RESEARCH COMMUNICATION

Interleukin 10 up-regulates elastin gene expression *in vivo* and *in vitro* at the transcriptional level

Sakari REITAMO, Anita REMITZ, Katsuto TAMAI, Isabel LEDO and Jouni Uitto*

Departments of Dermatology, and Biochemistry and Molecular Biology, Jefferson Medical College, and Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

In immune cells, such as T cells and monocytes, interleukin 10 (IL-10) has regulatory functions on a number of cytokines, including IL-1, IL-2, IL-8 and tumour necrosis factor- α expression. However, the effects of IL-10 have not previously been studied in detail in connective-tissue cells. In the present study, we show that recombinant human IL-10 at physiological concentrations has direct effects on the expression of the human elastin gene both *in vivo* and *in vitro*. Transgenic mice expressing a human elastin promoter/chloramphenicol acetyltransferase

(CAT) reporter gene construct were injected subcutaneously with IL-10 (1–100 ng) and the site of injection was biopsied after 24 h. CAT assay revealed an increase of up to 3.5-fold in the promoter activity with 10 ng of IL-10. Transforming growth factor- β 2 (TGF- β 2) is known to up-regulate elastin gene expression in cultured fibroblasts. When IL-10 was added to such cultures, the effects of TGF- β 2 on elastin mRNA levels were synergistically potentiated. These results suggest that IL-10 has an up-regulatory effect on elastin gene expression.

INTRODUCTION

Interleukin 10 (IL-10) was originally characterized as a factor secreted by activated mouse T helper (Th2) cell clones [1]. It was shown to inhibit synthesis of several cytokines by monocytes, and was therefore named cytokine-synthesis inhibitory factor [1]. IL-10 also suppresses the production of interferon- γ (IFN- γ) and other cytokines by Th1 cells [1,2], as well as antigen-specific proliferation of Th1 cells [3]. IL-10 is an acid-sensitive protein of 35–40 kDa [4] with structural features of a peptide segment deduced from an open reading frame in the Epstein–Barr virus genome, called BCRFI [2,5]. In man, IL-10 is produced by activated monocytes, in addition to T cells [6]. In contrast with mouse T cells, IL-10 is produced by various human T cell subsets, including Th0, Th1 and Th2 cell clones [7,8], as well as by other cell types.

The interactions of IL-10 with other cytokines have been studied in detail in human monocytes. For example, IL-10 is a powerful suppressor of the synthesis of IL-1 α , IL-1 β , IL-6, IL-8 and tumour necrosis factor- α (TNF- α), as well as granulocyte-macrophage and granulocyte colony-stimulating factors in activated monocytes [6,9]. Collectively, the results of these studies indicate that the effects of IL-10 in monocytes and T cells are predominantly down-regulatory.

Essentially nothing is known about the effect of IL-10 on the expression of extracellular-matrix genes. One of the predominant components of the extracellular matrix is elastin, the major component of elastic fibres, which provides elasticity and resilience to a variety of organs, including the lungs, blood vessels and the skin [10]. Previous studies have demonstrated that transforming growth factor- β (TGF- β) up-regulates elastin gene expression *in vitro*, as determined by assay of elastin mRNA steady-state levels, whereas TNF- α and IFN- γ counteract this up-regulation [11]. It was therefore decided to examine the effects of IL-10 on elastin gene expression in combination with TGF- β

and IFN- γ . For this purpose, the effects of recombinant human IL-10 on elastin gene expression were examined in dermal fibroblast cultures *in vitro*, as well as *in vivo* in transgenic mice which express a human elastin promoter/chloramphenicol acetyltransferase (CAT) reporter gene construct.

MATERIALS AND METHODS

Cell cultures

Adult human skin fibroblast cultures, established from tissue specimens obtained during cosmetic surgery, or neonatal foreskin fibroblast cultures were utilized in passages 4–10. The cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal-calf serum (FCS) pre-treated at 56 °C for 30 min, 2 mM glutamine, 50 μ g/ml streptomycin and 50 units/ml penicillin.

Preparations of IL-10, TGF- β 2 and IFN- γ

Human recombinant IL-10 was obtained from Pepro Tech Inc. (Princeton, NJ, U.S.A.) in freeze-dried form and dissolved first in distilled water, and subsequently diluted in DMEM. For cell cultures, IL-10 was used at a concentration of 10 ng/ml. For transgenic mice IL-10 was given subcutaneously in a total volume of 100 μ l containing 1–100 ng of IL-10. Recombinant human TGF- β 2 was kindly provided by Dr. David Olsen (Celtrix Laboratories, Santa Clara, CA, U.S.A.) and used for cell cultures in a final concentration of 10 ng/ml. IFN- γ was commercially obtained from Boehringer Mannheim (Indianapolis, IN, U.S.A.) and used for cell cultures in a final concentration of 1000 units/ml.

