Recombinant γ-interferon inhibits prostaglandin-mediated and parathyroid hormone-induced bone resorption in cultured neonatal mouse calvaria

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A role of γ-interferon in the bone remodeling process can be implicated from its interference with bone resorptive processes in cultured neonatal mouse calvaria The immune interferon is an efficient inhibitor of endogenous prostaglandin synthesis, particularly after stimulation by thrombin or arachidonic acid, and, in addition, has a calcitonin-like inhibitory effect on PTH-induced osteoclastic bone resorption

γ-Interferon Bone resorption Neonatal mouse calvaria Prostaglandin synthesis Parathyroid hormone Calcitonin

1. INTRODUCTION

Interleukins and lymphokines may play a role in the bone remodeling process [1], particularly through the bone resorbing activities of interleukin 1 [2] and of the so-called 'osteoclast activating factor' [3] which is elaborated by T-lymphocytes in response to various stimuli, among them probably also interleukin 1 [1]. γ-Interferon (IFN-γ) which like other lymphokines is produced by lymphocytes following proliferative or specific antigenic stimulation (cf. [4]), induces polykaryon formation in cultured human monocytes [5]. It is thus conceivable that IFN-γ could also induce the formation of multinuclear osteoclasts, which are believed to normally originate through fusion from monocyte precursor cells (cf. [1]), and could possibly thereby induce the resorption of mineralized bone. We therefore have utilized the organ culture system of neonatal mouse calvaria to study potential effects of IFN-γ on osteoclastic bone resorption.

2. MATERIALS AND METHODS

Recombinant DNA-derived murine IFN-γ (Genentech, CA, spec. act. 1.3×10^7 U/mg) was obtained through Ernst Boehringer-Institut fur Arzneimittelforschung, Vienna. Synthetic salmon calcitonin (spec. act. 100 MRC units/ml) was purchased from Sanabo (Vienna). Parathyroid hormone (PTH) was a synthetic human N-terminal fragment 1–34 from Bachem, Torrance, CA. Prostaglandin E_2 (PGE_2) was purchased as Prostin E_2® from Upjohn Bovine thrombin (Topostasin®) is a product of Hoffmann-LaRoche (Basle). Indomethacin was obtained from Merck, Sharp and Dohme.

Organ culture of neonatal mouse calvaria has been described in detail [7,8]. Briefly, calvariae were dissected from 4–6-day-old mice (strain HIM:OFI, SPF, Institute for Experimental Animal Research of the University of Vienna, Himberg) and cultured free-floating in roller tubes in Dulbecco's modified Eagle's medium (supplemented with 15% heat-inactivated horse serum). Treatments were begun at 0 h and con-

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tinued to 72 or 96 h, as appropriate. Medium was changed at 24 h. Bone resorption was quantitated by determination of calcium concentration in culture medium at 24 h intervals through fluorescence titration in a Corning 940 calcium analyzer.

Data are presented as means (from 5 calvariae) ± SE. Student's *t*-test was used for statistical analysis Significant differences between groups were assumed when $P < 0\ 05$

3. RESULTS

In bones maintained in organ culture without any treatment, the rate of resorption slightly exceeds that of formation of mineralized bone, as indicated by the continuous release of calcium into the medium (fig 1, controls). This process can be inhibited by indomethacin (fig.1, cf. [8]), and is therefore believed to be determined in its extent by the production rate of endogenous prostaglandins [8], which are potent inducers of osteoclast activity in bone culture [6] Fig.1 shows that addition of IFN-γ to the culture medium (at 100 U/ml) significantly reduces the amount of calcium mobilized from cultured calvariae In these experiments, the inhibitory action of IFN-γ on bone resorption could not be distinguished from that of salmon calcitonin (sCT) or indomethacin (fig 1).

