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Interleukin-4 (IL-4), but not IL-10, regulates the synthesis of IL-6, IL-8 and leukemia inhibitory factor by human bone marrow stromal cells

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Abstract

Leukemia inhibitory factor (LIF), interleukin 6 (IL-6) and IL-8 are important regulators of inflammation and hematopoiesis. Human bone marrow stromal cells regulate marrow hematopoiesis by secreting cytokines. By using reverse-transcriptase polymerase chain reaction (RT-PCR), we demonstrate that human bone marrow stromal cells constitutively express LIF, IL-6 and IL-8 transcripts. By using specific ELISAs, we found that their spontaneous productions of LIF, IL-6 and IL-8 are elevated in response to serum and after stimulation with the pro-inflammatory cytokines IL-1α and TNF-α. The anti-inflammatory cytokine IL-4 reduces their serum- and cytokine-induced LIF secretion. By contrast, IL-4 stimulates their serum- and IL-1α-induced IL-6 synthesis. IL-4 has no effect on the serum-induced IL-8 synthesis by marrow stromal cells, but stimulates their cytokine-induced IL-8 production. The anti-inflammatory cytokine IL-10 has no effect on the serum- and cytokine-induced LIF, IL-6 and IL-8 synthesis by bone marrow stromal cells. RT-PCR experiments reveal the presence of IL-4 receptor α-chain mRNA and IL-10 receptor mRNA in cultured bone marrow stromal cells. The differential regulation by IL-4 of two related cytokines, such as LIF and IL-6, and the enhanced effect of this 'anti-inflammatory' cytokine on IL-6 and IL-8 synthesis highlight the tightly controlled regulation and the complexity of the cytokine production within the human bone marrow.

Keywords: Bone marrow; Stromal cell; IL-4; IL-10

1. Introduction

Long-term bone marrow culture methods are characterized by the development of an adherent cell layer (mostly fibroblast-like cells) producing various cytokines anchored to cell membranes and/or released in the culture medium. These compounds interact together and are essential for the development of in vitro hematopoiesis [1,2]. Thus, bone marrow stromal cells regulate hematopoiesis by interacting directly (cell-to-cell contact) with hematopoietic cells and by secreting cytokines that modulate hematopoiesis either in a positive or a negative manner.

Human bone marrow stromal cells secrete numerous cytokines including leukemia inhibitory factor (LIF), interleukin 6 (IL-6) and IL-8 [3–5]. LIF is a pleiotropic cytokine identified by its growth and differentiation activities on hematopoietic cells [6–8]. LIF shares with IL-6 biological activities [9,10] and

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receptor components [11]. LIF promotes the survival and/or proliferation of primitive hematopoietic precursors [8,12]. IL-6 supports, in synergy with macrophage colony-stimulating factor, the formation of macrophage colonies from marrow progenitors [13]. IL-6, through a paracrine mechanism, is the major cytokine involved in the emergence of the tumor clone in patients with multiple myeloma [14]. IL-8 is reported to inhibit the growth of isolated $CD34^+$ stem/progenitor cells [15,16] and its intravenous injection induces a rapid mobilization of hematopoietic progenitor cells in rhesus monkeys [17].

Human bone marrow stromal cells produce LIF, IL-6 and IL-8 spontaneously, in response to serum and monocyte-derived cytokines such as IL-1α and TNF-α [3–5]. Conversely, no result is available concerning cytokines that down-regulate their LIF, IL-6 and IL-8 synthesis. IL-4 and IL-10 are classified as anti-inflammatory cytokines on the basis of their inhibitory effects on the production of several cytokines (including IL-6, IL-8 and LIF) from various cell types [18–22]. In this study we have investigated the effects of IL-4 and IL-10 on the spontaneous, serum- and cytokine-induced LIF, IL-6 and IL-8 production by human bone marrow stromal cells in vitro.

2. Materials and methods

2.1. Human bone marrow stromal cell cultures

Cultures of human bone marrow stromal cells were established from bone marrow aspirates harvested into heparinized tubes (Vacutainer system, Becton Dickinson, Meylan, France) and considered as normal by morphological analysis of the May Grunwald Giemsa stained smears. The procedure was performed according to the Helsinki recommendations on patients undergoing a myelogram as part of routine procedure. Mononuclear cells were separated on a Ficoll gradient ($400\times g$, 20 min), washed two times with Hanks' balanced salts solution ($400\times g$, 10 min), and seeded in culture flasks in RPMI 1640 with 20% FCS, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in 5% CO_2 in air [23]. Adherent cells were grown to confluence for 5–8 weeks with weekly changes of medium and were subcultured after trypsin treatment. As previously reported [4], in these experimental conditions, more than 99.8% of cells were $CD2^-$ and $CD22^-$ indicating the absence of T- and B-cells on the layers and 4% of cells were $CD14^+$ and $CD33^+$ indicating a monocytic lineage.

