Interleukin-10 induces interleukin-11 responsiveness in human myeloma cell lines

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Abstract Interleukin (IL)-6-dependent human myeloma cell lines (HMCL) can be reproducibly obtained from patients with multiple myeloma and terminal disease. The growth of some of these HMCL can also be supported by IL-11. We show that IL-11-responsive, but not -unresponsive, HMCL expressed the gene of human IL-11 receptor (IL-11R) and produced an autocrine IL-10. All HMCL expressed the IL-10 receptor. In addition, IL-10 induced IL-11R gene expression and conferred IL-11 responsiveness on unresponsive HMCL. The ability of HMCL to produce IL-10 was strictly correlated with the capacity of the original patient's myeloma cells to produce IL-10 or not, and with the presence or absence of IL-10 in the patient's plasma.

Key words: IL-10; IL-11; Multiple myeloma

1. Introduction

Interleukin (IL)-11, initially identified as an IL-6 unrelated growth factor for a murine plasma cell line [1], shares several biological activities with IL-6, i.e. as a thrombopoietic factor [2], inducer of acute-phase proteins [3], competence factor for hematopoietic stem cells [4] and bone-resorption factor [5]. These shared biological activities can be explained by the activation of the same transducer chain (gp130 transducer) by IL-6 and IL-11 [6]. The cloning of murine IL-11 receptor (IL-11R) cDNA indicated that IL-11 binds first to its IL-11R and that the IL-11/IL-11R complex then binds to gp130 and induces its dimerization in a way similar to that of IL-6 [7]. Two cDNA of human IL-11R, one coding for a membrane receptor and the other one for a soluble receptor, have been recently cloned [8]. The other 3 gp130-activating cytokines – leukemia inhibiting factor (LIF), oncostatin M (OM) and ciliary neurotrophic factor (CNTF) – involve another transducer chain, LIF receptor (gp190), forming a heterodimer with gp130 [9]. It is not presently clear whether the human IL-11/IL-11R complex induces an homodimerization of gp130 transducer [8].

We and others have previously reported that IL-6 is a major tumor growth factor in human multiple myeloma (reviewed in [10]), and that IL-6-dependent human myeloma cell lines (HMCL) can be obtained reproducibly from patients with terminal disease [11]. We recently found that the growth of some HMCL can be supported by other gp130-activating cytokines, including IL-11, whereas other HMCL are only sensitive to IL-6 [12]. In the present report, we show that IL-11-sensitive (but not -unsensitive) HMCL expressed IL-11R gene and produced IL-10. In addition, we show that IL-10 induced IL-11R gene expression and conferred IL-11 responsiveness on non-responsive HMCL. Thus, the ability of myeloma cells to produce IL-10 or not could be a crucial marker in distinguishing patients whose myeloma cells are responsive only to IL-6 from those whose myeloma cells may also respond to IL-11.

2. Patients, materials and methods

2.1. Patients

XG HMCL were obtained from three patients with plasma-cell leukemia (XG-1, XG-4 and XG-6) and one patient with pleural effusion (XG-2), as previously reported [11]. Myeloma cells were harvested from peripheral blood or pleural fluid. At the time of cell harvesting, plasma or pleural fluid was filtered and stored at −20°C until use.

2.2. Reagents

Recombinant IL-6 was provided by D. Stinchcomb (Synergen, Boulder, CO, USA); rIL-10 and anti-IL-10 IgG1 MoAb by Dr. J. Banchereau (Schering Plough, Dardilly, France) and Dr. J. Wijdenes (Diaclone, Besançon, France); and anti-IL-11 IgG1 MoAb by Dr. K. Turner (Genetics Institute, Boston, MA, USA). Anti-human CD3 MoAb, magnetic beads coupled to anti-murine IgG antibodies and control mouse purified IgG1 MoAb were purchased from Immunotech (Marseille, France).

2.3. Preparation of culture supernatants of fresh tumor cells

Mononuclear cells were separated on Ficoll Hypaque from peripheral blood samples of patients with plasma-cell leukemia. A virtually pure population of myeloma cells (>98%) was obtained by removing adherent cells and T cells using anti-CD3 MoAbs and magnetic beads. These cells were cultured at 10^6 cells/ml in RPMI 1640 supplemented with 2 mM of L-glutamine and 5% fetal calf serum (FCS). After 2 days of culturing, culture supernatants were filtered and stored at −20°C until use.

