Journal of Cellular Biochemistry 44:253–264 (1990)

Interleukin-1β- and Tumor Necrosis Factor-α-Independent Monocyte Stimulation of Fibroblast Collagenase Activity

David A. Tipton, Michael J. Pabst, and Mustafa Kh. Dabbous

Dental Research Center (D.A.T., M.J.P., M.K.D.) and Departments of Periodontology (D.A.T., M.J.P., M.K.D.) and Biochemistry (M.J.P., M.K.D.), The University of Tennessee, Memphis, Memphis, Tennessee 38163

To investigate the mechanism of cyclosporine (Cs)-induced fibrous gingival enlargement, the indirect effects of Cs on fibroblast collagenolysis via the drug's effect on the synthesis of the fibroblast regulatory monokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) have been studied. Peripheral blood monocytes stimulated with lipopolysaccharide (LPS) for 48 h produced conditioned media (MCM-LPS) that contained 665 pg/ml IL-1β and 16 pg/ml TNFα and significantly ($P < 0.001$) enhanced the collagenase activity of a fibroblast strain (GN 23) derived from a healthy individual with clinically normal gingiva. The concurrent addition of Cs (50, 100, or 150 ng/ml) with LPS to the monocytes (MCM-LPS-Cs) significantly diminished their ability to enhance GN 23 collagenase activity in a dose-dependent manner, with MCM-LPS-Cs (150 ng/ml) causing the greatest effect. Cs also significantly inhibited IL-1β and TNFα production. Although the greatest inhibition of both cytokines was at 50 ng/ml Cs, the corresponding MCM-LPS-Cs caused the *least* diminution (16%) of the collagenase stimulation caused by MCM-LPS (no Cs). This suggested that factor(s) other than or in addition to IL-1β and TNFα might be responsible for the stimulation of GN 23 collagenase activity. MCM-LPS depleted of IL-1β by affinity chromatography retained its stimulatory effect on GN 23 collagenolysis, and human recombinant IL-1β and TNFα, when tested alone or together at levels found in the stimulatory MCM-LPS and MCM-LPS-Cs, did not stimulate GN 23 collagenase activity as did the crude conditioned media. This evidence suggested that the conditioned media contained the complex mixture of cytokines necessary to stimulate collagenase activity of this fibroblast strain and that IL-1β and TNFα were not necessarily involved. Cs may alter the synthesis of other collagenase-stimulating cytokines, accounting for the diminished ability of Cs-treated monocytes to enhance collagenase activity of susceptible fibroblast strains. Decreased collagenase activity, therefore, resulting from Cs suppression of monokine production, may be an important factor in the development of fibrous gingival enlargement seen in some susceptible patients treated with Cs.

Key words: fibrosis, cell-cell interaction, cyclosporine, cytokines, gingival enlargement

Received August 13, 1990; accepted August 22, 1990.

Since the discovery of the immunosuppressant cyclosporine (Cs), the use of this drug has become common in solid organ transplantation to suppress allograft rejection [1,2]. Fibrous gingival enlargement has been recognized as one side effect of Cs therapy, occurring in about one-third of patients taking the drug [1,3–5]. Histological studies of Cs-enlarged gingiva have revealed the infiltration of lymphocytes (particularly T cells), macrophages, and plasma cells [4–6]. Indeed, chronic inflammatory activity in the pathogenesis of drug-induced gingival enlargements, including those caused by phenytoin [7] and nifedipine [8], is now generally accepted [9].

It is well established that many lymphokines and monokines alter fibroblast growth and activity including collagen metabolism [10–18]. Therefore, cytokines play an important role in tissue homeostasis by regulating connective tissue cells and the turnover of extracellular matrix proteins [19]. Because Cs can inhibit the production of some of these cytokines, the regulation of fibroblasts in the presence of infiltrating inflammatory cells may be altered or lost during Cs therapy, contributing to fibrous gingival overgrowth [6].

Tissue fibrosis may be a reflection of increased collagen synthesis, reduced collagenolytic activity, or both as well as disturbances in their regulation. The monokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) are key modulators in connective tissue remodeling and fibrosis because both are fibroblast mitogens and affect collagen synthesis and degradation [20–23]. Cs can directly inhibit IL-1β [24–29] and TNFα [30–33] production by monocytes, although there are conflicting reports in the case of IL-1β [29,34]. This in vitro study focused on the role of collagenolysis in Cs-induced gingival fibrosis. We studied the indirect effect of Cs on fibroblast collagenase activity via its effects on monokine secretion by lipopolysaccharide (LPS)-activated monocytes, and we have examined the roles of IL-1β and TNFα in this process.

MATERIALS AND METHODS

Fibroblasts

The fibroblast cell strain (designated GN 23) used in this study is a human gingival fibroblast strain derived from a gingival explant from a healthy individual with noninflamed gingiva. After excision of the tissue, it was minced using sterile techniques and washed several times in Hank's balanced salt solution (HBSS; Gibco, Grand Island, NY) supplemented with gentamicin (Gibco) at 100 μg/ml and fungizone (Gibco) at 2.5 μg/ml. Five to ten tissue pieces were placed into each of several 25 cm^2 flasks (Falcon, Oxnard, CA) and allowed to adhere to the flasks. Then 3 ml of complete medium [Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% newborn calf serum (NCS; Gibco) and gentamicin (100 μg/ml)] was added to each flask. The flasks were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells eventually were passaged by trypsinization with 0.25% trypsin (Enzart; Armour Pharmaceutical Co., Kankakee, IL) in HBSS containing 0.3 mM EDTA (Mallencrodt, Paris, KY) and eventually stored in liquid nitrogen. The fibroblasts used in experiments were taken from passages two through ten.

