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Up-regulation of interleukin (IL)-6 receptor gene expression in vitro and in vivo in IL-6 deprived myeloma cells

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Myeloma cells absolutely require interleukin-6 (IL-6) for growing in vivo in patients with multiple myeloma and exogenous IL-6-dependent myeloma cell lines have been reproducibly obtained. In this study we show a dramatic up-regulation of the IL-6 receptor (gp80 chain) gene expression in myeloma cell lines following the removal of exogenous IL-6. Such a regulation was also known to occur in IL-6-deprived myeloma cells in vivo in three patients who were treated with optimal doses of anti-IL-6 monoclonal antibodies. The direct effect of IL-6 on IL-6 receptor gene expression in myeloma cells was further confirmed by adding IL-6 to an autonomously growing myeloma cell line.

IL-6; IL-6 receptor; Multiple myeloma

1. INTRODUCTION

Interleukin-6 (IL-6) is a pleitotropic cytokine which is produced by various types of cells [1,2]. Among its numerous biological properties, IL-6 induces the terminal differentiation of B cells [3], the proliferation of murine hybridoma and plasmacytoma cells [4], and is a potent growth factor for human myeloma cells in vitro [5–8]. IL-6 is produced in large amounts in vivo in patients with active multiple myeloma (MM) [5,9,10], and we have recently shown that the injection of anti-IL-6 monoclonal antibodies (MAbs) could block IL-6 bioactivity and myeloma-cell proliferation in vivo in patients with terminal disease [11]. The IL-6 receptor (IL-6R) was shown to be expressed on a number of cells including myeloma cells [12–14], and the regulation of its expression by cytokines or other agents has already been studied in different types of cells [15–17]. By taking advantage of our recently obtained IL-6-dependent human myeloma cell lines (HMCL) and of the treatment of MM patients with anti-IL-6 MAbs, we report here that privation of IL-6 induces an up-regulation of IL-6R gene expression in myeloma cells in vitro and also in vivo.

Abbreviations: IL-6, interleukin-6; IL-6R, interleukin-6 receptor; MM, multiple myeloma; HMCL, human myeloma cell line; MAb, monoclonal antibody.

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2. MATERIALS AND METHODS

2.1. *Patients and cell lines*

Patients A and B had plasma cell leukemia with respectively 52% IgGλ and 65% IgAκ myeloma cells in the peripheral blood and patient C had Bence Jones myeloma with pleural effusion containing 82% myeloma cells. Patients A, B and C were treated with anti-IL-6 MAbs as described for one patient [11]. Peripheral blood or pleural effusion samples were taken before the first anti-IL-6 MAb injection and on each day during the first 4 days of treatment. Mononuclear cells were prepared by centrifugation of heparinized samples over Ficoll-Hypaque gradients. For enrichment in myeloma cells and mononuclear cells were depleted for monocytes, myeloid cells and T cells by incubation with a mixture of immunobeads (Dynabeads M-450, Biosys, France) coated with specific MAbs (anti-CD13, CD15, CD3) as already described [10]. The autonomously growing RPMI 8226 [18] HMCL was cultured in RPMI 1640 (Gibco BRL, France) containing 10% FCS. For XG-1, and XG-5 IL-6-dependent HMCL, 2 nM human recombinant (hr) IL-6 was added in the medium culture [19].

2.2. *Reagents*

The anti-CD13, anti-CD15, and anti-CD3 MAbs were purchased from Immunotech (Marseilles, France); the hrIL-6 was kindly provided by N. Vita and P. Ferrara (Sanofi, Labège, France). The anti IL-6 MAbs (B-E4 and B-E8, 20) by J. Wijdenes (CRTS, Besançon, France); the probe for human IL-6R (pBSF2R236, 14) by T. Hirano (University of Osaka, Japan), for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by M. Piechaczyk (CNRS Montpellier, France).

2.3. *Northern blot analysis and mRNA quantification*

Extraction of total RNA, electrophoretic conditions, blotting and membrane hybridizations with both IL-6R and GAPDH probes were done as already described [10]. Autoradiograms were scanned on a Dual-wavelength TLC scanner CS-390 (Shimatzu, Japan). The amount of IL-6R mRNA in each lane was evaluated in arbitrary units after standardization with the amount of GAPDH mRNA.