2.2. Cell stimulation

Human bone marrow stromal cells were used at their first passage. Cells (1×10^4 per well) were plated for 24 h in 96-well plates in 100 μl of RPMI 1640 with 20% FCS. Adherent cells were washed and 200 μl of serum-free medium was added to each well for 2 days. After washing, several experimental conditions were used to investigate bone marrow stromal cell cytokine production. In the first set of experiments, cells were reactivated with 200 μl of RPMI 1640 with 10% FCS and stimulated with IL-1α (10 ng/ml) (Tebu, Le Perray en Yvelines, France), TNF-α (10 ng/ml) (Tebu), IL-4 (10 ng/ml) (Shering Plough), IL-10 (10 U/ml) (a gift from Dr. J.C. Lecron, Poitiers, France) or IL-4 (10 ng/ml)+IL-10 (10 U/ml). Media were collected after 2, 4 or 7 days of growth and stored at −80°C until LIF, IL-6 and IL-8 assay. No significant level of endotoxin was found into FCS assessed by Limulus test (E-Toxate kit, Sigma). In another set of experiments, cells were reactivated with 200 μl of serum-free RPMI 1640 and stimulated with IL-1α (0.1–100 ng/ml), TNF-α (0.1–100 ng/ml), IL-4 (0.1–100 ng/ml), and IL-10 (0.1–300 U/ml). After 24 h, media were collected and stored at −80°C until LIF, IL-6 and IL-8 assay. In another set of experiments, stimulations with IL-1α (100 ng/ml), TNF-α (100 ng/ml), IL-1α (100 ng/ml)+TNF-α (100 ng/ml), IL-1α (100 ng/ml)+IL-4 (1 to 100 ng/ml), TNF-α (100 ng/ml)+IL-4 (1–100 ng/ml), IL-1α (100 ng/ml)+IL-10 (1–100 U/ml) or TNF-α (100 ng/ml)+IL-10 (1–100 U/ml) were made with 0% FCS in the culture medium. After 24 h, media were collected and stored at −80°C until LIF, IL-6 and IL-8 assay.

2.3. Measurements of LIF, IL-6 and IL-8 levels

LIF levels were measured by an enzyme-linked immunosorbent assays (ELISA) for human LIF as previously described [24]. IL-6 and IL-8 levels were

measured by a 'sandwich-type' ELISA (Pelikine compact human ELISA Kit, Tebu, France) following the manufacturer's instructions. The sensitivity of the assays enables the detection of levels as low as 2 pg/ml for IL-6 and IL-8 and 12 pg/ml for LIF. No significant cross-reactivity of the three ELISA was observed with the various cytokines used in this study to stimulate marrow stromal cells.

2.4. RT-PCR analysis of LIF, IL-6 and IL-8 transcripts

Human marrow stromal cells (1×10^6) were plated for 24 h in 25-cm^2 culture flasks in 5 ml of RPMI 1640 with 20% FCS. Adherent cells were then washed and 5 ml of serum-free medium were added to each flask for 3 days. Total RNA was then extracted with RNA NOW reagent (Biogentex, Ozyme, France) following the manufacturer's instructions. The first cDNA strand was synthesized using total RNA (2 μg) at 45°C for 30 min in a 100 μl reaction mixture containing 50 mM Tris-HCl (pH 8.3), 60 mM KCl, 10 mM dithiothreitol (DTT), 5 mM $MgCl_2$, 20 U of RNase inhibitor (Boehringer, Mannheim, Germany), 1 mM of each deoxynucleotide triphosphate (dNTP), 600 U of M-MLV reverse transcriptase (Gibco) and 2 μg of oligo(dT) 15 mer. One μg of the reaction mixture was made up to 25 μl using Taq polymerase buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) containing 10 pmol of each primer and 0.6 U of AmpliTaq DNA polymerase (Pharmacia Biotech, Uppsala, Sweden). Amplifications were performed using a thermal cycler for 35 cycles under the following conditions: denaturation for 1 min at 94°C, annealing for 45 s at 59°C and elongation for 1 min at 72°C using IL-8 primers: sense, 5′-TGGCTCTCTTGGCAGCCTTC and antisense, 5′-TCTCCACAACCCTCTGCACC; IL-6 primers: sense, 5′-TGGTGTTGCCTGCTGCCTTC and antisense, 5′-CTCATCTGCACAGCTCTGGC; LIF primers: sense, 5′-CCCTGCTGTTGGTTCTGCAC and antisense, 5′-CCAACACGCCGATGATCTGC. PCR products were separated in a 1% agarose gel and analyzed by ethidium bromide staining. Control PCR was performed using β-actin primers: 5′-GGCTACAGCTTCACCACCAC and antisense: 5′-GCACTGTGTTGGCGTACAGG to check RNA integrity.

