaluate whether the differentiation-associated increased expression of MCP-1, MIP-1alpha and MIP-1beta was associated with some enhancement in the expression of the corresponding

transcripts. As shown in Figure 4A, no significant changes in the expression of these mRNA were found during monocytes' differentiation to macrophages. In contrast, IFN-beta induced a clear-cut accumulation of MCP-1, MIP-1alpha and MIP-1beta mRNA in both 1 day-cultured monocytes and MDM (Figure 4A). The densitometric analysis of MCP-1 (Figure 4C) and MIP-1beta (Figure 4B) mRNA levels revealed IFN-beta-induced accumulation of these transcripts in both 1-day cultured monocytes and MDM. However, this effect was consistently higher in MDM with respect to 1-day monocytes. Similar results were obtained for the accumulation of MIP-1alpha mRNA following IFN-beta stimulation (3 fold of increase in 1-day cultured monocytes *versus* 5 fold of increase in MDM).

DISCUSSION

In this study, we report that IFN-beta markedly induced secretion of MCP-1, MIP-1alpha and MIP-1beta in peripheral blood monocytes. This effect was not observed for other beta-chemokines, as RANTES was neither spontaneously expressed nor induced by IFN-beta. Likewise, no significant changes were observed in the basal levels of MDC following IFN-beta treatment (data not shown). Interestingly, different patterns of induction were found depending on the chemokine considered and the monocyte differentiation stage. In fact, treatment of either 1 day-cultured monocytes or MDM with recombinant IFN-beta resulted in a dose-dependent increase of MCP-1 secretion, which was significantly higher in 1 day-cultured monocytes with respect to MDM. In contrast, enhancement of MIP-1alpha and MIP-1beta secretion was only detected in IFN-beta-treated MDM, but not in 1 day-cultured monocytes. A number of studies have shown that several cytokines, including LIF, IL-1beta, TNF-alpha, IL-4, IL-6 and IL-15, positively regulate the synthesis and secretion of MCP-1 in monocytes [32-35] as well as in other cell types [36-39]. Until now, limited information has been available on the effect of type I IFN on the modulation of chemokine production. Van Damme and colleagues [40] have shown the induction of MCP-1 and MCP-2 in mononuclear cells and in Hep-2 epithelial cells stimulated with high IFN-beta concentrations (300 IU/ml). Likewise, it has been demonstrated that IFN-alpha and IFN-beta are potent inducers of MCP-3 and to a lesser extent of MCP-1 in PBMC [41]. Moreover, it has been reported that IFN-beta synergizes with IL-1beta to induce MCP-1 and MCP-2 production in MG-63 osteosarcoma and Hep-2 epithelial cells [42]. However, negative effects of both type I and type II IFN on the production of some chemokines induced by LPS, cytokines or TCR triggering have also been

reported in human fibroblasts, monocytes and T cells [43-45]. Our article represents the first detailed study in which the activity of IFN-beta has been characterized with respect to the monocyte differentiation stage. In this regard, with respect to type I IFN, a previous study by Bug *et al.* [46], has reported that IFN-alpha induced MIP-1alpha mRNA expression in PBMC and purified monocytes. However, MIP-1alpha secretion was modestly (2-fold increase) enhanced by IFN-alpha only in LPS-activated monocytes, whereas no protein secretion was observed after a single treatment with IFN-alpha. A recent study by McManus *et al.* showed that IFN-beta treatment of microglia resulted in the induction of MIP-1alpha, MIP-1beta, MCP-1 and RANTES mRNA and protein, whereas IFN-gamma only induced MCP-1 [47]. In contrast with our results, induction of MIP-1alpha and MIP-1beta was also detected in 1-day monocytes treated with IFN-beta. The apparent discrepancy between these results and ours can be, at least in part, explained by the different experimental conditions used for monocyte isolation and culture. In fact, it is reasonable to assume that monocytes, purified by overnight adherence in the presence of other mononuclear cells [47] might have been primed for MIP-1alpha and MIP-1beta induction by cytokines/factors released in the culture medium.