3. RESULTS

We analyzed the effect of IL-6 on the gp80 IL-6R

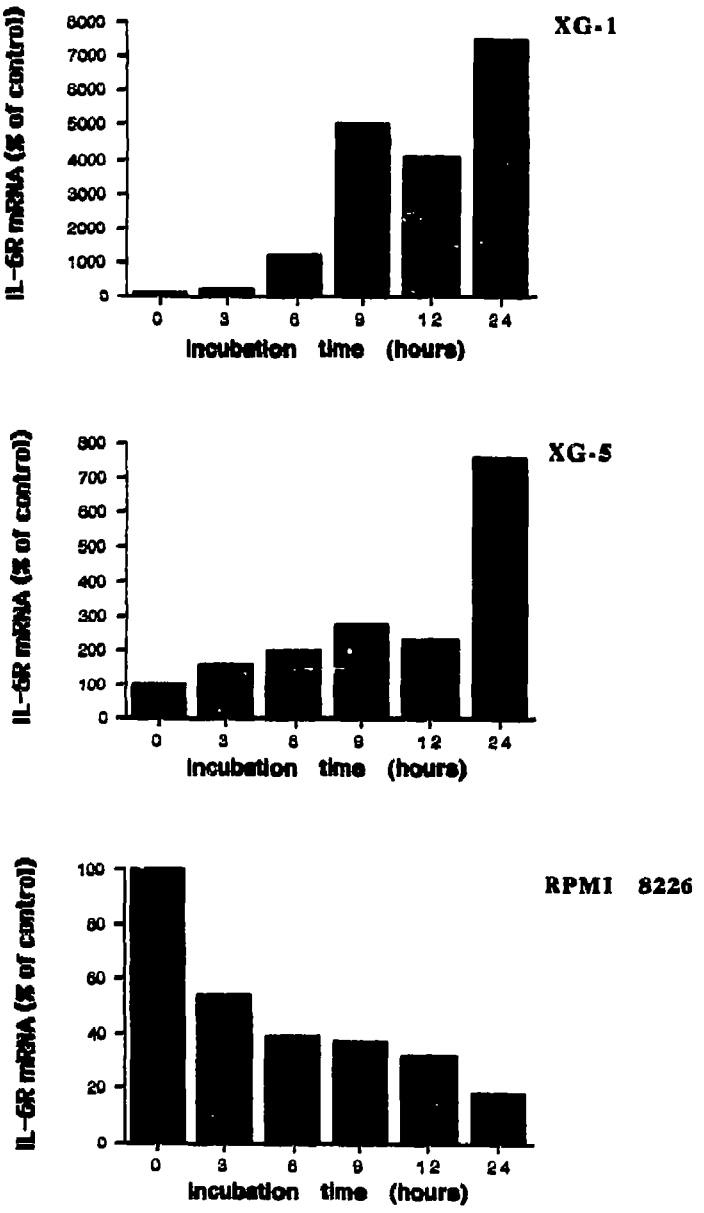

Fig. 1. Regulation of IL-6R gene expression in HMCL. XG-1, XG-5 and RPMI 8226 were cultured with or without 2 nM hrIL-6 for various lengths of time. Cells were harvested, total RNA were extracted and submitted to hybridization with both IL-6R and GAPDH specific probes. Autoradiograms were scanned and IL-6R mRNA levels were quantified. Results for each HMCL are expressed in percentage of the control group as detailed in the text.