Isolation of Monocytes From Human Peripheral Blood

Blood (200–400 ml) was drawn by venipuncture from healthy donors using 0.38% citrate as anticoagulant. Mononuclear cells and neutrophils were separated by a plasma-Percoll procedure with a two-step discontinuous gradient. Monocytes were

separated from lymphocytes by allowing the cells from the mononuclear cell layer to adhere to plastic tissue culture dishes. The monocytes adhered within 1 h, and the nonadherent lymphocytes were washed off. Alternatively, monocytes were separated from lymphocytes using the elutriator rotor (Beckman Instruments, Inc., Fullerton, CA). Monocytes were cultured for several days in the CO_2 incubator in a modified Earle's salt solution (EBSS). The solution contained 0.1% glucose and the proper amount of sodium bicarbonate to maintain pH at 7.3 in an atmosphere of 5% CO_2. Monocytes remained viable and functional for at least 4 days in this salt solution.

Monocyte Conditioned Medium

Monocytes were incubated with or without 50 ng/ml *Escherichia coli* lipopolysaccharide (LPS; Difco, Detroit, MI) and with or without Cs (50–150 ng/ml) for 48 h. Conditioned culture medium, consisting of EBSS plus cell products, was prepared by centrifuging culture supernatants to remove cells, followed by sterile filtration (Millex-GS 0.22 μm filter unit; Millipore, Bedford, MA). The direct effect of LPS on monocytes was monitored by measuring enhancement of f-Met-Leu-Phe (FMLP)-stimulated superoxide anion release from these cells.

IL-1β, TNFα, and Cs

Human recombinant IL-1β (hrIL-1β) was obtained from Cistron Biotechnology (Pine Brook, NJ). This preparation was >95% pure and had a specific activity of 10^7 U/mg. Human recombinant TNFα (hrTNFα) was obtained from Genentech (South San Francisco, CA) and had a specific activity of 5×10^7 U/mg. Cs was from Sandoz, Ltd. (Basel, Switzerland); a stock solution was prepared in ethanol containing 20% Tween 80 as described by Wiesinger and Borel [35].

Collagenase Activity Assay

Collagenase activity in the serum-free culture supernatants of normal human gingival fibroblasts was measured using reconstituted [^{14}C]proline- and [^{3}H]glycine-labeled collagen fibril gels as substrate [36]. Fibroblasts were seeded at 1×10^5 cells/well in 24-well tissue culture plates (Costar, Cambridge, MA) and cultured for 24 h at 37°C. The medium was removed and replaced with serum-free AMEM-F10 medium (Gibco) containing 100 μg/ml gentamicin or with this medium containing the MCM-LPS or MCM-LPS-Cs (diluted 1:4 v/v), hrIL-1β, hrTNFα, or combinations of the two recombinant cytokines. The recombinant cytokines were used at their levels present in the diluted conditioned media. Supernatants were collected at day 3. The latent collagenase in the medium was activated by incubation with 25 μg trypsin (Sigma, St. Louis, MO) for 10 min at 34°C. The trypsin was inactivated by adding a fivefold excess of soybean trypsin inhibitor (SBTI; Sigma). The supernatants were incubated on the collagen gels for 18–24 h at 34°C in 50 mM Tris Cl buffer with 0.01 M $CaCl_2$ and 0.2 M NaCl, pH 7.5, and counted in a liquid scintillation spectrometer. Results are expressed as units of collagenase per milligram protein. One unit degrades 1 μg collagen per minute at 34°C. Protein determination was performed by the method of Lowry et al. [37].

Assay of IL-1β and TNFα in MCM

IL-1β levels in the MCM-LPS and MCM-LPS-Cs were determined using an IL-1β ELISA Kit (Cistron). The anti-IL-1β antibody in this kit does not cross react

with TNFα, IL-1α, or IL-2. Levels of biologically active TNFα in the MCM-LPS and MCM-LPS-Cs were also determined by enzyme-linked immunosorbent assay (ELISA) using the Biokine TNF Test Kit (T-Cell Sciences, Inc., Cambridge, MA). The anti-TNFα antibody in this kit does not cross react with TNFβ (lymphotoxin), IL-1β, IL-2, or denatured TNFα.

Affinity Chromatography

Affinity chromatography was performed using an immunoaffinity agarose gel for IL-1β (Endogen, Inc., Boston, MA). This gel has a specific activity of greater than 10,000 neutralizing units per milliliter gel and does not bind TNFα, TNFβ, IL-2, IL-3, IL-4, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), or interferons (IFNs). Cross reactivity with IL-1α is $\leq 5\%$. The gel was washed with phosphate-buffered saline (PBS) and 100 μl of the gel was added to Eppendorf tubes containing the MCM samples. The tubes were incubated for 2 h at 37°C with slow end-over-end mixing. The samples were centrifuged for 1 min in a microfuge, and the supernatants were decanted and stored at −20°C until use in the ELISA or the collagenase assay. Since the amount of TNFα in the MCM-LPS was very low and was near the detection limit of the ELISA used to measure it, the decision was made not to attempt to remove it from the conditioned media.