In this study, we show that despite a clear-cut enhancement of MCP-1, MIP-1alpha and MIP-1beta secretion in MDM with respect to 1-day monocytes (Figure 2), the basal expression of the corresponding transcripts is not influenced by differentiation (Figure 4). Moreover, there is no correlation between the levels of MCP-1, MIP-1alpha and MIP-1beta transcripts accumulation after IFN-beta stimulation and the amount of protein that is indeed secreted (Figure 3 and 4). This apparent discrepancy is even more evident for MCP-1 expression, as a marked induction of mRNA accumulation is observed in IFN-beta-stimulated MDM under conditions in which MCP-1 secretion is only modestly increased. Although further studies are needed to precisely define the molecular events involved in the regulation of chemokine secretion, overall our results suggest that the regulation of beta-chemokine secretion in monocytes/macrophages by IFN-beta occurred through different mechanisms, involving a direct effect of this cytokine both on chemokine gene expression and translational/post-translational steps of regulation more likely linked to the differentiation process.

The monocyte/macrophage lineage represents heterogeneous cell populations characterized by major differences in phenotype and functional activities. The differential expression of chemokines during monocyte differentiation in

response to IFN-beta may be related to the diverse distribution, activities and functions of blood monocytes with respect to tissue macrophages. In light of our results and data from the literature, we can envisage the following scenario occurring during the course of a viral infection: i) circulating blood monocytes can be stimulated by the virus-induced type I IFN to produce MCP-1, a key chemokine in the recruitment of monocytes to sites of inflammation/infection [48, 49]. At the same time, the IFN-stimulated production of MCP-1 could contribute to the MCP-1-mediated inhibition of CCR2 expression thus negatively controlling the response of monocytes to this chemokine; ii) monocytes, recruited from the blood to tissues, proceed through their differentiation to macrophages characterized by the loss of response to MCP-1 [50] and the acquisition of response to IFN in terms of MIP-1alpha and MIP-1beta induction. The local production of these chemokines could promote further recruitment of monocytes/macrophages as well as of other cell targets (T cells), and activate NK cell functions [51].

The finding that IFN-beta can differentially modulate chemokine secretion reveals a novel role for this cytokine in the recruitment and activation of specific cell types during the immune response.

CONCLUSION

Acknowledgements. We are indebted to Sabrina Tocchio and Romina Tomasetto for excellent editorial assistance and to Roberto Gilardi for preparing graphs. This project was funded by a grant from the Italian Ministry of Health (40D.7).