Transgenic mice expressing the human elastin promoter

A homozygous transgenic mouse line expressing the human elastin gene was developed by isolating a 5.2 kb fragment of the

Abbreviations used: IL-10, interleukin 10; IFN- γ , interferon- γ ; TGF- β , transforming growth factor- β ; TNF- α , tumour necrosis factor- α ; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal-calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

* To whom correspondence should be addressed.

5'-flanking DNA of the human elastin gene and ligating it to a CAT reporter gene, followed by the polyadenylation signal [12]. The linearized construct was injected into fertilized oocytes of FOB/N mice, and the eggs were implanted into pseudopregnant foster mothers [13]. To test the offspring (FO) for the presence of the human elastin promoter construct, Southern blotting was used as described previously [12,14]. Mating of heterozygous animals resulted in the establishment of a homozygous mouse line. The homozygous animals express the human elastin promoter in a time-specific and developmentally regulated manner. Specifically, the highest promoter activity was noted in the lungs and aorta, tissues rich in elastin, whereas lower levels of expression were noted in the skin. Fetal and newborn animals showed higher levels of promoter activity than in adult animals [12]. Thus these mice provide a model to study the regulation of elastin gene expression at the promoter level. The animals were injected subcutaneously with IL-10 (see above), and a full-thickness 6 mm skin biopsy was obtained from the site of injection after 24 h. CAT assay of the skin biopsy was performed as described below and elsewhere [14].

CAT assays on transgenic animals

CAT activity in the skin of the transgenic animals was determined as an indicator of the expression of the elastin promoter/CAT reporter gene. The tissue specimens were homogenized in Tris/HCl, pH 7.5, with a Polytron tissue homogenizer. The homogenates were then centrifuged at 10000 *g* for 15 min at 4 °C. Protein content of the supernatants was determined with a commercial protein assay kit (Bio-Rad, Richmond, CA, U.S.A.). Portions containing 100 µg of protein were used for CAT activity determination, which was performed with [¹⁴C]chloramphenicol as a substrate. The acetylated and non-acetylated forms of radioactive chloramphenicol were separated by t.l.c. [14]. The CAT activity was determined as the radioactivity in the acetylated forms as a percentage of the total radioactivity in the sample.

Northern analyses

Total RNA was isolated from confluent human fibroblast cultures incubated with or without IL-10 in medium supplemented with 1% FCS for time periods ranging from 1 to 24 h. Total RNA (12 µg/lane) was fractionated on 0.8% agarose gels containing 2.2 M formaldehyde, transferred to nylon filters (Zeta Probe, Bio-Rad), and immobilized by heating at 80 °C for 30 min under vacuum. The filters were prehybridized and hybridized in 50% formamide/0.25 M NaH₂PO₄ (pH 7.2)/0.25 M NaCl/7% (w/v) SDS/1 mM EDTA, by using cDNA probes labelled by nick translation with both [^α-³²P]dGTP and [^α-³²P]dCTP [15,16]. After hybridization at 42 °C for 18 h, the filters were washed in solutions with decreasing ionic strength and increasing temperature, and the final stringency of the washes was 0.1 × SSC (SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1% SDS, at 65 °C. The cDNA-mRNA hybrids were detected by autoradiography, and the corresponding steady-state levels of mRNAs were quantified by scanning densitometry. A 3.2 kb human elastin cDNA [17] and a 1.3 kb rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (pRGAPDH13) [18] were used sequentially for hybridizations.

RESULTS AND DISCUSSION

IL-10 up-regulates elastin gene expression *in vivo*

When transgenic mice expressing the human elastin promoter linked to CAT reporter gene were injected with different amounts

of IL-10, an increase in CAT activity of up to 3.5-fold (Figure 1), and on average 3-fold (Table 1), was seen with 10 ng/ml.

Previous studies on IL-10 have mainly concentrated on the expression and effects of IL-10 in various immunocompetent cells, such as T lymphocytes and monocytes. In the present study we showed that recombinant human IL-10 is capable of up-regulating elastin gene expression *in vivo*, suggesting a possible role for IL-10 in extracellular-matrix synthesis and breakdown.