2.4. Human myeloma cell lines (HMCL)

Four HMCL were studied: XG-1, XG-2, XG-4 and XG-6. All had cytoplasmic Ig, expressed plasma-cell antigens (Ag) (CD38 and B-B4) and lacked the usual B-cell Ag (CD19 and CD20). They were not infected with EBV (lack of EBNA2 Ag), and their growth was completely dependent on addition of exogenous IL-6. Their detailed characteristics have been reported elsewhere [11]. To investigate their responsiveness to various cytokines, XG cells were washed once with culture medium, incubated for 5 h at 37°C in culture medium alone and washed again twice. The cells were then cultured at various concentrations in

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96-well flat-bottomed microplates for 5 days with either culture medium alone or graded concentrations of various cytokines. Tritiated thymidine (2.5 μCi, 25 Ci/mM, CEA, Saclay, France) was added for the last 8 hours of culture, and tritiated-thymidine incorporation was determined as reported elsewhere [13].

2.5. IL-10 assay by ELISA

The concentration of IL-10 in culture supernatants and patient plasma was evaluated using a sensitive ELISA (1 pg/ml) developed by J. Wijdenes [14]. This ELISA used the BN-10 anti-IL-10 MoAb as a catcher and biotinylated BT-10 anti-IL-10 MoAb and peroxidase avidin as a tracer.

2.6. RT-PCR analysis

Total RNA was isolated from human myeloma cell lines by the guanidium-CsCl method. cDNA was synthesized from 1 μg of total RNA using a reverse transcription kit (Promega, Madison, WI, USA). Thirty-five PCR cycles (60″ at 94°C, 45″ at 60°C, 60″ at 72°C), followed by a 3-min extension at 72°C, were carried out using primers corresponding to nucleotides 345–364 and 621–640 of the IL-10 sequence, 1141–1160 and 1423–1442 of the IL-10 receptor sequence, 1037–1060 and 1244–1267 of the IL-11R sequence, 6–37 and 269–300 of IL-11 sequence and 5–24 and 242–361 of the β2 m sequence. Fifteen microliters of each 25-μl PCR reaction were electrophoresed on 1.5% agarose gel in TAE buffer.

2.7. Statistical analysis

Results are the mean values determined on six replicates. The mean values were compared using a *t*-test from small samples.

3. Results

3.1. Interleukin-10 production by IL-11-responsive myeloma cell lines

As previously reported, XG-1, XG-2, XG-4 and XG-6 cells could not grow without addition of exogenous cytokines, and their growth required addition of IL-6 [11]. IL-11 also supported the proliferation of XG-4 and XG-6 cells but not XG-1 and XG-2 (Fig. 1). IL-11-responsive XG-4 and XG-6 HMCL, unlike IL-11-unresponsive XG-1 and XG-2 HMCL, expressed IL-11R gene (Fig. 2). Actually, two cDNA for IL-11R, one coding for the membrane receptor and the other one coding for a soluble form, were recently identified [8]. Our data show that these 2 cDNA were detected in the myeloma cell lines (Fig. 2).

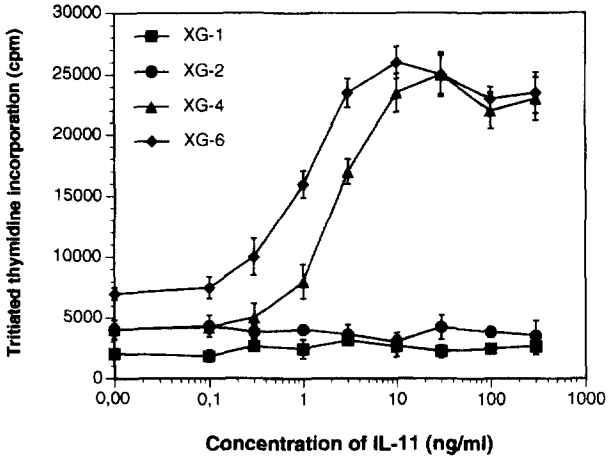

Fig. 1. Response of XG myeloma cells to IL-11. Ten thousand XG myeloma cells were cultured for 5 days in 150 μl RPMI 1640 cell culture medium and 10% FCS with various IL-11 concentrations. Results are means ± S.D. of tritiated thymidine incorporation determined in six separate culture wells.