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Figures

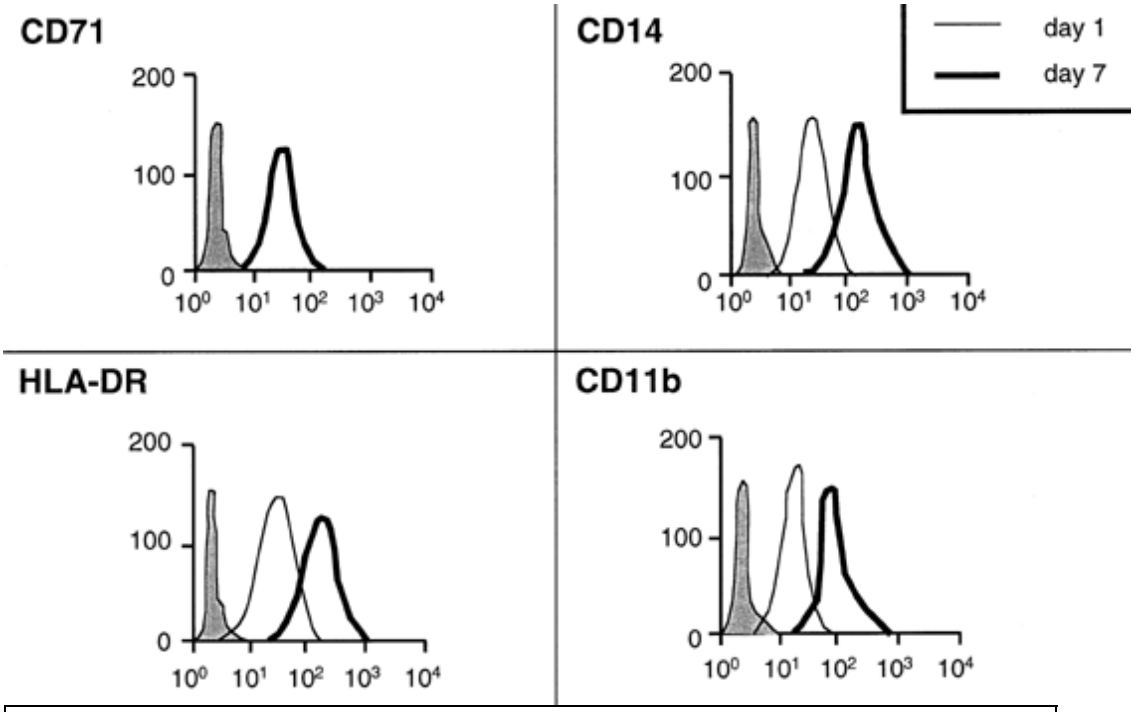


Figure 1
Immunofluorescence analysis of surface antigens during differentiation of monocytes to macrophages. Cells were harvested after 1 and 7 days of culture and processed for membrane fluorescence as described in Methods. The immunofluorescence profile obtained for each antibody was compared with that of its corresponding control isotype and the result is shown as a filled curve.

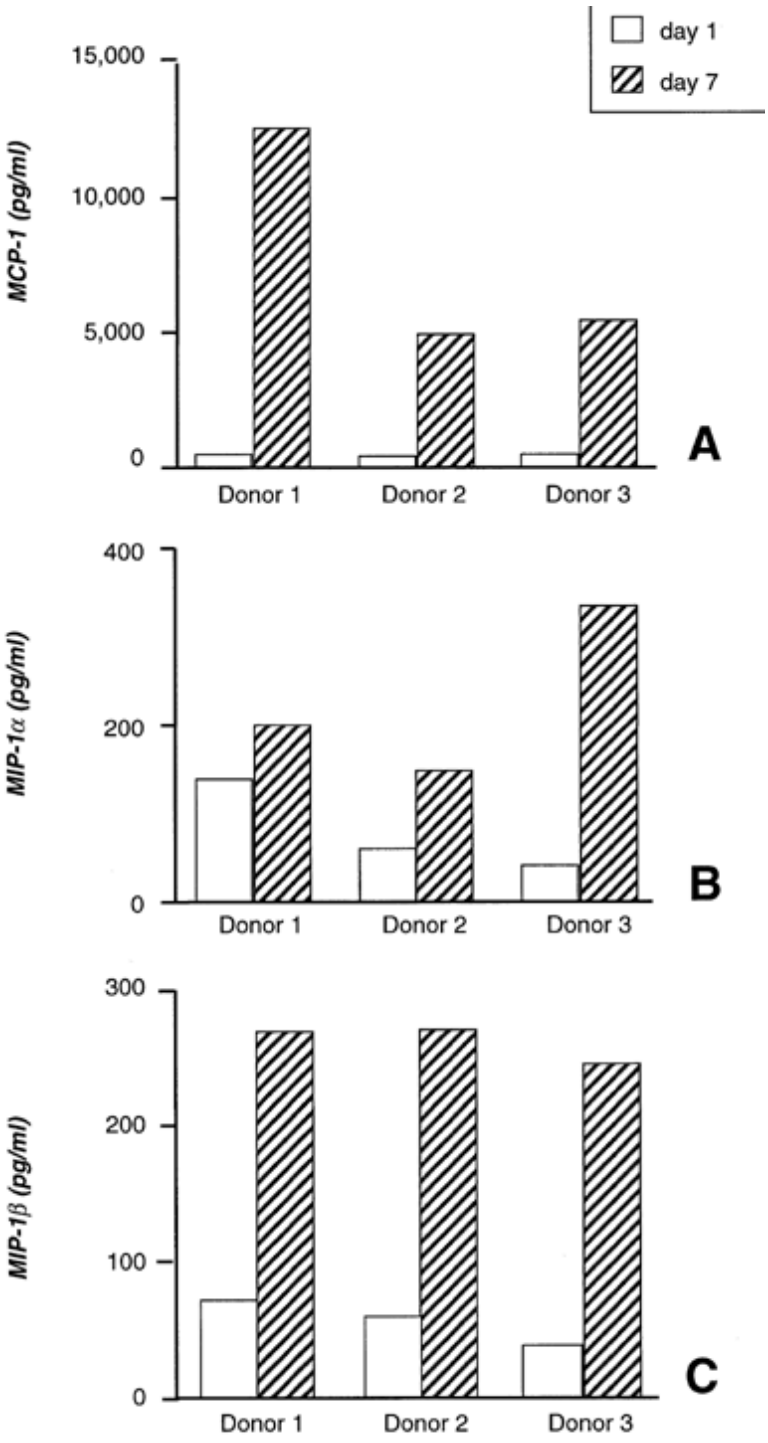


Figure 2

Constitutive expression of beta-chemokines during monocytes differentiation to macrophages.