2.5. RT-PCR analysis of IL-4 receptor α-chain (IL-4Rα) and IL-10R transcripts

Total RNA was extracted from bone marrow stromal cell cultures as reported above. RNA was reverse-transcribed as previously reported [25]. cDNA was boiled 5 min, quenched on ice and diluted with 80 μl of distilled water before PCR amplification. A 10-μl aliquot of the diluted cDNA mixture was amplified by PCR using recombinant Taq polymerase under the following conditions: 1 min, 94°C; 1 min, 53°C; 1.5 min, 72°C for 30 cycles. PCR reaction was performed in a total volume of 50 μl containing 75 mM of Tris-HCl (pH 9.0), 20 mM of $(NH_4)_2SO_4$, 0.01% of Tween 20, 1.5 mM of $MgCl_2$, 0.4 mM of each of dNTP, 0.15 μM of the two IL-4Rα, IL-10 or β-actin PCR primers, and 0.1 U/ml of DNA polymerase (Eurogentec, Belgium). The human IL-4Rα chain sense primer was 5′-GGAAGAGGGGTATAAGCCTTT-3′ and the antisense primer was 5′-CACGGAGACAAAGTTCACGAT-3′ spanning a 571-bp fragment [26]. The human IL-10R sense primer was 5′-CCATCTTGCTGACAACTTCC-3′ and the antisense primer was 5′-GTGTCTGATACTGTCTTGGC-3′ spanning a 439-bp fragment [27]. The β-actin sense primer was 5′-GAGACCTTCAACAACCC-3 and the antisense primer was 5′-GTGGTGGTGAAGCTGTAGCC-3′ spanning a 235-bp fragment [25]. PCR products were electrophoresed in a 2% agarose gel (Gibco) and visualized by ethidium bromide staining.

2.6. Statistical analysis

Results are presented as mean ± S.E.M. of four independent experiments. Differences between cytokine-treated groups and control groups were compared with the Mann–Whitney *U*-test. $P<0.05$ was considered to be significant.

3. Results

3.1. Presence of LIF, IL-6 and IL-8 transcripts in marrow stromal cells

As shown in Fig. 1, by using RT-PCR, we demonstrated that LIF, IL-6 and IL-8 transcripts were

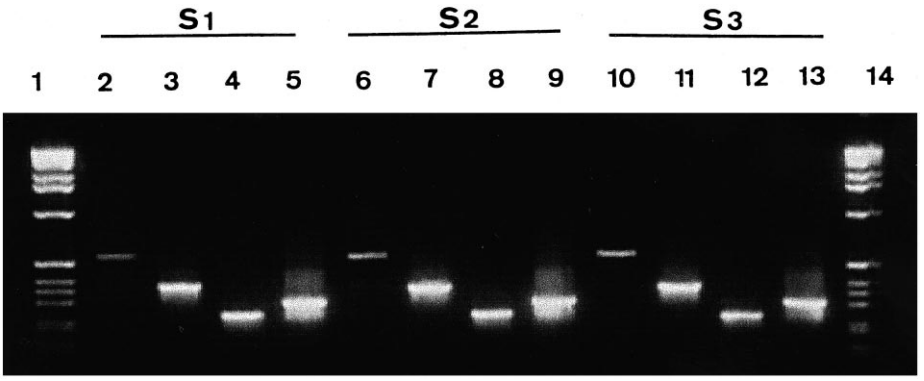

Fig. 1. Analysis of LIF, IL-6 and IL-8 transcripts in bone marrow stromal cells. Total RNA isolated from human bone marrow stromal cells were submitted to RT-PCR as described in Section 2 and amplification products were submitted to agarose gel electrophoresis and ethidium bromide staining. Lanes 2, 6 and 10, LIF transcripts (size of 575 bp) from three different bone marrow stromal cell samples; lanes 3, 7 and 11, IL-6 transcripts (size of 380 bp) from three different cell samples; lanes 4, 8, and 12, IL-8 transcripts (size of 250 bp) from three different cell samples; lanes 5, 9 and 13, β-actin transcripts (size of 310 bp) from three different cell samples; lanes 1 and 14, 1-kb DNA ladder.

constitutively expressed in 11/12, 12/12 and 12/12 of the different human marrow stromal layers, respectively (data not shown).

3.2. *Effects of cytokines on the production of LIF, IL-6 and IL-8 by bone marrow stromal cells cultured in serum-supplemented medium*

In accordance with their constitutive expression of LIF, IL-6 and IL-8 transcripts, marrow stromal cells cultured for 24 h in serum-free medium secreted LIF, IL-6 and IL-8 proteins in their culture supernatants (Table 1). These productions of LIF, IL-6 and IL-8 were significantly ($P < 0.05$) higher in serum-containing medium than in serum-free medium (Table 1). TNF-α (10 ng/ml) and IL-1α (10 ng/ml) enhanced in a time-dependent manner the production of LIF, IL-6 and IL-8 (Fig. 2A, B and C, respectively) by bone marrow stromal cells cultured in 10% FCS. IL-4 (10 ng/ml) significantly decreased their LIF secretion (Fig. 3A), increased their IL-6 production (Fig. 3B) and had no effect on their IL-8 synthesis (Fig. 3C). IL-10 (10 U/ml) had no effect on LIF, IL-6 and IL-8 secretions (Fig. 3A, B and C, respectively). When added to IL-4 (10 ng/ml), IL-10 (10 U/ml) did not modify the effect of IL-4 alone (data not shown). In these experiments, IL-1α, TNF-α and IL-4 did not change cell growth (data not shown).