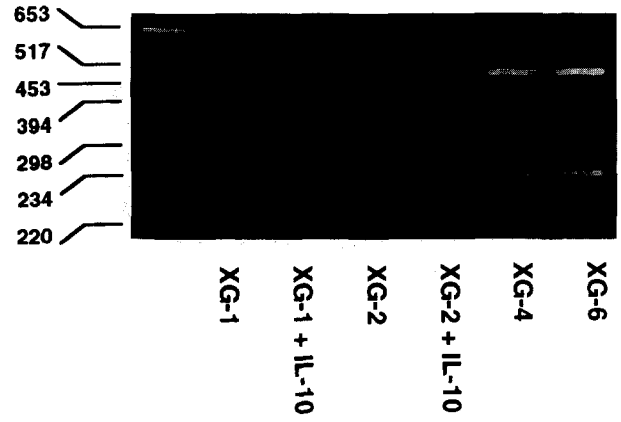

Fig. 2. IL-11 receptor gene expression in XG myeloma cells. RNA was isolated from XG cells harvested during exponential growth in the presence of 1 ng/ml of IL-6 (lanes XG-1, XG-2, XG-4, XG-6). In some experiments, 10 ng/ml of IL-10 was added for 12 h during exponential growth of XG-1 or XG-2 cells with 1 ng/ml of IL-6 and RNA was extracted (lanes XG-1 + IL-10 and XG-2 + IL-10). cDNA were obtained using reverse transcription and PCR were performed as described in section 2. Results are the bands visualized by ethidium bromide obtained with primers for IL-11R.

The 2 IL-11-responsive XG-4 and XG-6 HMCL also produced IL-10, as indicated in Fig. 3 which shows the presence of IL-10 mRNA by RT-PCR analysis in XG-4 and XG-6 cells but not in XG-1 and XG-2 cells. IL-10 protein was also detected in culture supernatants of XG-4 and XG-6 cells but not in those of XG-1 and XG-2 cells (Table 1). RT-PCR revealed the presence of IL-10 receptor mRNA in the 4 HMCL (Fig. 3). No IL-11 mRNA could be detected in the 4 HMCL by RT-PCR (results not shown).

3.2. Interleukin-10 induces IL-11 responsiveness in unresponsive cell lines

XG-1 and XG-2 cells failed to proliferate with IL-11. As indicated in Fig. 2, IL-10 was able to induce IL-11R gene expression in XG-1 and XG-2 cells. Moreover, in the presence of IL-10, IL-11 induced the proliferation of both HMCL in a concentration-dependent fashion. Maximal induction of IL-11 responsiveness was found with 10 ng/ml of IL-10. IL-10 induction of IL-11 responsiveness in XG-1 and XG-2 cells was inhibited by anti-IL-10 or anti-IL-11 antibodies (Fig. 4A and B).

As shown in Fig. 1 and Table 1, autocrine IL-10-producing XG-4 and XG-6 HMCL spontaneously responded to IL-11. Addition of exogenous IL-10, even at a high concentration (100 ng/ml), did not increase their sensitivity to IL-11, and addition of a neutralizing antibody to IL-10 failed to block the IL-11 responsiveness of these 2 HMCL (results not shown).

3.3. IL-10 may be involved in myeloma disease in vivo

IL-10 was detected in 2-day culture supernatants of purified primary tumor cells freshly explanted from XG-4 and XG-6 patients with plasma-cell leukemia (Table 1). IL-10 was also found in the plasma samples of these 2 patients. Conversely, no IL-10 was detected in culture supernatants of primary tumor cells or in plasma samples from XG-1 and XG-2 patients (Table 1).

Table 1
IL-10 activity* in culture supernatants of myeloma cells

	XG-1	XG-2	XG-4	XG-6
IL-10 activity in culture supernatant of HMCL	0	0	12	275
IL-10 activity in culture supernatant of patients' freshly expanted myeloma cells	0	0	111	227
IL-10 activity in plasma of XG myeloma patients	0	0	110	480

*IL-10 activity: (pg/ml).
XG cells (3×10^5 cells/ml) were cultured for 2 days with 1 ng/ml of IL-6, and culture supernatants were then harvested. Fresh myeloma cells from XG patients with plasma-cell leukemia or pleural effusion were harvested and purified. Purified myeloma cells (>98%) were cultured for 2 days at 10^6 cells/ml in RPMI 1640 and 10% fetal calf serum. The culture supernatants were frozen. Plasma from XG patients was harvested and frozen at the time cell lines were obtained.