Statistical Analysis

The data are expressed as mean ± standard deviation and were analyzed using a one-way analysis of variance (ANOVA).

RESULTS

Effect of MCM on Collagenase Activity

Medium from monocytes cultured for 48 h with 50 ng/ml LPS (MCM-LPS) (no Cs) significantly stimulated fibroblast collagenase activity ($P < 0.001$) (Fig. 1). To some monocyte cultures, Cs (50–150 ng/ml) was added at the time of LPS addition (MCM-LPS-Cs) and cultured for 48 h. Cs had no effect on unstimulated or FMLP-stimulated superoxide anion release from the monocytes (data not shown). Cs treatment of LPS-stimulated monocytes inhibited their ability to enhance collagenase activity. Although still significantly stimulated over control levels, the collagenase activities of fibroblasts exposed to MCM-LPS-Cs (50 ng/ml), MCM-LPS-Cs (100 ng/ml), and MCM-LPS-Cs (150 ng/ml) were significantly decreased [16% ($P < 0.05$), 43% ($P < 0.0005$), and 51% ($P < 0.001$), respectively] compared with the level of activity of fibroblasts incubated with MCM-LPS (no Cs).

IL-1β and TNFα Levels in MCM

The amounts of IL-1β and TNFα were measured in the MCM-LPS and MCM-LPS-Cs by ELISA (Fig. 2a,b). IL-1β was present in the MCM-LPS at 665 pg/ml, and Cs significantly decreased the levels of IL-1β released by LPS-stimulated monocytes at all concentrations tested (Fig. 2a). In contrast, TNFα production (16 pg/ml in the MCM-LPS) was significantly decreased by Cs only at 50 ng/ml ($P < 0.005$); there was no change at 100 ng/ml, and Cs at 150 ng/ml significantly increased TNFα production ($P < 0.01$) (Fig. 2b). Maximum TNFα levels (200–500 pg/ml) were found

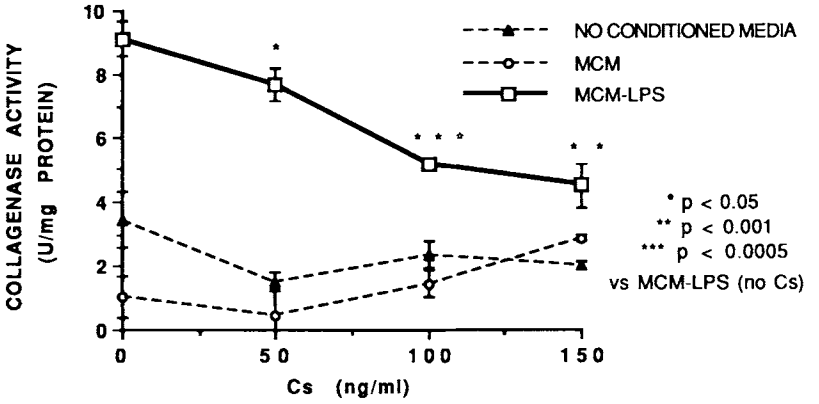

Fig. 1. Effect of MCM, MCM-LPS, and MCM-LPS-Cs on gingival fibroblast collagenolysis. Human gingival fibroblasts were cultured in the absence (no conditioned media) or presence of MCM, MCM-LPS, and MCM-LPS (containing Cs at 50, 100, or 150 ng/ml) diluted 1:4 (v/v) in serum-free AMEM-F10 medium for 3 days. Collagenase activity was determined using radiolabeled type I collagen gels as substrate. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment.

in conditioned media from monocytes stimulated with LPS for 12–18 h (data not shown). Because the MCM-LPS and MCM-LPS-Cs used in this study were prepared by stimulating monocytes for 48 h with LPS, the low levels of TNFα in these media were probably due to the decay of much of the cytokine by 48 h.

Effect of IL-1β-Depleted MCM-LPS on Collagenase Activity

IL-1β was removed from an MCM-LPS by affinity chromatography using an immunoaffinity agarose gel for IL-1β. Figure 3a shows that treatment of the MCM-LPS with the gel removed approximately 80% of the IL-1β. However, depletion of the cytokine did not diminish the ability of the MCM-LPS to stimulate collagenase activity significantly ($P < 0.001$) (Fig. 3b).

Effect of hrIL-1β and hrTNFα on Collagenase Activity

Human recombinant IL-1β and TNFα, alone and in combination, were tested for their effects on collagenase activity. Because the conditioned media were diluted 1:4 before addition to fibroblast cultures for the collagenase assay testing the effects of MCM-LPS and MCM-LPS-Cs (above), the recombinant cytokines were used at one-fourth the levels measured in the undiluted conditioned media (Fig. 2a,b). Therefore, hrIL-1β was used at 60, 115, 135, or 170 pg/ml, and TNFα was used at 0.1, 4, 7.5 or 4 pg/ml, corresponding to the levels in the diluted MCM-LPS-Cs (50 ng/ml), MCM-LPS-Cs (100 ng/ml), MCM-LPS-Cs (150 ng/ml), and MCM-LPS, respectively. Whereas the MCM-LPS and all MCM-LPS-Cs significantly stimulated collagenase activity over control levels (Fig. 1), neither hrIL-1β nor hrTNFα alone (Fig. 4a,b, respectively) or together, in the specific combinations found in the MCM-LPS and MCM-LPS-Cs (Fig. 5), were able to stimulate collagenase activity and duplicate the effect of the crude conditioned media.