Monocytes, isolated from the peripheral blood of healthy donors, were seeded in 48-well cluster plates at the cell concentration of 6.25 **x** 10^5/cm^2. After 1 or 7 days of culture, supernatants were collected for MCP-1 **(A)**, MIP-

1alpha **(B)** and MIP-1beta **(C)** determination. Results obtained with 3 different donors are shown.

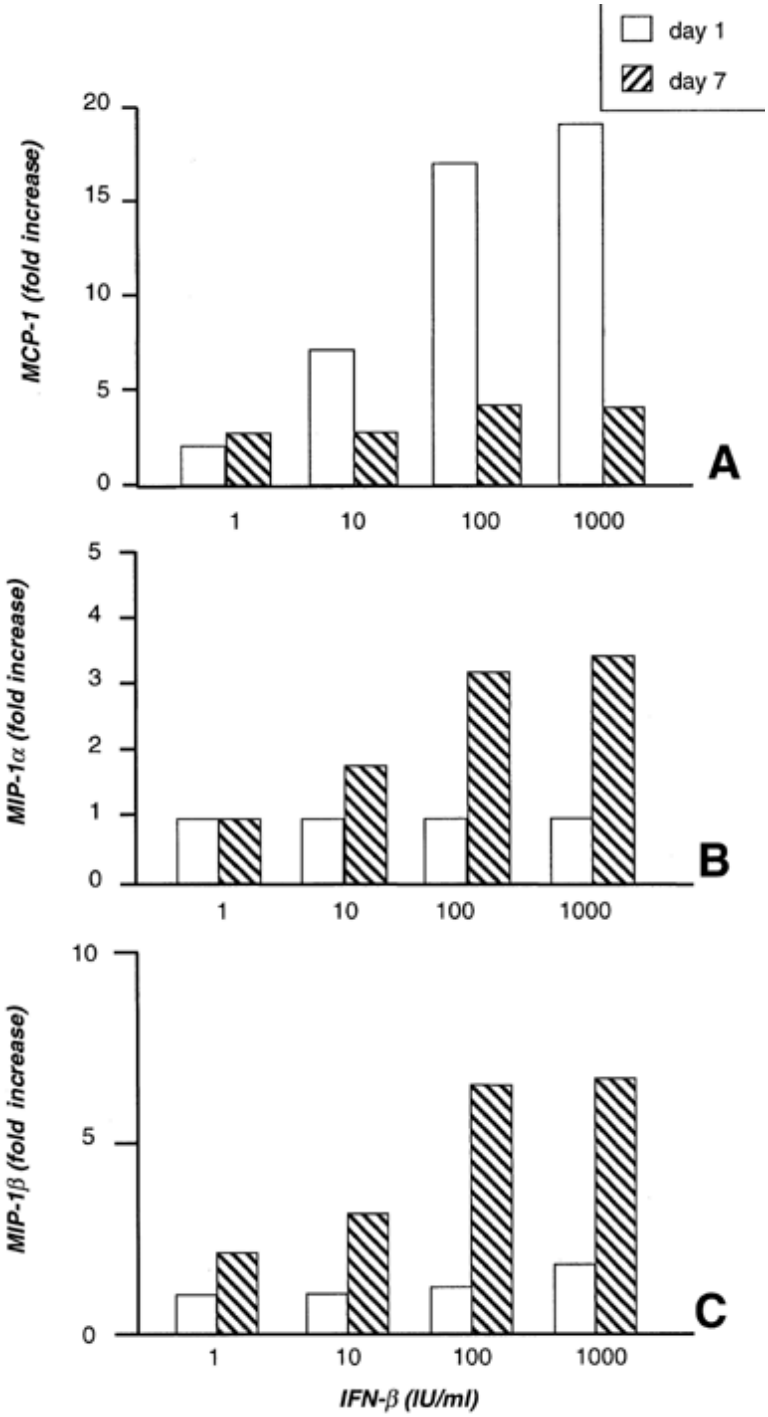


Figure 3

Dose-response of the IFN-gamma-induced beta-chemokine secretion in monocytes/macrophages. Monocytes were seeded as described in the legend to Figure 2. After 1 or 7 days of culture, cells were treated

with different concentrations of IFN-beta (ranging from 1 to 1,000 IU/ml) or left untreated. Twenty-four hours later, supernatants were collected for MCP-1 **(A)**, MIP-1alpha **(B)** and MIP-1beta **(C)** determination. The Figure shows the fold increase of chemokine release in treated cultures with respect to control cultures. Results are representative of 1 out of 3 independent experiments.