Table 1
Secretion of LIF, IL-6 and IL-8 by human bone marrow stromal cells

	0% FCS	10% FCS
LIF (pg/ml)	47 ± 15	565 ± 129[a]
IL-6 (pg/ml)	80 ± 10	1500 ± 810[a]
IL-8 (pg/ml)	45 ± 25	408 ± 144[a]

Cells (1×10^4) were cultured for 24 h in medium with 0 or 10% FCS. LIF, IL-6 and IL-8 were measured by ELISA and are expressed as mean ± S.E.M. of four independent experiments.
[a] $P < 0.05$ as compared to 0% FCS (Mann–Whitney *U*-test).

3.3. *Effect of cytokines on the production of LIF, IL-6 and IL-8 by bone marrow stromal cells cultured in serum-free medium*

Since FCS contained factors that stimulated LIF, IL-6 and IL-8 secretion by bone marrow stromal cells, we focused on the serum-free medium condition for the remainder of the experiments. As shown in Fig. 4A, IL-1α and TNF-α stimulated, in a dose-dependent manner, LIF secretion by human bone marrow stromal cells. In contrast, IL-4 and IL-10 had no effect. As shown in Table 2, the stimulatory effects of IL-1α (100 ng/ml) and TNF-α (100 ng/ml) on LIF production were additive. As shown in Fig. 4B, IL-1α, TNF-α and IL-4 stimulated, in a dose-dependent manner, IL-6 secretion by bone marrow stromal cells. The highest IL-10 tested concentration (100 U/ml) had a weak enhanced effect on their IL-6 secretion. As reported in Table 2, the co-addition of

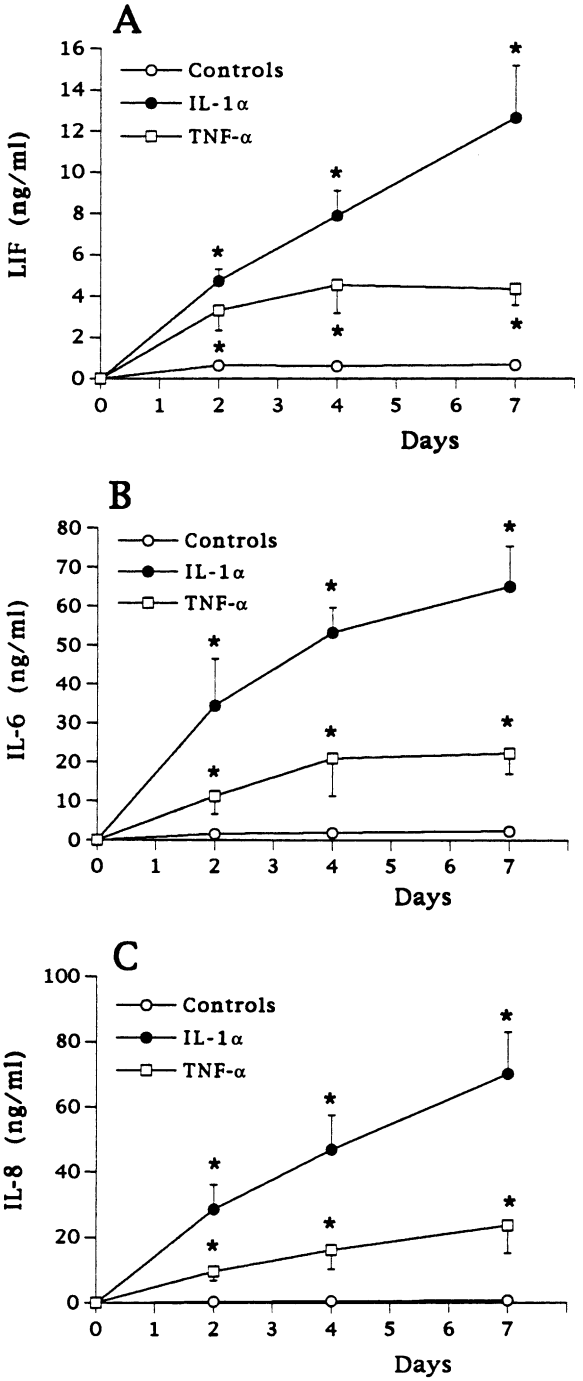

Fig. 2. Kinetics of LIF (A), IL-6 (B) and IL-8 (C) secretion by human bone marrow stromal cells in response to IL-1α and TNF-α. Cells (1×10^4) were grown in medium with 10% FCS in the absence (control) or the presence of IL-1α (10 ng/ml) or TNF-α (10 ng/ml). At various times, supernatants were harvested and assayed for LIF, IL-6 or IL-8. Mean ± S.E.M. of four experiments. *$P<0.05$ as compared to controls (Mann–Whitney *U*-test).