In a series of experiments we therefore addressed the question as to whether IFN-γ could interact

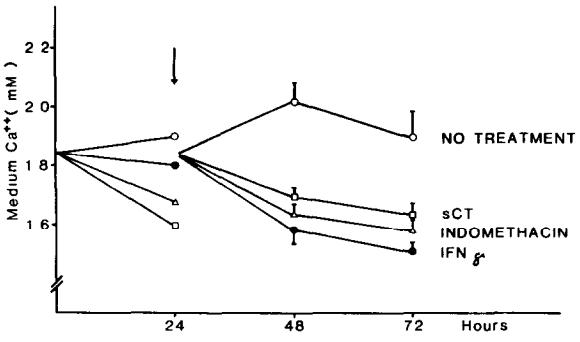

Fig 1 Effect of IFN-γ on calcium release from cultured neonatal mouse calvaria Medium concentrations IFN-γ, 100 U/ml, salmon calcitonin (sCT), 20 mU/ml, indomethacin, 5×10^{-7} M Arrow indicates change of culture medium Treated groups were significantly different from untreated controls at least at $P < 0\ 05$ at 48 and 72 h

with prostaglandin (PG) synthesizing reactions in bone. The latter can be efficiently stimulated through continuous exposure of bone to moderate activities of thrombin in organ culture [9]. In this experimental condition, which has been designed to evaluate specifically the biopotency of inhibitors of prostaglandin synthesis in bone (unpublished), IFN-γ proves to be as potent an inhibitor of prostaglandin-mediated bone resorption as, e.g., indomethacin [9] (fig.2). That IFN-γ thereby does not interfere with thrombin activation of prostaglandin synthesis but rather with the cyclooxygenase system itself can be concluded from the observation that IFN-γ displays its inhibitory potency when resorption of cultured bones by prostaglandins is facilitated by addition of their precursor arachidonic acid (5×10^{-5} M) to the culture medium [8] (fig.2)

It should be noted that calcitonin had no effect on calcium release from bones stimulated by thrombin or arachidonic acid (not shown).

Consistent with its proposed mechanism of action on prostaglandin synthesis, IFN-γ, at the 100 U/ml level, did not substantially reduce bone resorption evoked by exogenous PGE_2 ($5 \times$

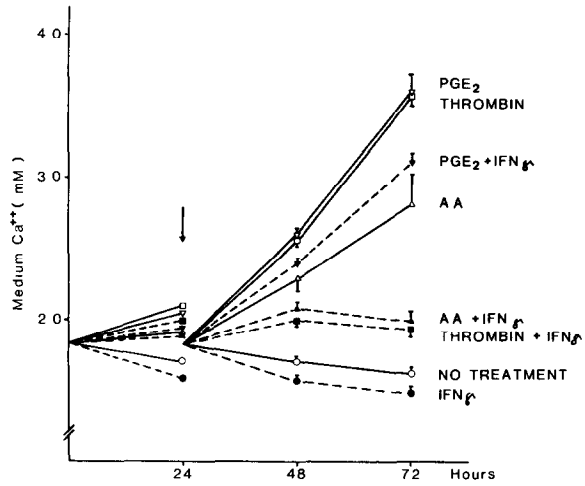

Fig 2 Effect of IFN-γ on calcium release from cultured bone stimulated by thrombin, arachidonic acid (AA), or PGE_2 Medium concentrations IFN-γ, 100 U/ml, thrombin, 14 U/ml, arachidonic acid, 5×10^{-5} M, PGE_2, 5×10^{-7} M Medium indicates change of culture medium IFN-γ treatment resulted in significant differences from respective control groups ($P < 0\ 05$) at 48 and 72 h

10^{-7} M), though in the presence of IFN-γ less calcium was mobilized by PGE_2 than could have been expected if IFN-γ inhibited endogenous PG formation only (fig.2).

This observation led us to pursue the question of whether IFN-γ, eventually at higher concentrations, could inhibit osteoclastic bone resorption, possibly by interference with the recruitment and/or activation of osteoclasts This phenomenon occurs either as result of PG action [6] or through an independent effect of PTH on osteoclasts and their mononuclear precursors in cultured bone [10,11]. In fact, IFN-γ, particularly at higher medium levels, substantially reduced calcium release induced by 10^{-8} M PTH, which was shown to be the maximally effective concentration in the mouse calvaria system [7]. This effect of IFN-γ was quite comparable to the antagonistic action of calcitonin on PTH-induced bone resorption [12] (fig.3).

