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Interleukin 1 modulates collagen accumulation by rat granulation tissue cells both in vivo and in vitro

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Summary. In in vivo studies 0.5 U human interleukin 1 (IL-1) was inoculated daily into a subcutaneously implanted viscose cellulose sponge. IL-1 significantly decreased the dry weight (7.8%) and the hydroxyproline content (24.2%) of granulomas. When the cultured rat granulation tissue cells were exposed to IL-1 (0.5–2.0 U/ml) their collagen production decreased to 80% of that in controls. No effect on cell proliferation was detected.
Key words. Interleukin 1; granulation tissue.

Interleukin 1 (IL-1) is a 17.5 kDa polypeptide factor. It has been suggested that it regulates connective tissue breakdown in chronic inflammation. In cell culture conditions, IL-1 is known to stimulate prostaglandin E_2 (PGE_2) [1], collagenase [2] and plasminogen activator synthesis [3] by fibroblasts and it also enhances the synthesis of enzymes degrading proteoglycan complexes [4] and extracellular fibronectin [5]. Recent studies have suggested that IL-1 may induce matrix reformation by increasing the synthesis of fibronectin [6] and hyaluronic acid [7]. The studies on collagen synthesis suggest that experimental procedure, and the origin of the cell line, affect the results. IL-1 seems to stimulate collagen production in skin fibroblasts by elevating collagen mRNA levels [8, 9]. Especially in synovial cells and chondrocytes IL-1 may decrease collagen synthesis [8, 10, 11]. This may by due to indirect effects mediated via PGE_2. IL-1 has also been reported to induce cellular proliferation in skin fibroblast and synovial cell cultures [12, 13]. In the present study, the effects of IL-1 were tested both in cell cultures and in vivo in the viscose cellulose sponge model of experimental granulation tissue. We have previously shown that the same model is suitable to test the in vivo effects of human epidermal growth factor (EGF) [14–16].

Material and methods. A standardized experimental wound model described by Niinikoski, Heughan and Hunt was used [17]. Cylindrical viscose cellulose sponges (Säteri Oy, Valkeakoski, Finland) were implanted under the skin in the dorsal midline of Male Sprague-Dawley rats. Each rat received one sponge cylinder. Altogether 12 rats were studied in two groups of 6 animals. In the control group the implants were treated immediately after implantation by injecting 0.05 ml of 0.9% saline solution into the central tunnel of the implant. The implant of the test group was injected correspondingly with 0.05 ml saline solution containing 0.5 U of IL-1. Ultrapure human IL-1 purified from glass-adhered human monocytes was obtained from Genzyme Corporation (Boston, MA, USA). Injections of the two groups were repeated daily under strictly aseptic conditions. Seven days postoperatively rats were sacrificed, and the implants were dissected free from the surrounding tissue. Bacteriological examinations of wound fluid were performed in both groups at the end of the experiments and no infections were observed. Nucleic acids were extracted from the implants according to the method of Schmidt and Thannhauser [18]. DNA was determined by the diphenylamine reaction [19] and RNA was assayed as RNA-ribose by the method of Ceriotti [20]. Aliquots were taken for the determination of nitrogen [21], hydroxyproline [22], hexosamines [23], and uronic acids [24].

Granulation tissue cells were isolated from experimental granulomas induced in adult rats. The cells were detached from the cellulose sponge matrix slices by digestion with collagenase and trypsin [25] and then cultured in Dulbecco's modification of Eagle's minimum essential medium supplemented with 10% fetal calf serum [26].

The rate of collagen synthesis was measured using [^{3}H]proline (Amersham) as the precursor. Confluent cultures were first preincubated for 24 h in the presence of 1.0 U/ml IL-1 and ascorbic acid (50 µg/ml). After preincubation fresh ascorbic acid and IL-1 were added with the isotope and the hydroxyproline bound radioactivity was measured after 24 h [27].

In the cell proliferation assays the number of cells in culture dishes was counted with a Bürker's hemocytometer, after the cells had been detached by trypsin treatment.

Results. Data for the effects of IL-1 on various wound healing parameters in experimental granulation tissue are shown in the table. After daily application of 0.5 U of IL-1, statistically significant reductions were observed in the try weight of the sponge (– 7.8%) and hydroxyproline (– 24.2%). Considering the mean amounts of DNA, RNA and protein, measured as total nitrogen, no significant reductions were observed between the groups. The mean amounts of hexosamines and uronic acids, reflecting the amounts of glycosaminoglycans, were almost similar in both groups.

The collagen production in cultured granulation tissue cells exposed to IL-1 was about 80% of that in controls (fig. 1). In the presence of 14 µM indomethacin, IL-1 had no effect on the rate of collagen synthesis (fig. 1).

IL-1 had no effect either on the early or on the late proliferation of granulation tissue cells when this was measured as a change in the number of cells (fig. 2).