IL-10 transiently up-regulates elastin mRNA steady-state levels *in vitro*

Human skin fibroblasts in culture have been previously shown to express the elastin gene, as determined at the mRNA level. To study the effects of IL-10 on elastin gene expression, human skin fibroblast cultures maintained in 1% FCS were incubated for up to 24 h in the presence of IL-10 at a concentration of 10 ng/ml. When different time points of incubation were studied, IL-10 showed an up-regulatory effect on elastin mRNA steady-state levels at 6 h of incubation, which was 2-fold compared with control cultures incubated without IL-10 (results not shown).

Figure 1 Enhancement of elastin promoter/CAT reporter gene activity in the skin of transgenic mice by IL-10

Transgenic mice (4 days old) were injected subcutaneously without or with 10 ng of IL-10; 24 h later, biopsy specimens of the skin were obtained from the injection site, and CAT activity was determined. An autoradiogram of the separation of the acetylated (AC) and non-acetylated (C) forms of [¹⁴C]chloramphenicol by t.l.c. is shown.

Table 1 Enhancement of elastin promoter/CAT reporter gene activity by IL-10 *in vivo*

Transgenic mice (4 days old) were injected subcutaneously with IL-10; 24 h later, biopsy specimens of the skin were obtained from the injection site, and CAT activity was determined by assay of acetylated forms of [¹⁴C]chloramphenicol as a percentage of total radioactivity, as shown in Figure 1.

Dose of IL-10 (ng)	No. of mice studied	CAT activity (percentage acetylation) (mean ± S.E.M.)	Relative CAT activity (% of control)
0	4	4.09 ± 0.27	100
0.1	2	4.43 ± 0.27	108
1	2	7.31 ± 4.58	178
10	4	12.07 ± 2.79	295
100	4	5.66 ± 1.02	138

Figure 2 Effects of TGF- β 2 and IFN- γ on IL-10-induced changes in elastin gene expression

TGF- β 2 at 5 ng/ml and IL-10 at 10 ng/ml were added consecutively to confluent fibroblast cell strains in medium containing 1% FCS. Total RNA (12 μ g/lane) was extracted after 24 h and analysed by Northern hybridizations with cDNA probes for elastin and GAPDH. Quantification of elastin mRNA levels by scanning densitometry indicated an approx. 2-fold increase by TGF- β , after correction for GAPDH mRNA levels.

TGF- β synergistically potentiates IL-10 effects *in vitro*

When dermal skin fibroblasts were incubated with IL-10 (10 ng/ml), together with TGF- β 2 (10 ng/ml) or IFN- γ (1000 units/ml) for 24 h, TGF- β 2 alone clearly up-regulated elastin gene expression, as shown previously [11] (Figure 2). An approximately 2-fold increase in elastin mRNA levels was seen when IL-10 was added to such cultures, based on scanning laser densitometry (Figure 2). In contrast, IL-10 had no effect on the down-regulation of elastin mRNA steady-state levels caused by IFN- γ .

At present it is not clear whether there are cells in the skin which are capable of IL-10 synthesis and secretion. As shown recently by Enk and Katz [19], murine keratinocytes secrete IL-10 when appropriately stimulated. The proximity of keratinocytes to dermal fibroblasts could result in induction of elastin by keratinocyte IL-10. Rivas and Ullrich have shown that murine keratinocytes secrete IL-10 after exposure to u.v. radiation; u.v. exposure has been shown to result in impairment of delayed-type hypersensitivity, which has been shown to be mediated, at least in part, by IL-10 [20]. Chronic exposure to u.v. also leads to changes in the composition of the matrix molecules, namely to a decrease in the amount of collagen and an increase in the amount of elastic fibres in the dermis [21,22]. The mechanisms of the u.v.-induced changes in the matrix are poorly understood. The present studies suggest that IL-10 could play a role in the pathogenesis of solar elastosis.

Both TGF- β and IL-10 have a broad spectrum of functions and have both inhibitory and stimulatory effects on diverse cells. On inflammatory murine macrophages TGF- β and IL-10 have a predominantly deactivating effect, but they suppress macrophage

cytokine production under different conditions and by different mechanisms [23]. Previous studies have shown that TGF- β up-regulates elastin gene expression in pig smooth-muscle cells [24] and in human skin fibroblasts [11]. In the present study, IL-10 enhanced the up-regulatory effect of TGF- β on elastin gene expression in human dermal fibroblasts, but it did not alter the IFN- γ -elicited down-regulation of the elastin gene expression. In monocytes, IFN- γ and IL-10 antagonize each other's function [25]. The differences in the antagonistic effects of IL-10 and IFN- γ on different cell types may reflect differential signal-transduction pathways, or may be due to different *cis*-elements in the target genes.