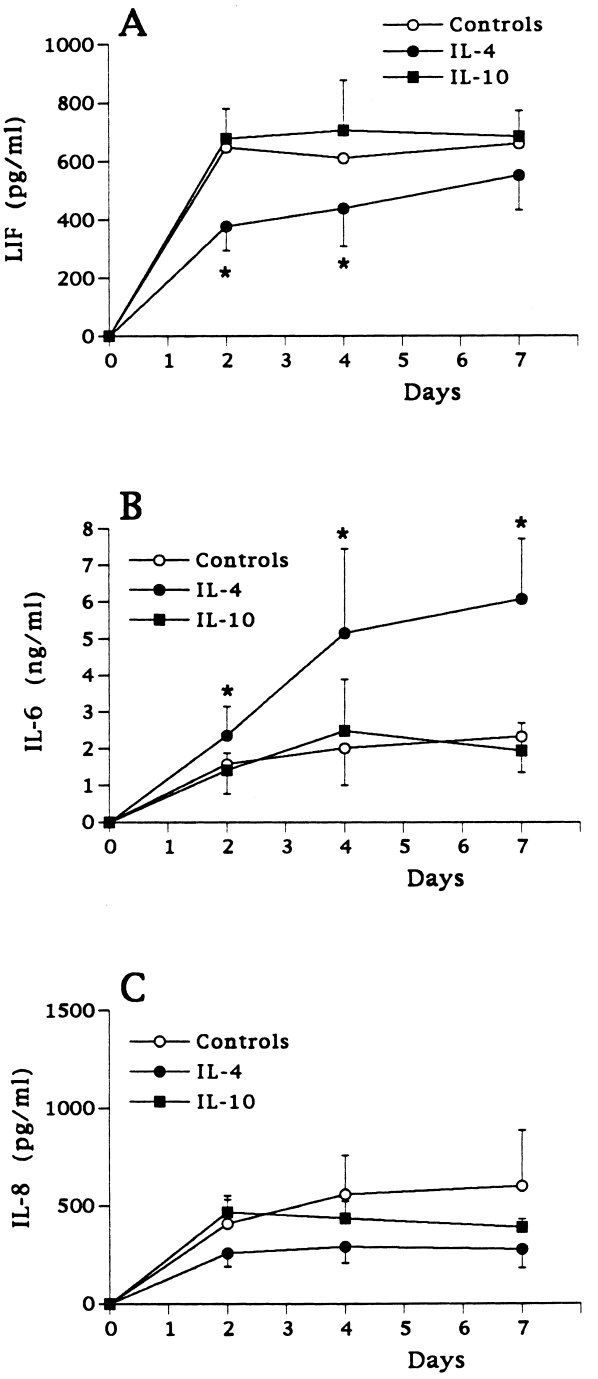

Fig. 3. Kinetics of LIF (A), IL-6 (B) and IL-8 (C) secretion human bone marrow stromal cells in response to IL-4 and IL-10. Cells (1×10^4) were grown in medium with 10% FCS in the absence (control) or the presence of IL-4 (10 ng/ml) or IL-10 (10 U/ml). At various times, supernatants were harvested and assayed for LIF, IL-6 or IL-8. Mean ± S.E.M. of four experiments. *$P<0.05$ as compared to controls (Mann–Whitney *U*-test).

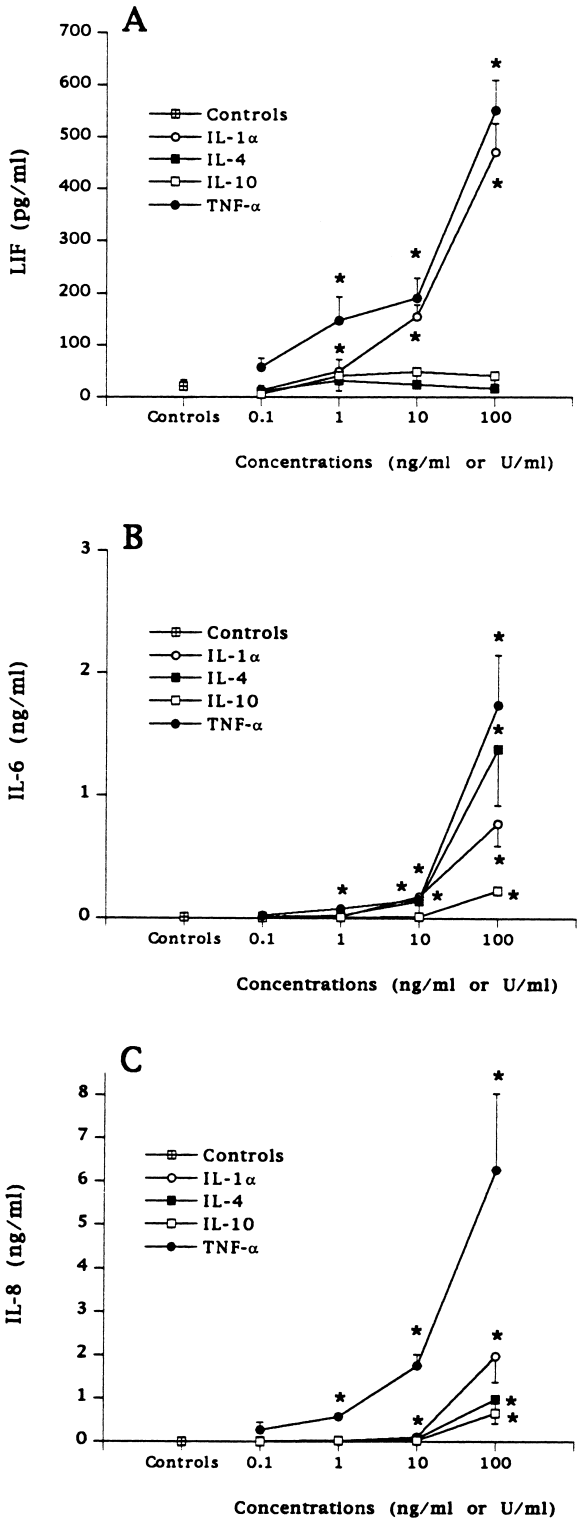

Fig. 4. Dose-dependent effect of IL-1α, TNF-α, IL-4 and IL-10 on LIF (A), IL-6 (B) and IL-8 (C) secretion by human bone marrow stromal cells. Cells (1×10^4) were stimulated for 24 h in serum-free medium. Mean ± S.E.M. of four experiments. *$P<0.05$ as compared to controls (Mann–Whitney *U*-test).

