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Regulation of Collagen Expression by Interleukin-1β Is Dependent on Donor Age

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Cultured skin fibroblasts derived from old (>60 years) donors differ in various morphological and functional aspects from cells obtained from young (<20 years) donors. We were interested in whether fibroblasts obtained from old and young donors differ in their cellular response to interleukin-1β, a cytokine which has been shown to affect the synthesis of extracellular matrix proteins in human dermal fibroblasts. Therefore the expression of interstitial collagen (type I), minor collagen (type VI) and interstitial collagenase genes was investigated, using fibroblasts derived from either old or young donors.

Fibroblasts were incubated in the absence or presence of interleukin-1β (5 units/ml, 50 units/ml, 500 units/ml) for 48 h. Total RNA was isolated and mRNA levels were determined by Northern and dot blot analysis. Comparing the synthetic response of fibroblasts obtained from young and old donors, we observed a marked increase of the inhibition of type I and type VI collagen expression and the stimulation of interstitial collagenase in the aged fibroblasts. These results suggest that physiological ageing in human fibroblasts is associated with an altered responsiveness to interleukin-1β. *Key words: ageing; fibroblasts; collagen type I; collagen type VI.*

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Characteristic alterations of the ageing skin, like wrinkling, loss of elasticity and atrophy, are mainly caused by metabolic, morphological and functional changes occurring in the dermis (1). Furthermore, in ageing skin the remodelling of the extracellular matrix, as it takes place e.g. during wound healing, appears to be altered (2). When skin fibroblasts are investigated in vitro, various morphological and functional differences can be observed when fibroblasts from old donors are compared to cells obtained from young donors (3, 4). Skin fibroblasts cultured from old donors display a reduced proliferation rate (5), a reduced chemotactic activity (6) and a decreased capacity to contract collagen gels (7).

Cytokines and growth factors play an important role in the metabolism of the extracellular, taking part in the control of the coordinated synthesis of new matrix components as well as its degradation. Several investigators could demonstrate that cytokines and growth factors are involved in the altered metabolism of ageing fibroblasts. They describe a decreased responsiveness of fibroblasts to growth factors like platelet-derived-growth-factor (PDGF), epidermal growth factor (EGF) and insulin (8), as well as an increased cytokine synthesis of interleukin-1β (Il-1β), interleukin-6 and tumour-necrosis-factor-α (TNF-α) in mononuclear cells of healthy elderly people (9–11).

We were interested in studying whether fibroblasts derived from old and young donors differ also in their expression of extracellular matrix proteins when exposed to cytokines. IL-1β is a cytokine that is produced by monocytes, macrophages and also by nonphagocytic cells such as fibroblasts and keratinocytes (12). Recently it was demonstrated that hr-IL-1β modulates the synthesis of extracellular matrix proteins in human dermal fibroblasts. Several investigators reported that IL-1β can exert a positive but also negative regulatory effect on α_1(I) collagen expression and protein synthesis (13–15). These contradicting results could be explained by the different concentrations of IL-1β and the various culture conditions used in these studies (16).

In our study we therefore investigated the effect of various concentrations of IL-1β (5 units/ml, 50 units/ml, 500 units/ml) under different culture conditions on the expression of extracellular matrix proteins in fibroblasts explanted from the skin of young donors, compared to fibroblasts obtained from old donors. The expression of the most abundant interstitial collagen in the skin, type I collagen, and the expression of the α_1-and α_3-chains of the minor type VI collagen were determined by Northern and dot blot analysis. As IL-1β had repeatedly been shown to act as a potent enhancer of the expression of interstitial collagenase (17), we used the induction of mRNA for the enzyme as a control for the biological activity of IL-1β, whereas hybridization with GAPDH was carried out as an internal control.