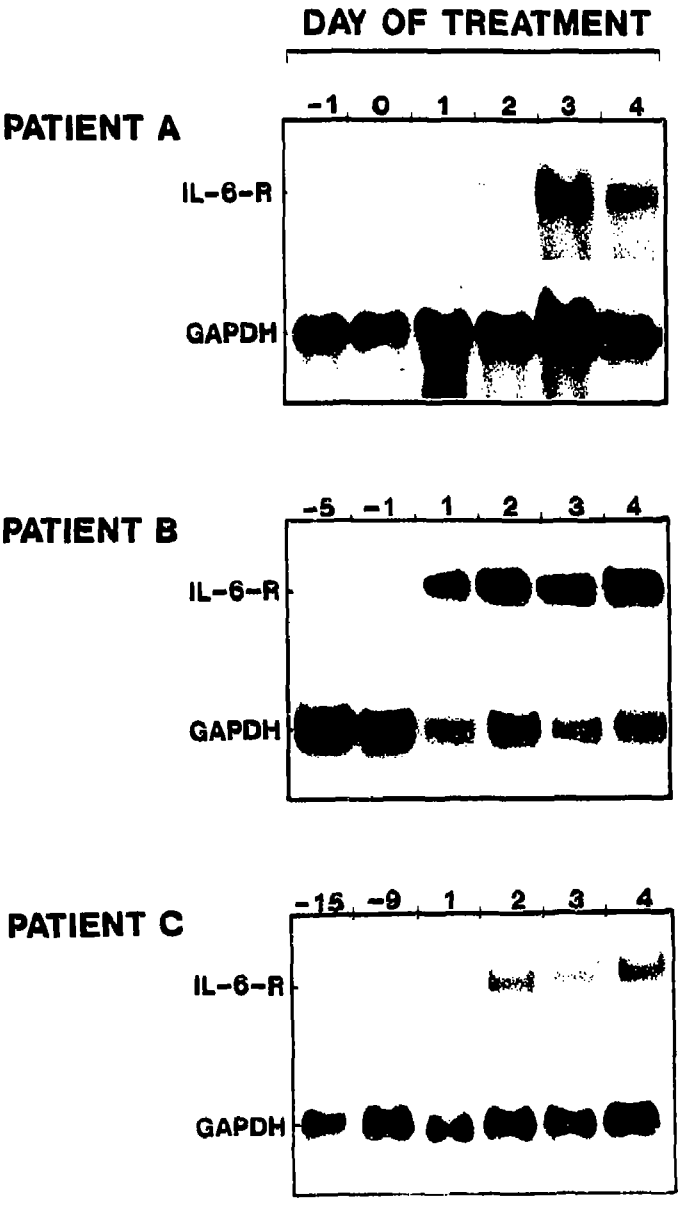

Fig. 2. IL-6R gene expression in myeloma cells from patients treated with anti-IL-6 MAbs. Patients A, B and C were treated with anti-IL-6 MAbs. Samples were taken before the beginning and on each day during the first 4 days of treatment. Myeloma cells were enriched, total RNA were extracted and submitted to hybridization with both IL-6R and GAPDH probes.

gene expression in 3 HMCL by Northern blot hybridization. The amounts of IL-6R mRNA at each time incubation and for each HMCL are expressed in percentage of the control group, i.e. cells incubated *with* hrIL-6 for the IL-6-dependent HMCL, and *without* hrIL-6 for the autonomously growing HMCL. Results presented in Fig. 1 show that a 7.4–72.0-fold increase of IL-6R mRNA level was seen after 24 h of privation of IL-6 for XG-5 and XG-1, respectively, and that a 24-h incubation with hrIL-6 resulted in a 82% decrease of IL-6R mRNA level for RPMI 8226.

The regulation of IL-6R expression in myeloma cells in vivo was studied during treatment of terminal myeloma patients with anti-IL-6 MAbs. IN 3 patients with extramedullary MM, myeloma cells were harvested and purified each day during the course of anti-IL-6 therapy, and analyzed for IL-6R gene expression. For the 3 patients, a dramatic increase of IL-6R mRNA in myeloma cells was found following neutralization of exogenous IL-6 by the anti-6 MAb (Fig. 2). Quantification