Effects of interleukin 1 on experimental granulation tissue in rats 7 days after operation

Group	Dry weight (mg)	DNA (mg)	RNA-ribose (mg)	RNA-ribose/DNA (mg)	Nitrogen (mg)	Hydroxy-proline (mg)	Hexos-amines (mg)	Uronic acids (mg)
Control	509 ± 12	6.0 ± 0.5	0.9 ± 0.1	0.16 ± 0.01	29.6 ± 2.0	3.3 ± 0.3	2.3 ± 0.1	2.3 ± 0.1
IL-1	469 ± 8**	5.2 ± 0.2	0.8 ± 0.1	0.15 ± 0.01	26.7 ± 1.4	2.5 ± 0.2*	2.2 ± 0.1	2.3 ± 0.1

Means ± SEM are indicated; each group consisted of six rats. * $p < 0.05$; ** $p < 0.01$, Wilcoxon Rank-sum test for two groups.

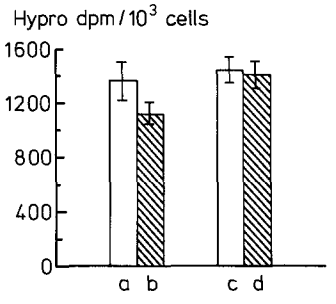

Figure 1. The rate of collagen production by granulation tissue fibroblasts in culture. Cells were preincubated with 1.0 U/ml IL-1 and 50 µg/ml ascorbic acid for 24 h and then labeled with 10 µCi/ml [^{3}H]proline in the presence of fresh IL-1 and ascorbic acid. *a* Control culture; *b* IL-1 treated culture; *c* control culture with 14 µM indomethacin; *d* IL-1 treated culture with 14 µM indomethacin. Means and ranges are indicated (*a* and *b* contain 3 parallel experiments; *c* and *d* 2 experiments).

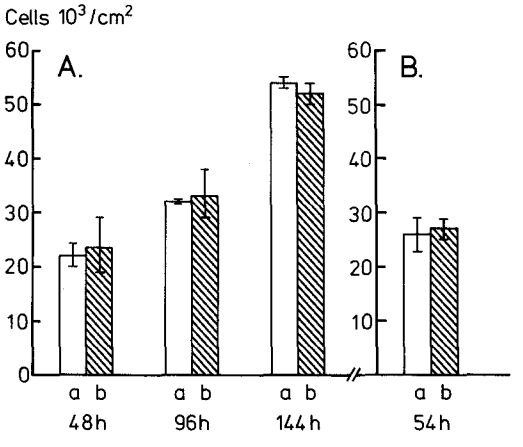

Figure 2. Proliferation of rat granulation tissue fibroblasts in control cultures (a) and in the presence of IL-1 (b).
A Cultures were exposed to 0.5 U/ml IL-1 throughout the experiment and the number of cells was counted after 48, 96 and 144 h in culture. Fresh IL-1 was added every other day. *B* Cultures were exposed to 2.0 U/ml IL-1 and the number of cells was counted after 54 h in culture. Means and ranges are indicated (*A* contain 2 parallel experiments; *B* contain 5 experiments).

Discussion. Several mononuclear cell factors are known to have an effect on collagen synthesis and cellular proliferation in wound healing, fibrosis and chronic inflammation [28]. Interferon gamma decreases collagen synthesis in fibroblast cultures [29], whereas transforming growth factor beta stimulates it both in vivo and in vitro [30]. EGF increases collagen accumulation mainly by enhancing cellular proliferation but it does not increase collagen synthesis per cell [16, 30].
IL-1 may modulate collagen accumulation in several different ways. First, it stimulates collagen synthesis by increasing collagen mRNA levels [8, 9]. Second, it decreases collagen synthesis by elevating PGE_2 production [1, 31]. Third, it increases synthesis of collagenase [2]. Fourth, IL-1 increases the synthesis of plasminogen activators [3], which can activate the latent collagenase [32].
Previously, no in vivo studies of the effects of IL-1 on collagen accumulation have been published. Our results indicate that in the formation of experimental granulation tissue IL-1 decreases the amount of collagen in viscose cellulose sponges. The effect may be caused by either decreased collagen production or by increased collagenase activity.
Earlier in vitro studies indicate that IL-1 can stimulate the collagen production in skin fibroblasts [8, 9]. It can also stimulate the proliferation of human fibroblasts of either adult or fetal origin [11, 12, 33]. However, under identical conditions, IL-1 lacked significant mitogenic effects on human, bovine, rabbit, or canine arterial smooth muscle cells [33]. In another study, IL-1 did not stimulate DNA synthesis in quiescent fibroblasts [34]. Our in vitro results suggest that in cell culture the granulation-tissue derived cells decrease their collagen production when exposed to IL-1. This effect could be caused by elevated prostaglandin synthesis in culture because no difference in collagen synthesis between IL-1 treated cells and controls was seen in the presence of indomethacin.
No effect caused by IL-1 on cellular proliferation was detected either in vivo or in vitro, suggesting that granulation tissue cells differ also in this respect from skin fibroblasts. Our results indicate that IL-1 is a limiting factor for accumulation of collagen in the formation of experimental granulation tissue. Thus, cellular proliferation and increase in collagen production is caused by other mediators and growth factors. These results, of course, do not negate the possibility that IL-1 may in other tissues, for example in skin, induce the formation of collagenous matrix also in vivo.