MATERIAL AND METHODS

Cell culture conditions

Human skin fibroblasts obtained from 5 healthy young (<15 years old) and 5 old (>60 years) donors were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with sodium ascorbate (50 μg/ml), glutamine (300 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml) and 10% fetal calf serum (FCS, Gibco) at 37°C in the moist atmosphere of 5% CO_2. The experiments were carried out with cells in passages 5 to 8. Confluent fibroblast monolayer cultures were incubated with various concentrations of hr-IL-1β (H. Biermann GmbH) (5 units/ml, 50 units/ml, 500 units/ml) in DMEM, supplemented with 1% or 10% FCS for 48 h. As control, fibroblast monolayer cultures obtained from the same donor were cultured under identical conditions in the absence of IL-1β.

RNA isolation, Northern and dot blot analysis

Total RNA was isolated by ultracentrifugation using the GTC/CsCl method (18). Purified RNA was directly applied to Gene Screen membranes in serial concentrations (0.25–2 μg) using a dot blot apparatus, or transferred to Gene Screen following electrophoretic separation in denaturing agarose gels (19). RNA was immobilized by UV cross linking (Stratagene, 1,200 kJ). Prehybridization and hybridization with ^{32}p-labeled cDNA probes specific for the mRNA of α_1(I) procollagen, the α_1- and α_3-chains of type VI collagen, interstitial collagenase and interleukin-1 receptor type I (IL-1 Rt I) were performed in 50% formamide, 5 × SSC, 5 Denhardt's and 100 μg/ml herring sperm DNA at 42°C. The cDNA was labelled using a multiprime labelling kit (Amersham-Buchler) and ^{32}P-dATP (spec.

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act. 3000 Ci/mMol), according to the supplier's protocol. Hybridization with cDNA complementary to glyceraldehyde-phosphate-dehydrogenase (GAPDH) was used as an internal control. Signal intensity was quantified by densitometry and calculated relative to the signals obtained for GAPDH.

cDNA clones

The following human sequence specific cDNA clones were used for RNA hybridization: a 1.4 kb cDNA (Hf 677) for α_1 (I) procollagen (20); a 1.8 kb cDNA (P 18) for α_1-chain of type VI collagen (21); a 1.8 kb cDNA (P 24) for α_3-chain of type VI collagen (21); a 2 kb cDNA (PX 7) for interstitial collagenase I (22); a 2.2 kb cDNA for IL-1-RT I (23) and a 1.3 kb cDNA for rat-glyceraldehyde-3-phosphate-dehydrogenase (24).

Statistics

Rates of collagen mRNA expression were expressed as the percentage of the value for the untreated control group. Statistical differences were determined using the unpaired-samples Student's *t*-test. $p<0.05$ was taken to be significant.

RESULTS

In order to investigate the influence of Il-1β on the expression of extracellular matrix proteins in aged fibroblasts, we first determined the effect of exposure time of IL-1β on the expression of extracellular matrix proteins. Treatment of human dermal skin fibroblasts with 50 units/ml hr-IL-1β for 24 and 48 h resulted in a biphasic alteration of the expression of α_1 (I) procollagen mRNA and a time-dependent decrease of α_1- and α_3-chains of type VI collagen. Compared to untreated cells, the expression of α_1(I) procollagen mRNA increases after 24 h by about 33%, whereas after 48 h the expression of α_1(I) procollagen gene is reduced (inhibition 21%). In contrast, after incubation with IL-1β the expression of the α_1- and α_3-chain of type VI collagen shows a distinct time-dependent decrease (see Fig. 1). Therefore we chose the incubation time of 48 h, when comparing the effect of IL-1β on the expression of extracellular matrix proteins by human dermal fibroblast obtained from young and old donors.

Treatment of human dermal fibroblasts with increasing concentrations of hr-IL-1β resulted in a dose-dependent inhibition of type I procollagen and α_1-and α_3-chains of type VI collagen in DMEM with 10% FCS, as analyzed by quantitative densitometry of autoradiographs from Northern and dot blot analysis (Table I). However, a less marked effect is seen after incubation of the same cells with increasing concentrations of hr-IL-1β in DMEM with 1% FCS (data not shown).