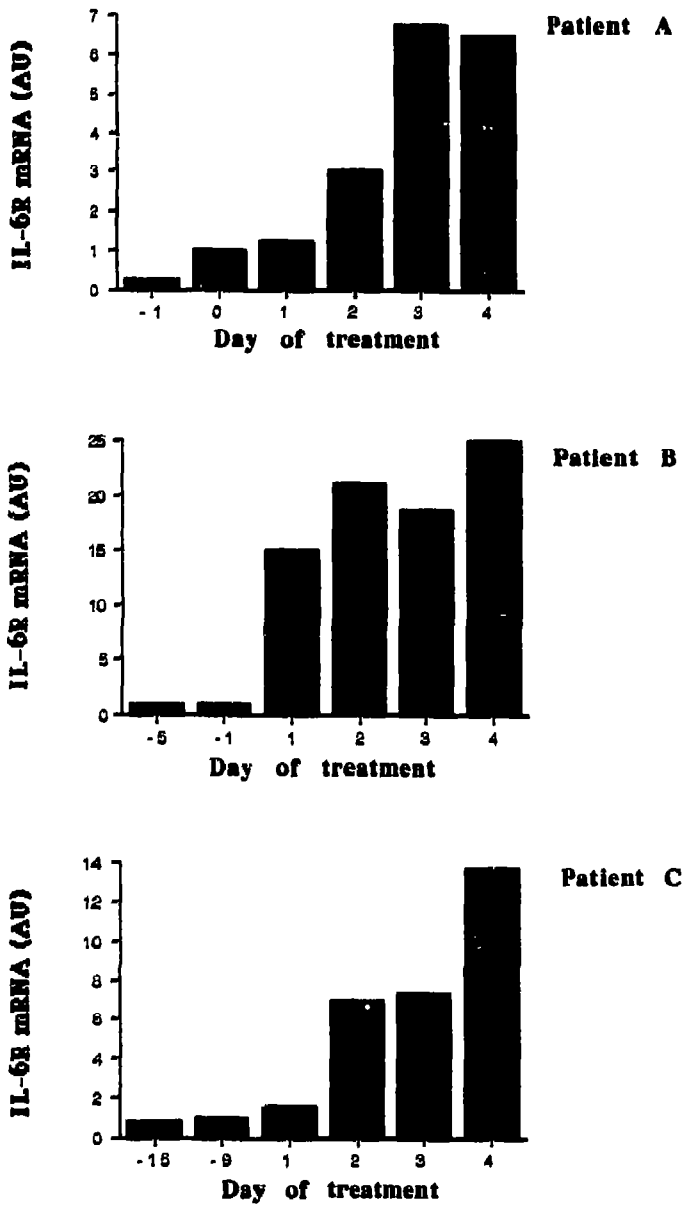

Fig. 3. Quantification of IL-6R mRNA in myeloma cells in course of anti-IL-6 therapy. Results for each patient are expressed in arbitrary units (AU), assuming the '1' value to initial content of IL-6R mRNA in myeloma cells before the beginning of treatment.

of autoradiograms showed that this increase was at maximum after 3–4 days of treatment and ranged from 6.8 (patient A) to 24.0 (patient B) (Fig. 3).

4. DISCUSSION

In the present study we report the regulation of IL-6R gene expression by IL-6 in myeloma cells in vitro and in vivo. First, we showed that privation of IL-6 for 2 IL-6-dependent HMCL induced a dramatic increase of their amount of IL-6R mRNA. These HMCL are a priviliged model of study since they have retained an absolute requirement of exogenous IL-6 for growing in vitro, similarly to freshly-explanted myeloma cells [6,8,10]. Second, we analyzed IL-6R gene expression in myeloma cells from 3 MM patients treated with anti-IL-6 MAbs. The efficiency of the anti-IL-6 therapy to prevent binding of IL-6 to its cell surface receptor was shown, in these patients, by the inhibition of both the in vivo myeloma-cell proliferation and the production of C-reactive protein by hepatocytes [11]. Indeed the production of this acute phase protein by human hepatocytes in primary cultures has recently been shown to be solely under the control of IL-6 [21]. Results presented here show that a significant increase of IL-6R mRNA occurred during the treatment of the 3 patients, the maximum being at 3–4 days. This relatively late effect can be explained by the fact that the maximal serum concentration of anti-IL-6 MAb was obtained only after 2 days of treatment in these patients (manuscript in preparation). Finally, a down-regulation of IL-6R gene expression was observed by adding hrIL-6 to an autonomously growing HMCL, which does not produce IL-6 and whose growth is not affected by it [6]. Such a down-regulation has already been reported for monocytes [15,16].

In conclusion, the present study clearly demonstrates a dramatic up-regulation of IL-6R gene expression in IL-6-deprived myeloma cells in vitro and, interestingly, also in vivo. As high levels of IL-6 are produced in MM patients and as they reflect the disease activity, these results suggest that IL-6 continuously represses the IL-6R gene expression in myeloma cells in vivo. Our finding of an up-regulation of IL-6R gene expression during anti- IL-6 therapy is of importance for the future of this therapy. Indeed we have recently demonstrated that high levels of IL-6 in the form of immune complexes accumulated in the plasma of patients treated with anti-IL-6 MAbs (manuscript in preparation). The up-regulation of IL-6R might lead to the emergence of IL-6 hypersensitive tumoral subclones which are able to displace these immune complexes, thus leading to a progressive resistance to treatment. Such a hypothesis may explain why a significant index of myeloma cell proliferation was still found after one patient had been treated for 2 months, whereas the tumoral cell proliferation was still dependent on exogenous IL-6, and whereas the IL-6 MAb was still present at this period and able to neutralize the biological activity of IL-6 on hepatocytes in vivo [11].

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