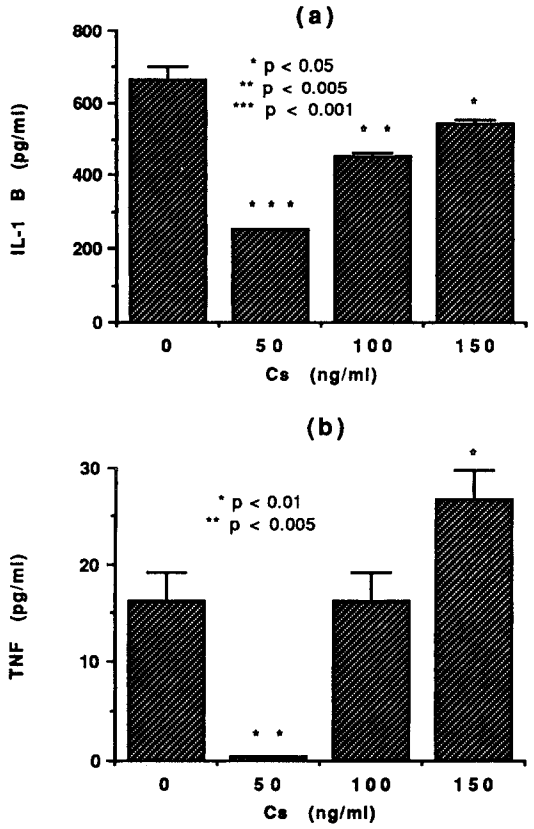

Fig. 2. Effect of Cs on IL-1β (a) and TNFα (b) production by human peripheral blood monocytes. Monocytes were incubated with 50 ng/ml LPS and with or without Cs for 48 h. The amounts of IL-1β and TNFα in the conditioned media were determined by ELISA. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment.

DISCUSSION

This study demonstrating that supernatants of LPS-activated monocytes stimulate collagenase activity of fibroblasts supports others showing that mononuclear cells (including monocytes/macrophages) secrete soluble products that alter a variety of fibroblast functions [18,38–43]. We show here that concurrent addition of Cs and LPS to monocyte cultures diminishes their stimulatory effect on fibroblast collagenolysis. There are at least two pathways through which Cs may affect fibroblasts: 1) by direct action after uptake by the fibroblasts or 2) indirectly via the drug's effect on the synthesis of lymphokines and monokines that regulate fibroblast functions. The direct effects of Cs on fibroblasts have been demonstrated in this laboratory [44–46] and others [47–50]. Hassell et al. [51] have demonstrated lymphocyte-mediated stimulatory effects of Cs on total protein and collagen synthesis by human gingival fibroblasts (dependent on the Cs dose and the donor from which the fibroblast strain was derived). That supernatants of Cs-treated immune cells can both stimulate collagen synthesis and inhibit its breakdown by gingival fibroblasts (as described in this study)

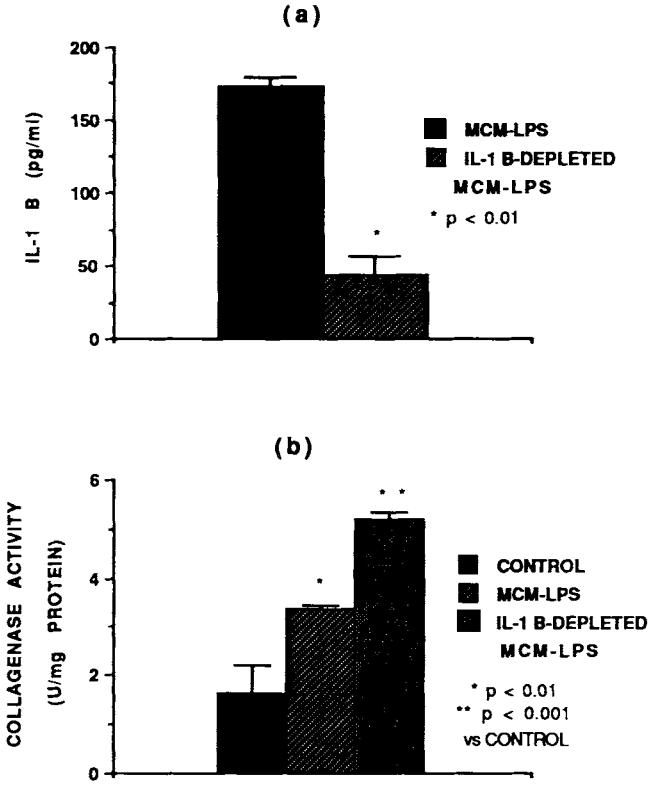

Fig. 3. IL-1β removal from MCM-LPS and effect of IL-1β-depleted MCM-LPS on gingival fibroblast collagenolysis. **a:** MCM-LPS was prepared as described in Materials and Methods, and the IL-1β level was measured by ELISA. The MCM-LPS was subjected to affinity chromatography using an immunoaffinity agarose gel for IL-1β. The gel-treated MCM-LPS was assessed for IL-1β removal by ELISA. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment. **b:** The MCM-LPS and the IL-1β-depleted MCM-LPS were diluted 1:4 (v/v) in serum-free AMEM-F10 medium and incubated with human gingival fibroblasts for 3 days. Collagenase activity was determined using radiolabeled type I collagen gels as substrate. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment.

suggests an important role for the indirect effect of Cs in the pathogenesis of gingival fibrosis in susceptible individuals.