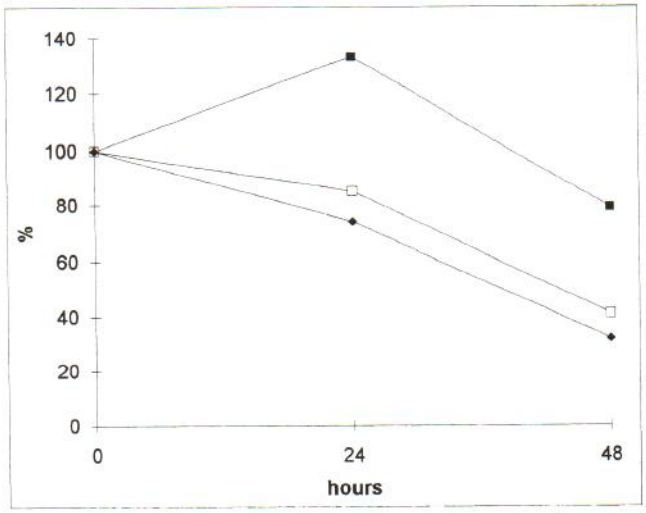

Fig. 1. Time-dependent effect of IL-1β on the expression of α_1(I) procollagen mRNA (*black square*), α_1(VI)-chain of type VI collagen (*white square*) and α_3-chain of type VI collagen (*black rhomb*). Following autoradiography the relative amounts of specific mRNAs were quantified by densitometric scanning ±SD of 3 parallel experiments of one donor.

Compared to untreated cells, high concentrations of hr-IL-1β (500 units/ml) reduced the expression of procollagen type I in old donors by about 70%, whereas the decrease in fibroblasts derived from young donors was only 23% (Table I and Figs. 2 and 3). A typical Northern blot analysis of type I collagen expression is shown in Fig. 2. This difference is even more pronounced when the levels of type I collagen expression are calculated relative to the levels obtained in young fibroblasts (Fig. 3). The expression of α_1 type VI collagen mRNA was inhibited by increasing concentrations of hr-IL-1β. This inhibition was most marked in fibroblasts incubated with 500 units/ml hr-IL-1β, revealing a more pronounced response in cells obtained from old donors compared to young donors (to 43% and to 76% of the control, respectively) (Table I). Similar effects are shown in the expression of α_3 chain of type VI collagen. Increasing concentrations of hr-IL-1β decrease the expression of the α_3 chain of type VI collagen mRNA. This inhibition was more pronounced in cells obtained from old donors (to 23% and to 43% of the control, respectively) (Table II). In all experiments (DMEM with 10% and 1% FCS) hr-IL-1β induced a strong increase of the expression

Table I. *Influence of hr-IL-1β on mRNA steady-state levels of α(I) procollagen, α_1 and α_3 chains collagen (VI) in human dermal fibroblasts obtained from young and old donors, incubated for 48 h in increasing concentrations*

Following autoradiography, the relative amounts of specific mRNAs were quantified by densitometric scanning ±SD of 3 parallel determinations. Values are expressed as percentage of the control without hr-IL-1β. They present the average of two (5 units/ml and 50 units/ml) and five (0 units/ml and 500 units/ml) donor pairs (i.e. young and old). NS = not significant.

Concentration of hr-IL-1β units/ml	α_1(I) procollagen			α_1(VI) collagen			α_3(VI) collagen		
	young	old	*p*	young	old	*p*	young	old	*p*
0	100	100		100	100		100	100	
5	101±2,3	110±0,5	NS	90,5±8,2	84,8±10,2	NS	79,3±7,3	65,2±12,5	NS
50	83,7±2,7	59,3±2,7	<0,02	58,7±8,7	52,2±8,8	NS	51,5±4,5	55,7±8,7	NS
500	76,7±7,7	30,6±13,1	<0,001	76,2±17,3	42,6±12,7	<0,009	43,1±3,6	23,3±4,7	<0,001