4. Discussion

We showed that the IL-11 responsiveness of human myeloma cell lines was associated with their ability to express IL-11R gene and produce an autocrine IL-10, as assayed by RT-PCR and an ELISA. We also showed that exogenous IL-10 induced IL-11R gene expression and IL-11 responsiveness in myeloma cells initially unresponsive to IL-11 and unable to produce IL-10. These findings may explain why some myeloma cell lines which produce an autocrine IL-10 respond spontaneously to IL-11. As the biological activity of an autocrine IL-10 is difficult to inhibit, it is hardly surprising that no inhibition of IL-11 responsiveness was found when an anti-IL-10 mAb was used on IL-10-producing cell lines (results not shown).

The ability of some HMCL to produce an autocrine IL-10 was associated with IL-10 production by the patient's purified primary tumor cells and the presence of circulating IL-10 in plasma. This suggests that the ability of primary myeloma cells to produce IL-10 could be useful in identifying patients whose tumor cells are sensitive only to IL-6 in vivo or to IL-6 and IL-11. This might be important for IL-10-producing patients since IL-11 is a bone cytokine produced by osteoblast cells [1], and myeloma cells preferentially proliferate close to bone tissue.

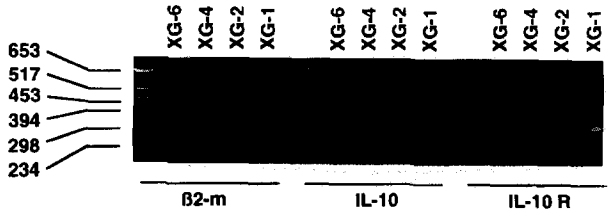

Fig. 3. IL-10 and IL-10 receptor gene expression in XG myeloma cell lines. RNA was isolated from cells harvested during exponential growth in the presence of IL-6. cDNA was obtained using reverse transcription. The different PCR were performed on the same cDNA, as described in section 2. Results are the bands visualized by ethidium bromide obtained with primers for IL-10, IL-10R or β2m.

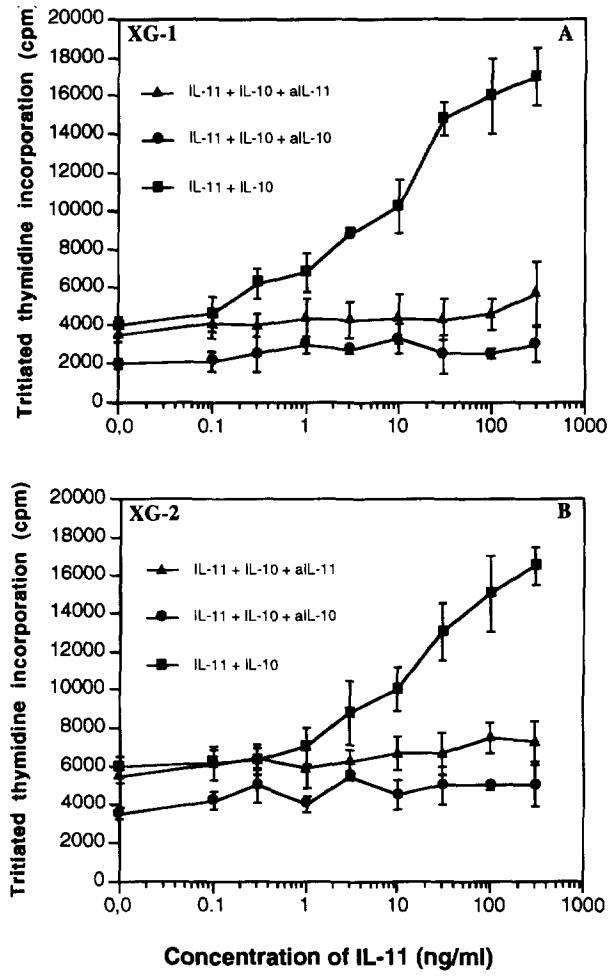

Fig. 4. IL-10 induces IL-11 responsiveness in XG-1 and XG-2 cell lines. Ten thousand XG myeloma cells were cultured for 5 days in 150 μl RPMI 1640 cell culture medium and 10% FCS together with various IL-11 concentrations and with 10 ng/ml of IL-10 and 20 μg/ml of a control murine IgG1 MoAb in the IL-11 + IL-10 group (■), or 10 ng/ml of IL-10 and 20 μg/ml of an anti-IL-10 IgG1 MoAb in the IL-11 + IL-10 + anti-IL-10 group (●), or 10 ng/ml of IL-10 and 20 μg/ml of an anti IL-11 IgG1 MoAb in the IL-11 + IL-10 + anti-IL-11 group (▲). Results are means ± S.D. of tritiated thymidine incorporation determined in six separate culture wells.

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