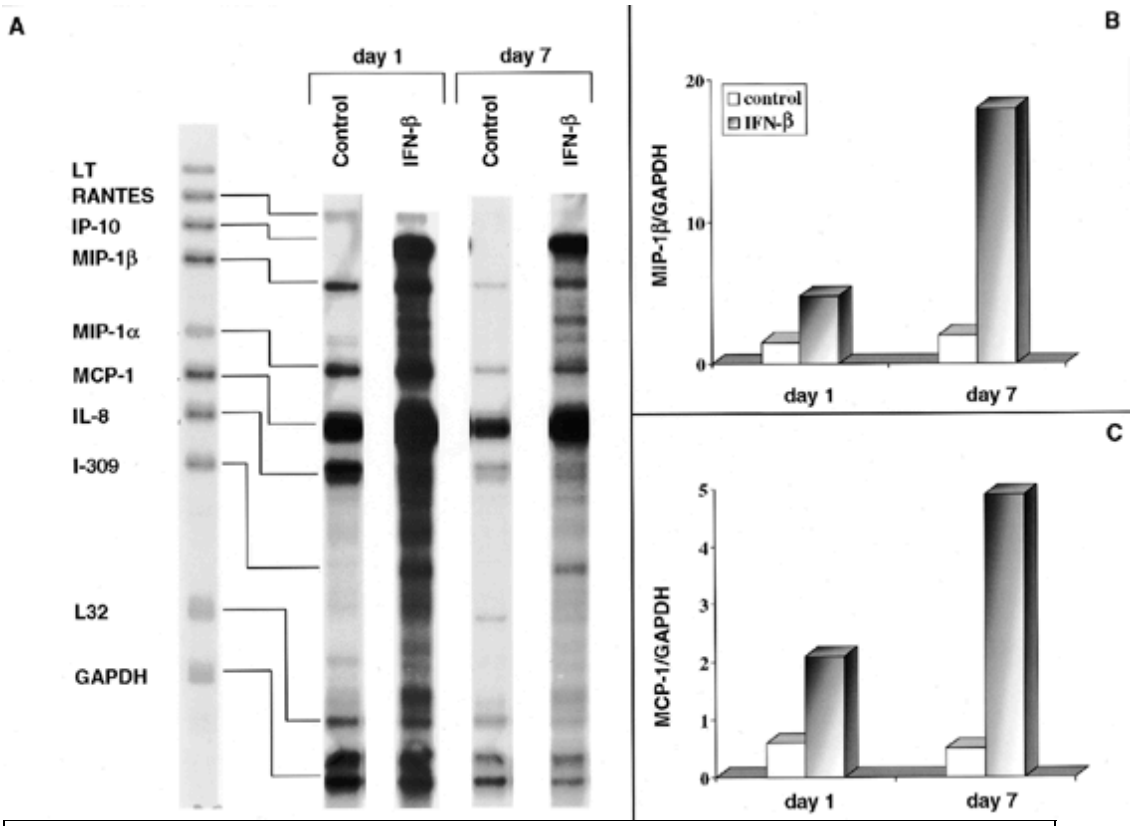


Figure 4

Effect of IFN-beta on beta-chemokine mRNA expression in monocytes/macrophages. Monocytes were seeded in 6-well cluster plates at the cell concentration of 2 **x** 10^5/cm^2. After 1 or 7 days of culture, cells were treated with IFN-beta (100 IU/ml) or left untreated. Four hours later, total RNA was extracted and beta-chemokine mRNA expression was analyzed by RNase protection **(A)**. The densitometric analyses of MCP-1 **(C)** and MIP-1beta **(B)** mRNA levels are shown. Values are given as chemokine/GAPDH mRNA ratio. Results are representative of 1 out of 3 independent experiments.