This work was supported by U.S. Public Health Service, National Institutes of Health grants R01-AR28450 and T32-AR07561, The Academy of Finland and Finska Läkaresällskapet. S.R. was recipient of a Fellowship from the Jefferson Center for International Dermatology at Jefferson Medical College.

REFERENCES

- Fiorentino, D. F., Bond, M. W. and Mosmann, T. R. (1989) *J. Exp. Med.* **170**, 2081–2095
- Viera, P., de Waal-Malefyt, R., Dang, M.-N., Johnson, K. E., Kastelein, R., Fiorentino, D. F., de Vries, J. E., Roncarolo, M.-G., Mosmann, T. R. and Moore, K. W. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1172–1176
- de Waal-Malefyt, R., Haanen, J., Spits, H., Roncarolo, M.-G., te Velde, A., Figdor, C., Johnson, K., Kastelein, R., Yssel, H. and de Vries, J. E. (1991) *J. Exp. Med.* **174**, 915–924
- Howard, M. and O'Garra, A. (1992) *Immunol. Today* **13**, 198–200
- Moore, K. W., Viera, P., Fiorentino, D. F., Trounstein, M. L., Khan, T. A. and Mosmann, T. R. (1990) *Science* **248**, 1230–1234
- de Waal-Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G. and de Vries, J. E. (1991) *J. Exp. Med.* **174**, 1209–1220
- Yssel, H., de Waal-Malefyt, R., Roncarolo, M.-G., Abrams, J. S., Lahesmaa, R., Spits, H. and de Vries, J. E. (1992) *J. Immunol.* **149**, 2378–2384
- Del Prete, G., de Carli, M., Almerigogna, F., Giudizi, M. G., Biagiotti, R. and Romagnani, S. (1993) *J. Immunol.* **150**, 353–360
- Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W. and O'Garra, A. (1991) *J. Immunol.* **146**, 3444–3451
- Rosenbloom, J., Bashir, M., Kähäri, V.-M. and Uitto, J. (1991) *Crit. Rev. Eukaryotic Gene Expression* **1**, 145–156
- Kähäri, V.-M., Olsen, D. R., Rhudy, R. W., Carrillo, P., Chen, Y. Q. and Uitto, J. (1992) *Lab. Invest.* **66**, 580–588
- Hsu-Wong, S., Katchman, S., Ledo, I., Wu, M., Khillan, J., Bashir, M. M., Rosenbloom, J. and Uitto, J. (1994) *J. Biol. Chem.* **269**, 18072–18075
- Hogan, B., Costantini, F. and Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratories Press, Cold Spring Harbor, NY
- Kähäri, V.-M., Fazio, M. J., Chen, Y. Q., Bashir, M. M., Rosenbloom, J. and Uitto, J. (1990) *J. Biol. Chem.* **265**, 9485–9490
- Thomas, P. S. (1980) *Proc. Nat. Acad. Sci. U.S.A.* **77**, 5201–5205
- Fazio, M. J., Olsen, D. R., Kauh, E. A., Baldwin, C., Indik, Z., Ornstein-Goldstein, N., Yeh, H., Rosenbloom, J. and Uitto, J. (1988) *J. Invest. Dermatol.* **91**, 458–464
- Fort, P., Marty, L., Piechaczyk, M., El Sabrouy, S., Danz, C., Jeanteur, P. and Blanchard, J. M. (1985) *Nucleic Acids Res.* **13**, 1431–1442
- Enk, A. H. and Katz, S. I. (1992) *J. Immunol.* **149**, 92–95
- Rivas, J. M. and Ullrich, S. E. (1992) *J. Immunol.* **149**, 3865–3871
- Mitchell, R. E. (1967) *J. Invest. Dermatol.* **43**, 203–230
- Bernstein, E. F., Chen, Y. Q., Tamai, K., Shepley, K. J., Resnik, K. S., Zhang, H., Tuan, R., Mauviel, A. and Uitto, J. (1994) *J. Invest. Dermatol.* **103**, 182–186
- Bogdan, C., Paik, J., Vodovotz, Y. and Nathan, C. (1992) *J. Biol. Chem.* **267**, 23301–23308
- Liu, J. and Davidson, J. M. (1989) *Biochem. Biophys. Res. Commun.* **154**, 895–901
- Chomarat, P., Rissoan, M.-C., Banchereau, J. and Miossec, P. (1993) *J. Exp. Med.* **177**, 523–527