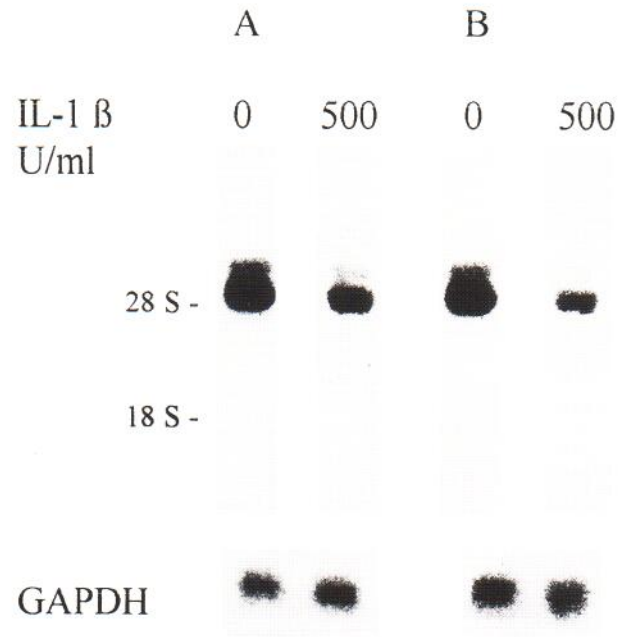

Fig. 2. Northern blot analysis of RNA isolated from skin fibroblasts obtained from young (A) and old (B) donors treated with or without 500 units/ml Il-1β. Two micrograms of total RNA per panel was electrophoresed on a 1% agarose gel and transferred. The filter was hybridized to a radiolabelled human cDNA specific for α_1 (I) procollagen and processed by autoradiography. GAPDH levels served as a standard.

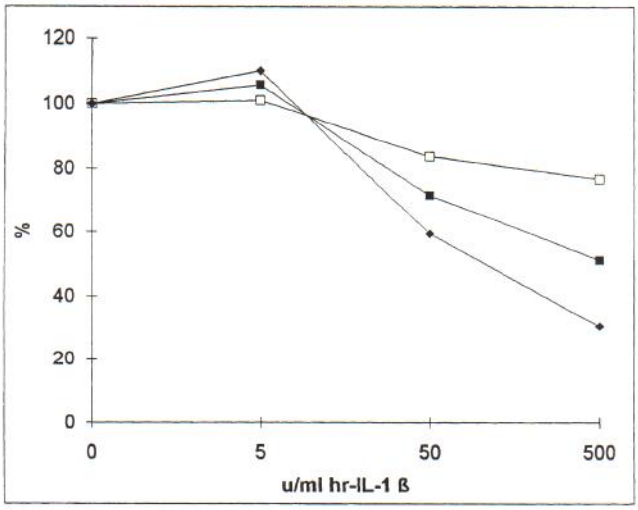

Fig. 3. Quantification of α_1(I) procollagen mRNA after incubation with increasing concentrations of hr-IL-1β. Confluent human skin fibroblasts derived from young (*white square*) and old (*black rhomb*) donors were incubated for 48 h with increasing concentrations of hr-IL-1β. One line (*black square*) shows the expression of total mRNA in all samples studied (i.e. young and old).

of interstitial collagenase mRNA. Treatment of fibroblasts with 500 units/ml hr-IL-1β in DMEM with 10% FCS resulted in a 2.5-fold higher increase of collagenase expression in cells obtained from old donors compared to young donors (Table II).

In order to investigate the mechanism of the differential regulation of extracellular matrix protein expression in human dermal fibroblasts obtained from young and old donors, we examined the steady-state level of IL-1 receptor I mRNA by Northern blot hybridization. Treatment of human dermal fibroblasts with increasing concentrations of IL-1β (5 units/ml, 50 units/ml, 500 units/ml) resulted in a decreasing expression of IL-1 Rt I, as previously described (data not shown). No significant differences were observed in fibroblasts obtained from young and old donors.