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In this issue

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TGF-beta1, cytokine, knockout mice, gene therapy, adenovirus.

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Salvatore Cuzzocrea, Emanuela Mazzon, Laura Dugo, Ivana Serraino, Domenico Britti, Massimo De Maio, Achille P. Caputi
collagen, inflammation, nitric oxide, peroxynitrite, superoxide dismutase, IL-10.

Reduced adrenal response and increased mortality after systemic Klebsiella pneumoniae infection in interleukin-6-deficient mice

Frank H.J. van Enckevort, C.G.J. Sweep, Paul N. Span, Mihai G. Netea, Ad R.M.M. Hermus, Bart Jan Kullberg
corticosterone, cytokine, IL-6-deficient mice, granulocyte recruitment, survival.

Gamma-interferon induces apoptosis of the B lymphoma WEHI-279 cell line through a CD95/CD95L-independent

mechanism

K.-E. Ben Jilani, K. Akarid, D. Arnoult, F. Petit, E. Baert, J.-P. Gaillard, J.-C. Ameisen, J. Estaquier
IFN-g, B cells, apoptosis, CD95, caspases, cytokines.

IFN-beta stimulates the production of beta-chemokines in human peripheral blood monocytes. Importance of macrophage differentiation

Laura Fantuzzi, Irene Canini, Filippo Belardelli, Sandra Gessani
MCP-1, MIP-1alpha, MIP-1beta, IFN-beta, monocytes/macrophages, differentiation.

Relationship between cell kinetics and apoptotic effects in TNF-alpha and IFN-gamma-treated human tumour cell lines

Heinz Baisch
apoptosis, c-Myc, cell cycle, cytokines, dual pathways.

L-Buthionine-(S,R)-sulfoximine, an irreversible inhibitor of gamma-glutamylcysteine synthetase, augments LPS-mediated pro-inflammatory cytokine biosynthesis: evidence for the implication of an IkappaB-alpha/NF-kappaB insensitive pathway

John J. Haddad
antioxidant, chemioxyexcitation, cytokines, glutathione disequilibrium, pharmacotherapy, reactive oxygen species, redox/thiol regulation.

Endotoxin regulates the maturation of sterol regulatory element binding protein-1 through the induction of cytokines

Luisa Diomede, Diego Albani, Marco Bianchi, Mario Salmona
SREBP-1, LPS, HepG2, liver, mice.

Regulation of Staphylococcus aureus-mediated activation of interleukin-18 in peripheral blood mononuclear cells

Ulrich Böcker, Tobias Manigold, Joanna M. Watson, Manfred V. Singer, Siegbert Rossol
human, cytokine, bacterial antigen, CD14, signal transduction.

Interleukine-6 (IL-6) synthesis and gp130 expression by human pericardium

Emmanuelle Menet, Pierre Corbi, Cécile Ancey, Franck Morel, Adriana Delwail, Martine Garcia, Anne-Marie Osta, John Wijdenes, Daniel Potreau, Jean-Claude Lecron
IL-6, gp130, pericardium.

Plasmodium falciparum liver-stage antigen-1 peptide-specific interferon-gamma responses are not suppressed during uncomplicated malaria in African children

Adrian J.F. Luty, Martina Bongartz, Philipp Rezbach, Jean-Francois Faucher, Michael R. Hollingdale, Peter G. Kremsner
malaria, cytokine, interferon-gamma, liver.

Immunoexpressions of p21, Rb, mcl-1 and bad gene products in normal, hyperplastic and carcinomatous human prostates

Mar Royuela, María Isabel Arenas, Fermín R. Bethencourt, Manuel Sánchez-Chapado, Benito Fraile, Ricardo Paniagua
p21, Rb, mcl-1, bad, human prostate, benign prostatic hyperplasia, prostatic carcinoma.

Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic myelodepression in patients with malignant tumors I. Phase I-II clinical trials of recombinant human interleukin-1 beta as a leukopoiesis stimulator in cancer patients receiving combination chemotherapy

M.L. Gershanovich, L.V. Filatova, S.A. Ketlinsky, A.S. Simbirtsev
human recombinant interleukin-1 beta, myelopoiesis, myelodepression, cancer chemotherapy.

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human recombinant interleukin-1 beta, leukopoiesis, myelodepression, cancer chemotherapy.

French society of immunology, cytokines

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