Activated monocytes secrete an array of cytokines, including IL-1β, TNFα, G-CSF, M-CSF, IL-6, and TGFβ, which have a broad range of regulatory effects on several different cell types [52–57]. IL-1β and TNFα are two of the major fibroblast regulatory cytokines and among their effects on fibroblasts is the stimulation of collagenase activity [21]. For these reasons they were chosen for further study as potential mediators for the stimulatory effect of the MCM-LPS on collagenase activity, as well as the effects of the MCM-LPS-Cs. For example, if IL-1β and TNFα were responsible, Cs-induced inhibition of their production might account for the diminished ability of the MCM-LPS-Cs to stimulate collagenolysis.

Gingival fibroblast collagenase activity was significantly stimulated ($P < 0.001$) by the MCM-LPS (Fig. 1), supporting the findings of others [58]. As expected, there

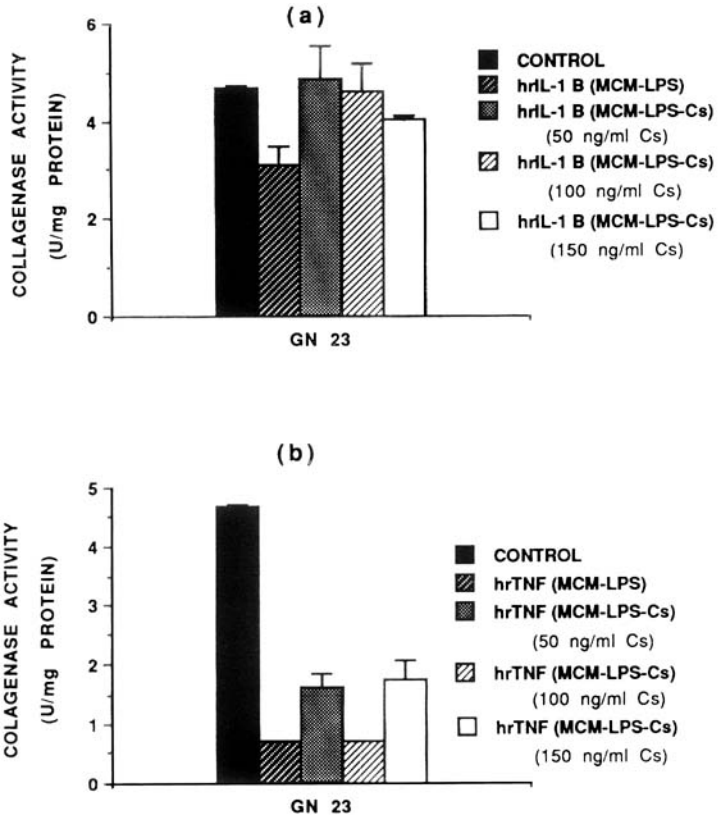

Fig. 4. Effect of hrIL-1β or hrTNFα on gingival fibroblast collagenolysis. **a:** Human gingival fibroblasts were cultured in the absence or presence of hrIL-1β in serum-free AMEM-F10 medium for 3 days. The amounts of hrIL-1β used were 60, 115, 135, or 170 pg/ml and corresponded to the amounts in the diluted MCM-LPS-Cs (50 ng/ml), MCM-LPS-Cs (100 ng/ml), MCM-LPS-Cs (150 ng/ml), and MCM-LPS, respectively. Collagenase activity was determined using radiolabeled type I collagen gels as substrate. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment. **b:** The fibroblasts were incubated with hrTNFα and collagenase activity was determined as described above. The amounts of hrTNFα used were 0.1, 4, and 7.5 pg/ml, corresponding to the amounts in the diluted MCM-LPS-Cs (50 ng/ml), MCM-LPS-Cs (100 ng/ml)/MCM-LPS, and MCM-LPS-Cs (150 ng/ml), respectively. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment.

was a high level of IL-1β (665 pg/ml) in the MCM-LPS, although the amount of TNFα was relatively low (16 pg/ml). Because the maximum level of TNFα produced by LPS-stimulated monocytes occurs at 12–18 h (data not shown), the low level of TNFα was probably due to the decay of much of it by the time the MCM-LPS was harvested (48 h). IL-1β production was significantly inhibited by all concentrations of Cs tested; TNFα production, on the other hand, was inhibited by Cs at 50 ng/ml only. The inhibition of IL-1β and TNFα production by Cs supports work from other laboratories [24–33], some of which has demonstrated that Cs inhibits the extracellular release of both cytokines without inhibiting their mRNAs [30–32,59]. In this study, the pattern of the effect of Cs on the production of both cytokines was the same: The lowest concentration tested (50 ng/ml) caused the greatest inhibition, and, as the Cs concen-