DISCUSSION

Skin ageing is characterized by specific changes as a loss of elasticity and a decrease in dermal thickness reflecting an altered metabolism of the connective tissue (1). Previous studies could demonstrate that in vitro this correlates with a decrease in size (3, 4), a decrease in proliferation rate (5) and a decrease in the synthesis of several extracellular matrix proteins of human dermal fibroblasts (25, 26).

Several studies indicate that cytokines play a major role in the regulation of connective tissue metabolism. A pleiotropic cytokine having a profound influence on the regulation of connective tissue metabolism is Il-1β (12), which is a potent inducer of interstitial collagenase (17), whereas an induction as well as an inhibition of collagen synthesis by this cytokine (13–15) has been described. Recent studies could demonstrate that cytokines, in particular Il-1 and Il-6, may play a role in ageing. Therefore we investigated whether the altered biosynthetic behaviour of fibroblasts obtained from old donors is accompanied by an altered responsiveness to Il-1β. The expression of the most important interstitial collagen, type I collagen, the minor collagen type VI, and the interstitial collagenase was therefore analysed at different incubation periods and Il-1β concentrations. In this study fibroblasts derived from several donors of different age were investigated ('in vivo ageing'). It has the advantage of making it possible to control the interindividual variability of the parameters studied.

This study confirms previous results reported by Mauviel et al. (14) showing a biphasic response of type I collagen expression to Il-1β with an increase after 24 h and a decrease after 48 h. This first study investigating the influence of Il-1β on the expression of type VI collagen, however, fails to show a biphasic response of type VI collagen expression to Il-1β. Our results indicate a clear time and dose dependent inhibition of type VI collagen expression by Il-1 β in dermal fibroblasts. Comparing the synthetic response of fibroblasts obtained from young and old donors, a marked increase of the inhibition of type I and type VI collagen expression and the stimulation of interstitial collagenase in the fibroblasts obtained from the old donors is observed. This difference was most pronounced at 10% FCS and the higher concentrations of Il-1β used.

A possible explanation for the altered responsiveness of the aged fibroblasts might be a differential regulation of the Il-1β receptor. On the surface of fibroblasts, as on keratinocytes, endothelial cells or T-cells, the Il-1 type I receptor is expressed (12). We therefore tested whether the increased responsiveness of the fibroblasts obtained from the old donors could be due to an increased expression of this receptor. However, on the transcriptional level no difference was found in the expression of Il-1 type I receptor. Recently it was reported that even an occupation of 5% of the receptors induces intracellular signalling (27), indicating that the sensitivity of a cell to Il-1β presumably is not regulated on the transcriptional level.

Table II. *Quantification of interstitial collagenase mRNA in human dermal fibroblasts obtained from young and old donors, incubated for 48 h with increasing concentrations of hr-IL-1β in DMEU with 1% and 10% FCS*

Specific mRNAs were quantified by densitometric scanning ±SD of 3 parallel determinations. Values are expressed as percentage of the control without hr-IL-1β. They present the average of two donor pairs (i.e. young and old). NS = not significant.

Concentration of hr-IL-1β units/ml	Collagenase in DMEM with 1% FCS			Collagenase in DMEM with 10% FCS		
	Young	Old	*p*	Young	Old	*p*
0	100	100		100	100	
5	831 ± 593	1775 ± 510	NS	nd	nd	
50	2276 ± 387	2269 ± 17	NS	nd	nd	
500	3203 ± 186	6616 ± 431	NS	1644 ± 343	4181 ± 776	0.02

However, glycosylation of the Il-1 receptor induces enhanced binding of Il-1 (12, 28), which could explain the increased responsiveness of the aged fibroblasts to Il-1.

In summary, these results underline the importance of cell donor characteristics, i.e. age, when studying primary cells and their response to cytokines in vitro. Furthermore it is suggested that physiological ageing in human fibroblasts, in addition to the previously reported changes in synthesis and proliferation, is also associated with an altered responsiveness to cytokines, i.e. Il-1β.

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