Table 2
Secretion of LIF, IL-6 and IL-8 by IL-1α- and TNF-α-stimulated human bone marrow stromal cells

	LIF (ng/ml)	IL-6 (ng/ml)	IL-8 (ng/ml)
Controls	0.06 ± 0.02	0.08 ± 0.01	0.02 ± 0.00
IL-1α	1.20 ± 0.13	2.68 ± 0.61	2.70 ± 0.90
TNF-α	0.69 ± 0.07	1.21 ± 0.15	4.70 ± 0.15
IL-1α+TNF-α	2.03 ± 0.5	14.25 ± 2.40[a]	20.36 ± 5.33[a]

Cells (1×10^4) were cultured for 24 h in serum-free medium in the absence or presence of IL-1α (100 ng/ml) and TNF-α (100 ng/ml) or a combination of IL-1α and TNF-α. LIF, IL-6 and IL-8 were measured by ELISA and are expressed as mean ± S.E.M. of three independent experiments.
[a] $P<0.05$ (Mann–Whitney *U*-test) as compared with the sum of the effect of IL-1α alone and TNF-α alone.

IL-1α (100 ng/ml) and TNF-α (100 ng/ml) gave a greater than additive increase for the induction of IL-6 ($P<0.05$, Mann–Whitney *U*-test) suggesting a synergistic effect. As shown in Fig. 4C, IL-1α and TNF-α stimulated IL-8 secretion in serum-free conditions. The highest IL-4 (100 ng/ml) and IL-10 (100 U/ml) tested concentrations had a weak enhanced effect on the IL-8 secretion. As reported in Table 2, the co-addition of IL-1α (100 ng/ml) and TNF-α (100 ng/ml) gave a greater than additive increase for the induction of IL-8 ($P<0.05$, Mann–Whitney *U*-test) suggesting a synergistic effect.

3.4. Effect of IL-4 and IL-10 on IL-1α- and TNF-α-induced production of LIF, IL-6 and IL-8 by bone marrow stromal cells

We next assessed the effects of various concentrations of IL-4 and IL-10 on the TNF-α- and IL-1α-induced LIF, IL-6 and IL-8 secretion by marrow stromal cells cultured in serum-free medium. As shown in Fig. 5A, IL-4 (10 ng/ml) significantly ($P<0.05$) reduced by 50% and 68% the LIF secretion by cells stimulated with TNF-α and IL-1α, respectively. In contrast, IL-4 (100 ng/ml) increased by 3.9-fold their IL-6 production in response to IL-1α (Fig. 5B). Finally, IL-4 (100 ng/ml) enhanced the IL-8 secretion by TNF-α and IL-1α-stimulated marrow stromal cells by 1.8- and 4.5-fold, respectively (Fig. 5C). In similar experimental conditions, IL-10 (1–100 U/ml) had no significant ($P>0.1$) effect on the LIF, IL-6 or IL-8 production by cytokine-stimu-