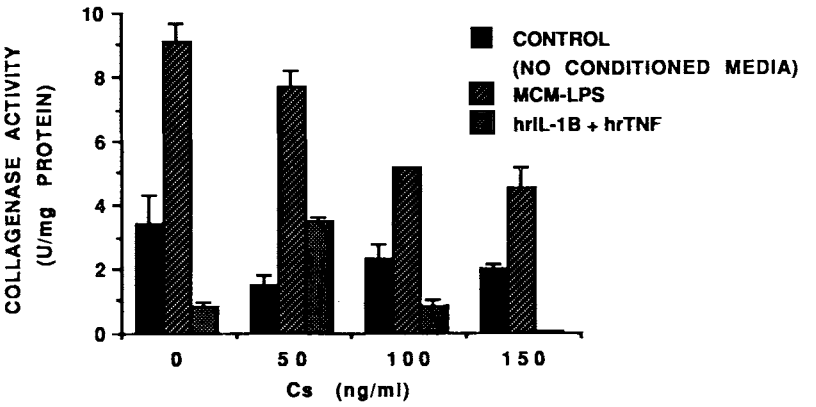

Fig. 5. Effect of combinations of hrIL-1β and hrTNFα on gingival fibroblast collagenolysis. Human gingival fibroblasts were cultured in the absence or presence of hrIL-1β and hrTNFα in serum-free AMEM-F10 medium for 3 days. The amounts of hrIL-1β and hrTNFα used ranged from 60 to 170 pg/ml and 0.1 to 7.25 pg/ml, respectively, and corresponded to the specific combinations of the cytokines found in the diluted MCM-LPS and MCM-LPS-Cs, measured by ELISA. Collagenase activity was determined using radiolabeled type I collagen gels as substrate. The data are expressed as means and standard deviations of triplicate cultures of a representative experiment.

tration increased, a lesser degree of inhibition (or, in the case of TNFα, no change or stimulation) of cytokine production was seen (Fig. 2a,b).

Although 50 ng/ml Cs caused the greatest inhibition of IL-1β and TNFα production, the corresponding MCM-LPS-Cs caused the least diminution (16%) of the collagenase stimulation caused by the MCM-LPS (no Cs) (Fig. 1). This suggested that a factor(s) other than, or in addition to, IL-1β and TNFα was responsible for the stimulation of GN 23 collagenase activity and raised the possibility that the synthesis and/or release of this factor(s) might also be altered by exposure of the monocytes to Cs.

To investigate further the roles of IL-1β and TNFα in the stimulation of gingival fibroblast collagenase activity, we tested hrIL-1β and hrTNFα, alone and together, for their effects on collagenase activity. Neither hrIL-1β nor hrTNFα, alone or in specific combinations found in the MCM-LPS and MCM-LPS-Cs, was able to stimulate collagenase activity or alter it as did the crude MCM-LPS and MCM-LPS-Cs (Figs. 1, 4, 5). In other studies, IL-1β stimulated collagenase activity in gingival fibroblasts [58], skin fibroblasts [21], and synovial fibroblasts [60], at levels from as low as 25 pg/ml to as high as 2,500 pg/ml, and Dayer et al. [61] showed that TNFα stimulated dermal fibroblast collagenase activity when used at approximately 50 ng/ml.

The lack of collagenolytic response to IL-1β and TNFα of the GN 23 strain could be due to several factors. For example, it may be caused by a strain-specific lack of susceptibility to these cytokines, since there are variations among individuals in cellular response to other agents, such as Cs [62], in terms of response/nonresponse and also in terms of magnitude and direction [51]. The amounts of IL-1β to which this strain was exposed were comparable to levels that stimulated collagenase in other studies [21,58], although the levels were much lower than those used in studies in which IL-1β stimulated gingival fibroblast hyaluronic acid synthesis [63], plasminogen activator [64], or prostaglandin E_2 [65]. The amounts of TNFα used in this study were

much lower (pg/ml level) than the amount used in a study in which TNFα stimulated dermal fibroblast collagenase (ng/ml level) [61]. The GN 23 strain may indeed respond collagenolytically to much higher levels of IL-1β and TNFα than those used in this study, but nevertheless it did not exhibit increased collagenase activity when exposed to the recombinant cytokines at levels present in the stimulatory MCM-LPS or MCM-LPS-Cs.

Human fibroblasts have relatively high numbers of IL-1β receptors compared with lymphoid cells [66]. Two human gingival fibroblasts strains were found to have from 5,000 to 15,000 IL-1β receptors/cell [67], and it has been suggested that IL-1 action on fibroblasts is regulated at the level of receptor expression [68]. Tsujimoto et al. [69] reported the presence of high-affinity ($K_d = 3.2 \times 10^{-10}$ M) receptors for human TNFα on human fibroblasts, with approximately 7,500 binding sites/cell. The number and affinities of IL-1β and TNFα receptors on the GN 23 strain is unknown, but a reduction in the numbers and/or affinities of these receptors, compared with susceptible fibroblast strains, might account for the lack of GN 23 response to IL-1β and TNFα. Whereas the GN 23 strain as a whole may be nonresponsive to IL-1β and TNFα, it may contain clone(s) that, when isolated, might respond collagenolytically to these cytokines. Korn et al. [38] have demonstrated fibroblast clonal heterogeneity in response to mononuclear cell mediators, and clonal interactions within the mass strain may suppress the effects of IL-1β on the responsive clones [38,70]. We are currently deriving clones of normal gingival fibroblasts to test their responses to Cs-treated monocyte supernatants and recombinant cytokines.