4. DISCUSSION

This study presents experimental evidence for a potential inhibitory effect of IFN-γ on bone resorption in vivo Immune interferon might thus play a role in the bone remodeling process, primarily by suppression of PG formation, though an additional, calcitonin-like effect on PTH-induced osteoclast activation must also be considered a likely possibility. With respect to PG synthesis, IFN-γ could counteract the osteolytic activity of other immunoregulatory factors such as interleukin 1 and osteoclast activating factor, which both are effective, at least in part, via stimulation of PG formation [2,13].

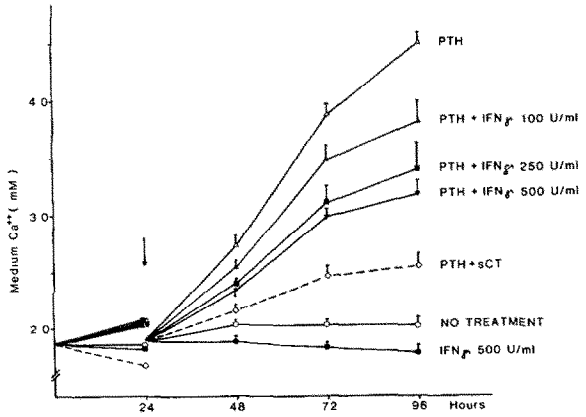

Fig 3 Effect of IFN-γ on PTH-induced bone resorption in cultured neonatal mouse calvaria Medium concentrations: PTH, 10^{-8} M, sCT, 20 mU/ml Arrow indicates change of culture medium IFN-γ and sCT-treated groups showed statistically significant difference from PTH-treated group between 48 and 96 h ($P <$ 0 05)

Until now it has been generally assumed that interferons, particularly those of the α- and β-type, would increase rather than decrease PG synthesis in various tissues [14]. Notably, recombinant human α- and β-interferon have been shown to increase PGE_2 levels in the hypothalamus [15], thus providing an explanation why fever is a common side effect of interferon therapy. However, a different situation might prevail in bone. Jilka and Hamilton [16] did not observe any bone resorbing activity when neonatal mouse calvaria in organ culture was treated with a human leukocyte interferon. In addition, Nilsson et al. [18] obtained no indication for a bone resorbing effect of mouse C-243 cell interferon on heterotopic bone implants in mice

The calcitonin-like effect of IFN-γ is difficult to interpret if one assumes that the immune interferon actually brings about the fusion of monocytes into osteoclasts If this actually occurred in bone this would force the conclusion that these cells cannot be activated in the presence of IFN-γ This could also explain the reduced effectivity of PTH in the presence of the immune interferon observed in our experiments Similar conclusions pertain to a comparable action of a recombinant human leukocyte IFN on PTH-induced bone resorption [16]

The ability of IFN-γ to block PG biosynthesis in bone could be exploited for treatment of tumors that stimulate bone resorption by either increasing circulating levels of PGs or more directly their formation in bone (cf. [18]). IFN-γ might also be useful in the treatment of various inflammatory diseases associated with localized PG-mediated bone destruction, particularly rheumatoid arthritis. However, the therapeutical value of IFN-γ could be diminished by the possibility that in this autoimmune-related disorder, IFN-γ, under certain conditions, could even enhance antibody production as well as delayed type hypersensitivity reactions (cf. [4]). Treatment of osteoporosis with IFN-γ instead of or in combination with calcitonin

might also be promising for the following reason. In osteoporosis, bone resorption undoubtedly exceeds the formation of new bone [19] There is some evidence that this might be partially due to an enhanced local production of PGE_2 in bone [20] Thus, IFN-γ, unlike calcitonin, could certainly block any PG-mediated bone destruction and, in addition, through its calcitonin-like effect even further reduce the rate of osteoclastic bone resorption

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