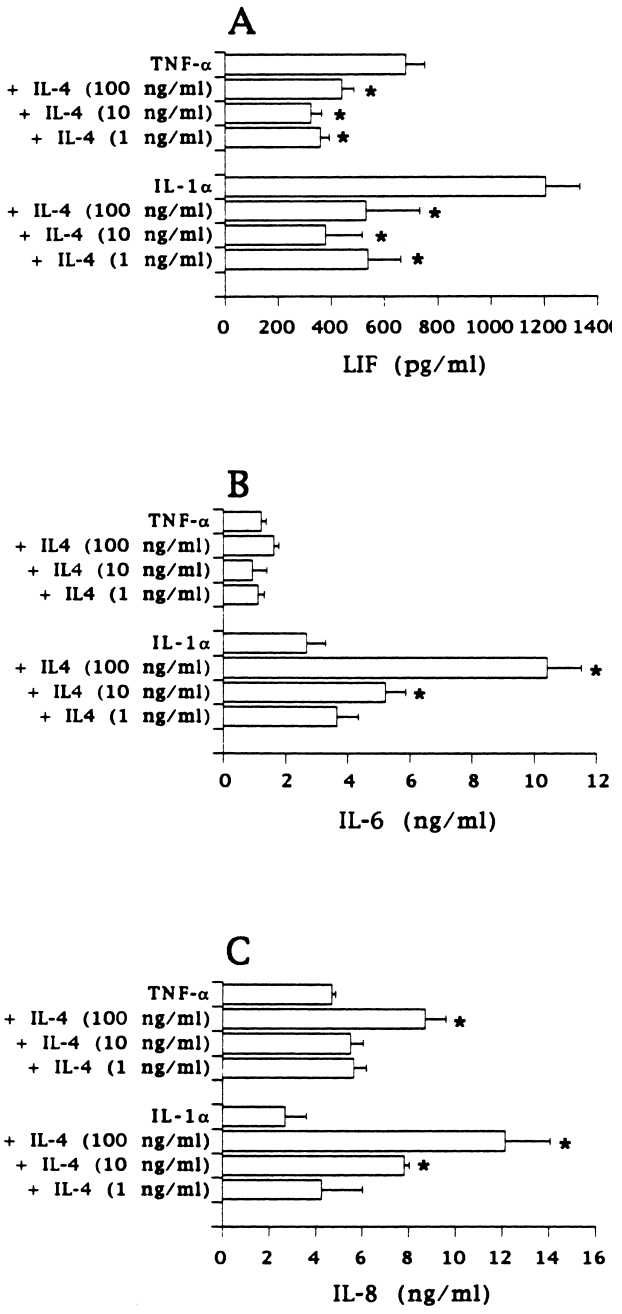

Fig. 5. Effects of IL-4 on TNF-α- or IL-1α-induced LIF (A), IL-6 (B) and IL-8 (C) secretion by human bone marrow stromal cells. Cells (1×10^4) were stimulated for 24 h in serum-free medium by TNF-α (100 ng/ml) or IL-1α (100 ng/ml) in the absence or presence of the indicated IL-4 concentrations. Mean ± S.E.M. of four experiments. $^*P<0.05$ as compared to controls (Mann–Whitney *U*-test).

lated human bone marrow stromal cells (data not shown).

3.5. *Presence of IL-4Rα and IL-10R transcripts in marrow stromal cells*

Because the lack of effect of IL-10 on bone marrow stromal cells might be related to the absence of IL-10R, we studied the expression of IL-10R mRNA in these cells. As shown in Fig. 6, RT-PCR experiments reveal the presence of IL-10R mRNA and IL-4Rα chain mRNA in human bone marrow stromal cells and in blood mononuclear cells used as a positive control.

4. Discussion

LIF, IL-6 and IL-8 are important regulators of marrow hematopoiesis [8–17]. While inflammatory cytokines stimulate human bone marrow stromal cells to produce LIF, IL-6 and IL-8 [3–5,28], no result has highlighted cytokines that reduce their synthesis. To further our understanding of the regulation of LIF, IL-6 and IL-8 synthesis in the bone marrow, we have investigated the effect of the two anti-inflammatory cytokines IL-4 and IL-10 on the serum- and cytokine-induced LIF, IL-6 and IL-8 production by human bone marrow stromal cells.

Human bone marrow stromal cells constitutively express LIF, IL-6 and IL-8 transcripts and enhanced their LIF, IL-6 and IL-8 secretions in response to serum and inflammatory cytokines, such as TNF-α and IL-1α. IL-4 markedly reduces their serum- and cytokine-induced LIF production. Our results, that agree with a previous study reporting that IL-4 decreases the LIF synthesis from long-term adherent layer cultures grown in serum [28], show that IL-4 acts on marrow fibroblast-like cells, the major component of the long-term bone marrow culture. In contrast to IL-4, IL-10 does not change their serum- and cytokine-induced LIF synthesis. This differential effect of IL-4 and IL-10 on LIF synthesis is similar to that found in isolated human synoviocytes (another local stromal cell population) [21].

IL-10 does not change the serum- and cytokine-induced IL-6 production by human bone marrow stromal cells. While IL-4 reduces marrow stromal