Finally, the lack of collagenolytic effect of hrIL-1β and hrTNFα may be a reflection of culture conditions. All the media used in supernatant production and collagenase activity determination were serum free. Bartold has shown that IL-1β stimulates fibroblast proliferation, but this effect was not evident in the absence of serum, indicating that the serum contained some factor(s) that augment the biological effect of IL-1β [71]. Singh et al. [72] likewise suggested that the collective action of two macrophage-derived monokines (IL-1β and macrophage-derived growth factor) may constitute a system for the proliferative response of fibroblasts to IL-1β. This may be the case with the stimulation of GN 23 collagenase activity by MCM-LPS. It appears that cytokines have an extremely complex and interrelated action network, with multiple overlapping actions, induction of other cytokines, and synergistic or inhibitory effects with one another [73, and references therein]. The MCM-LPS apparently contain the complex mixture of these cytokines necessary to stimulate GN 23 collagenase activity.

The removal of IL-1β from this mixture by affinity chromatography did not diminish and indeed somewhat enhanced the stimulatory effect of the MCM-LPS on collagenase activity. We also obtained two batches of MCM-LPS, which, though activated to similar extents, differed tenfold and threefold in their levels of IL-1β and TNFα, respectively. Both, however, significantly ($P < 0.005$) stimulated GN 23 collagenase actively to about the same degree (data not shown). These data further suggest that among the cytokines and factors necessary for this stimulation, IL-1β and TNFα are not required with this fibroblast strain. This would explain the observation that the MCM-LPS-Cs in which there was the greatest inhibition of IL-1β and TNFα production had almost the same stimulatory effect as the IL-1β-rich MCM-LPS, as well as the observation that two MCM-LPS, which differed in their levels of IL-1β and TNFα, both significantly stimulated collagenase activity to about the same extent.

Therefore, in addition to IL-1β and TNFα, Cs may affect an additional cytokine(s) that acts to stimulate collagenase activity. The inhibition of the production of this cytokine(s) may be responsible for the diminished ability of Cs-treated monocytes to stimulate collagenase activity of susceptible fibroblast strains, and we are currently investigating this possibility. Such strain-specific collagenolytic responses of gingival fibroblasts to direct effects of Cs, as well as differences in response to regulatory cytokines whose production is affected by Cs, may in part account for the observation that only a portion of patients on Cs therapy develop fibrous gingival enlargement.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the expert technical assistance of Karen Pabst. The expert secretarial assistance of Kathy Walker is also acknowledged. This work was supported by U.S. PHS grant DE-05455 from the Natural Institute of Dental Research (D.A.T.), U.S. PHS Biomedical Research Support Grant RR-05994 (D.A.T.), U.S. PHS grant DE-05494 (M.J.P.), and U.S. PHS grant CA-25617-10 from the National Cancer Institute (M.Kh.D.)

REFERENCES

1. Daley TD, Wysocki GP: J Periodontol 55:708–712, 1984.
2. Borel JF: Transplant Proc 15:2219–2229, 1983.
3. Calne R, Rolles K, White DJ, et al.: Lancet 2:1033–1036, 1979.
4. Rateitschak-Pluss E, Hefti A, Lortscher R, et al.: J Clin Periodontol 10:237–246, 1983.
5. Rostock MH, Fry HR, Turner JE: J Periodontol 57:294–299, 1986.
6. Bartold PM: J Oral Pathol 16:463–468, 1987.
7. Hassell TM: In: "Epilepsy and the Oral Manifestations of Phenytoin Therapy." Vol. 1, p 116, 1981.
8. Lucas RM, Howell LP, Wall BA: J Periodontol 56:211–215, 1985.
9. Savage NW, Seymour GJ, Robinson MF: J Periodontol 58:475–480, 1987.
10. Wahl SM, Wahl LM: In: "Lymphokines," 1981, Vol 2, p 153.
11. Atkins FN, Freedman MM, Subba-Raio PV, et al.: Int Arch Allergy Appl Immunol 77:96–102, 1985.
12. Wahl SM, Gately CL: J Immunol 130:1226–1230, 1982.
13. Postlethwaite AE, Smith GN, Mainardi CL, et al.: Immunology 132:2470–2477, 1984.
14. Schmidt JA, Mizel SB, Cohen D, et al.: J Immunol 128:2177–2182, 1982.
15. Krane SM, Dayer JM, Simon LS, et al.: Coll Rel Res 5:99–117, 1985.
16. Beutler B, Cerami A: Nature 320:584–589, 1986.
17. Mornex J-F, Martinet Y, Yamamuchi K, et al.: J Clin Invest 78:61–66, 1986.
18. Narayanan AS, Voss T, Page RC: Biochem Biophys Res Commun 131:1028–1032, 1985.
19. Kahari V-M, Sandberg M, Kalimo H, et al.: J Invest Dermatol 90:664–670, 1988.
20. Vlassara G, Brownlee M, Manogue KR, et al.: Science 240:1546–1548, 1988.
21. Postlethwaite AE, Raghow R, Stricklin GP, et al.: J Cell Biol 106:311–318, 1988.
22. Mochan E, Armor L, Sporer R: J Periodont Res 23:28–32, 1988.
23. Vilcek J, Tsujimoto M, Palumbella VJ, et al.: J Cell Physiol Suppl 5:57–61, 1987.
24. Bunjes D, Hardt C, Rollinghoff M, et al.: Eur J Immunol 11:657–661, 1981.
25. Yoshimura N, Oka T, Ohmori I, et al.: Transplant Proc 19:1194–1198, 1987.
26. Yoshimura N, Kahan BO, Matsui S, et al.: Transplant Proc 20 [Suppl 2]:69–74, 1988.
27. Andrus L, Lafferty K: Scand J Immunol 15:449–458, 1982.
28. Yoshimura N, Matsui S, Hamashima T, et al.: Transplantation 45:157–162, 1988.
29. Abbud-Filho M, Kupiec-Weglinski J, Araujo J, et al.: J Immunol 133:2582–2586, 1984.
30. Espevik T, Figari IS, Shalaby MF, et al.: J Exp Med 166:571–576, 1987.
31. Remick D, Nguyen D, Eskandari M, et al.: Biochem Biophys Res Commun 161:551, 1989.
32. Nguyen D, Eskandari R, DeForge L, et al.: J Immunol 10:3822–3828, 1990.