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Effect of protein kinase C activation and depletion on insulin stimulation of glycogen synthesis in cultured hepatoma cells

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Summary. Insulin stimulation of glycogen synthesis was nearly abolished in hepatoma cells shortly treated with 4 β-phorbol 12 β-myristate, 13 α-acetate (protein kinase C activation) but remained unmodified in cells chronically treated with the phorbol ester (protein kinase C depletion). Thus, although exogenous activation of protein kinase C results in an inhibition of insulin action, protein kinase C depletion has no influence on this process. The results suggest that, in hepatoma cells, no endogenous activation of protein kinase C may occur in response to the signal triggered by insulin.
Key words. Glycogen synthesis; insulin; protein kinase C; Zajdela hepatoma cells.

Calcium-activated, phospholipid-dependent protein kinase (protein kinase C, PKC) has recently emerged as a key cellular enzyme which appears to be involved in both the transduction and the modulation of the signals triggered by several growth factors and hormones including insulin[1]. Supporting this dual role of PKC is the finding that 4 β-phorbol 12 β-myristate, 13 α-acetate (PMA), a potent tumor promoter which directly activates PKC, exhibited in rat adipocytes insulin-like as well as insulin-antagonizing effects on metabolic processes such as glucose transport and lipogenesis[2-4]. However, when tested on rat liver glycogen synthesis, the effect of PMA proved quite different, since the phorbol ester was reported either to decrease the basal activity of glycogen synthase[5-7] or to be ineffective[8, 9]. In any case, PMA was found to antagonize insulin stimulation of glycogen synthase in Fao hepatoma cells, indicating that PKC is able to counteract the hormone bioactivity on glycogen synthesis when exogenously activated by the phorbol ester.

The present study was designed to determine whether, in Zajdela hepatoma cultured cells (ZHC cells), the signal triggered by insulin for stimulation of glycogen synthesis might be modulated through a mechanism involving endogenous activation of PKC. Thus, we measured the hormone stimulation of this process in ZHC cells where PKC had been either exogenously activated by a short exposure to PMA or largely depleted by a chronic exposure to the phorbol ester, in comparison to the stimulation observed in untreated cells.

Materials and methods. Stock cultures of ZHC cells, derived from the Zajdela rat ascite hepatoma (strain D), were subcultured into 25-ml flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% calf serum and glucose (45 mM final concentration)[11]. Down-modulation of PKC was obtained by preincubating confluent ZHC cells for 17 h in serum-free DMEM either in the absence (control cells) or the presence of 500 ng/ml of PMA (PKC-deficient cells). After 3 extensive washings, cells were used for the determination of either glycogen synthesis from [U-^{14}C] glucose, or insulin binding or PKC activity. Cell viability was estimated by the Trypan blue exclusion test.

Incorporation of [U-^{14}C] glucose into glycogen was measured as described previously[12]. Briefly, control and PKC-deficient ZHC cells, once treated as described in the legends of the figure and table 2, were incubated for 1 h at 37 °C in 2 ml serum-free DMEM containing 45 mM glucose and a tracer amount of 2.5 μCi of [U-^{14}C] glucose (240 mCi/mmol, Commissariat à l'Energie Atomique, France). Glycogen was then extracted and its radioactivity was determined as described[12]. Data are given as nanomoles of glucose incorporated into glycogen/mg protein/h. Protein content was evaluated by the Bradford dye method, with the use of Bio-Rad reagent and bovine serum albumin as the standard.

Insulin binding was measured by incubating (17 h at 4 °C) control or PKC-deficient ZHC cells in suspension (6×10^5 cells/ml) with 100 pM [^{125}I]-labeled insulin (80–100 μCi/μg, New England Nuclear Corp., USA) in the presence of increasing concentrations (0–10^4 ng/ml) of native insulin, as previously described[12].

Protein kinase C activity was measured in total cellular extracts. In short, control or PKC-deficient ZHC cells were suspended in 2 ml of extraction buffer (Tris-HCl 20 mM pH 7.5, 2 mM EDTA, 5 mM EGTA, 0.25 M sucrose, 50 mM β-mercaptoethanol, 0.2 mM PMSF, 0.1% Triton X 100) and