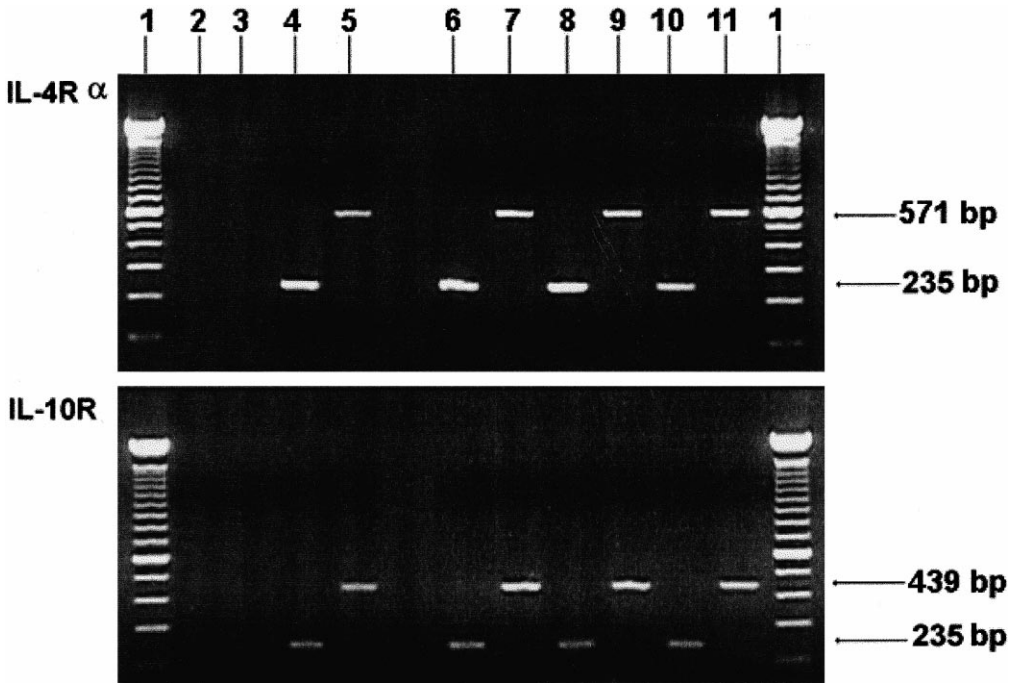

Fig. 6. IL-4Rα chain and IL-10R mRNA expression by human bone marrow stromal cells by RT-PCR. Lane 1, 100-bp DNA size ladder; lane 2, β-actin blank; lane 3, IL-4Rα (or IL-10R) blank; lane 4, β-actin from blood mononuclear cells; lane 5, IL-4Rα (or IL-10R) from blood mononuclear cells; lanes 6, 8 and 10, β-actin from three different marrow stromal cell cultures; lanes 7, 9 and 11, IL-4Rα (or IL-10R) from three different marrow stromal cell cultures. The size of the PCR products is indicated with arrows.

cell LIF synthesis, it markedly stimulates their IL-6 production in response to serum and IL-1α. This dichotomy between IL-6 and LIF synthesis in response to IL-4 was already reported for human synoviocytes [21]. Moreover, as for human bone marrow stromal cells, IL-4, but not IL-10, is found to stimulate human synoviocytes IL-6 secretion [29]. The similarities between bone marrow stromal cells and synoviocytes concerning the regulation of their LIF and IL-6 production in response to TNF-α, IL-1α, IL-4 and IL-10 might be related to their fibroblast-like cell status.

Similarly to that observed for LIF and IL-6 synthesis, IL-10 has no significant effect on IL-8 production by bone marrow stromal cells. This later result is consistent with the absence of IL-10 effect on the synthesis of IL-8 by pulmonary stromal cell populations [30], skin and synovial fibroblasts [31]. While IL-4 does not change the serum-induced secretion of IL-8 by marrow stromal cells, it stimulates their IL-8 synthesis in response to TNF-α and IL-1α. IL-4 is reported to stimulate IL-8 synthesis by endotoxin-stimulated endothelial cells [32]. However, opposite results have been found [33].

Cultured bone marrow stromal cells and marrow stromal cell lines express LIF, IL-6 and IL-8 mRNA [3–5,34,35]. In contrast, Cluitmans et al. [36] have documented that LIF and IL-8 mRNA are not present in bone marrow biopsy specimens from the iliac crest of normal healthy individuals suggesting that LIF and IL-8 are not expressed by bone marrow stromal cells in steady-state condition. The pattern of cytokine production from cultured bone marrow stromal cells and cells inside marrow biopsy specimens might be different due to new cellular interactions during the development of the bone marrow stromal cell layer in vitro. For example, data have reported that freshly isolated monocytes are rapidly induced to produce IL-8 mRNA and protein by adherence to plastic [37]. Alternatively, it might also be suggested that, in the study of Cluitmans et al. [36], the LIF and IL-8 mRNA levels in marrow biopsy specimens are below their detection limit. Clearly, further studies are required to ensure these discrep-

ancies and the molecular signals mediating the positive effect of IL-4 on IL-6 and IL-8 secretion by cultured bone marrow stromal cells and its negative effect on their LIF synthesis.

Human bone marrow stromal cells express IL-4Rα chain transcripts and IL-4 modulates their LIF, IL-6 and IL-8 secretions suggesting the presence of functional IL-4R on human bone marrow stromal cells. By contrast, despite the presence of IL-10R mRNA in human bone marrow stromal cells, IL-10 has no effect on their LIF, IL-6 and IL-8 secretion. It could, thus, be argued that the high sensitivity of the RT-PCR procedure might reveal levels of mRNA expression that could be physiologically irrelevant and that the presence of IL-10R mRNA does not implicate the production of a functional membrane IL-10R.

In summary, human bone marrow stromal cells produce LIF, IL-6 and IL-8 in response to serum or inflammatory cytokines. IL-4, but not IL-10, reduces their serum- and cytokine-induced LIF synthesis. Conversely, IL-4, but not IL-10 increases their IL-6 and IL-8 production. The differential regulation by IL-4 of two related cytokines, such as LIF and IL-6, highlights the tightly controlled regulation of cytokine productions within the human bone marrow. These results are the basis for future investigations examining the consequences of IL-4-mediated inhibition of LIF and stimulation of IL-6 and IL-8 expression on marrow hematopoiesis. We suggest that IL-4, by increasing bone marrow IL-6 and IL-8 concentrations, might lead to the selective recruitment of cells, such as monocytes/macrophages and T-lymphocytes, contributing to the progression and/or resolution of the inflammatory response and immune reaction.

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