33. McKenna R, Szturm K, Jefferson J, Rush D: Transplantation 47:343–348, 1989.
34. Wagner H: Transplant Proc 15:523–526, 1983.
35. Weisinger D, Borel JF: Immunobiology 156:454–463, 1979.
36. Dabbous MKh, El-Torky M, Haney L, et al.: Int J Cancer 31:357–364, 1983.
37. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265–275, 1951.
38. Korn JH, Halushka PV, LeRoy EC: J Clin Invest 65:543–554, 1980.
39. Kelley J, Trombley L, Kovacs E, Davis G, Absher M: J Cell Physiol 109:353–361, 1981.
40. Korn JH, Torres D, Downie E: Arthritis Rheum 27:174–179, 1984.
41. Austgulen R, Nissen-Meyer J, Degré M, Espevik T: Scand J Immunol 26:175–185, 1987.
42. Elias J, Rossman M, Zurier R, Daniele R: Am Rev Respir Dis 131:94–99, 1985.
43. Jordana M, Newhouse M, Gauldie J: J Leuk Biol 42:51–60, 1987.
44. Tipton D, Dabbous MKh: J Dent Res (Special Issue) 65:331, 1989.
45. Tipton D, Stricklin G, Dabbous MKh: J Dent Res (Special Issue) 66:312, 1987.
46. Tipton D, Dabbous MKh: J Dent Res (Special Issue) 68:361, 1989.
47. Fan X-F, Scott PG: J Dent Res (Special Issue) 68:288, 1989.
48. Sobhani S, Hassell T, Stone C: J Dent Res (Special Issue) 68:416, 1989.
49. Hassell T, Buchanan J, Cuchens M, Douglas R: J Dent Res (Special Issue) 67:273, 1988.
50. Coley C, Jarvis K, Hassell T: J Dent Res (Special Issue) 65:353, 1986.
51. Hassell TM, Romberg E, Sobhani S, Lesko L, Douglas R: Transplant Proc 20 [Suppl 3]:993–1002, 1988.
52. Dinarello C, Savage N: Clin Rev Immunol 9:1–20, 1989.
53. Le J, Vilcek J: Lab Invest 56:234–248, 1987.
54. Chervenick P, Lobuglio A: Science 178:164–166, 1972.
55. Hamblin AS: In: Male D, Rickwood D (eds). "Lymphokines," Washington, DC., IRL Press, Ltd., 1988, pp 1–12.
56. Bendtzen K: Immunol Lett 19:183–192, 1988.
57. Assoian R, Fleurdelys B, Stevenson P, et al.: Proc Natl Acad Sci USA 84:6020–6024, 1987.
58. Richards D, Rutherford R: Arch Oral Biol 33:237–243, 1988.
59. Granelli-Piperno A, Keane M: Transplant Proc 20 [Suppl 2]:136–142, 1988.
60. Dayer J, Rochmonteix B de, Burrus B, et al.: J Clin Invest 77:645–648, 1986.
61. Dayer J, Beutler B, Cerami A: J Exp Med 162:2163–2168, 1986.
62. Sander B, Brigati C, Möller E: Scand J Immunol 23:435–440, 1986.
63. Bartold PM: J Periodont Res 23:139–147, 1988.
64. Mochan E, Armor L, Sporer R: J Periodont Res 23:28–32, 1988.
65. Newton R, Covington M: Cell Immunol 110:338–349, 1987.
66. Dower S, Kronheim S, March C, et al.: J Exp Med 162:501–515, 1986.
67. Qwarnstrom E, Page R, Gillis S, Dower S: J Biol Chem 263:8261–8269, 1988.
68. Bonin P, Singh J: J Biol Chem 263:11052–11055, 1988.
69. Tsujimoto M, Yip Y, Vilcek J: Proc Natl Acad Sci USA 82:7626–7630, 1985.
70. Woodruff M, Ansell J, Forbes GM, et al.: Nature 299:822–824, 1982.
71. Bartold PM: Connect Tissue Res 17:287–304, 1988.
72. Singh J, Adams L, Bonin P: J Cell Biol 106:813–819, 1988.
73. Rosenblum M, Donato N: Crit Rev Immunol 9:21–44, 1989.