Murine fibroblasts synthesize and secrete kininogen in response to cyclic-AMP, prostaglandin E₂ and tumor necrosis factor

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Abstract

Fibroblasts prepared from the meninges of newborn mice or from mouse embryos, as well as fibroblast L929 cells, secreted an immunoreactive material (ir-kininogen) against rabbit anti-mouse low-molecular-weight kininogen antibody in response to dibutyryl cAMP. Western blots using a bradykinin-directed monoclonal, as well as a polyclonal anti-mouse low-molecular-weight kininogen antibody, showed that ir-kininogen had a molecular weight of 80 000 and that it contained a kinin moiety. N-terminal amino acid sequence of the ir-kininogen was consistent with that of mouse L-kininogen. The ir-kininogen produced by fibroblasts released a kinin by incubating with trypsin and mouse submandibular gland kallikrein, and it was identified as bradykinin by means of high-performance liquid chromatography, indicating that mouse fibroblasts produce and secrete a kininogen. Forskolin, prostaglandin E₂ and tumor necrosis factor α stimulated the production of ir-kininogen by meningeal fibroblasts, whereas neither dibutyryl cAMP nor these agents influenced kininogen production by mouse hepatocytes in primary cultures. These results demonstrated that fibroblasts are a source of kininogen in the mouse, and that it is regulated by the inflammatory mediators, prostaglandin E₂ and tumor necrosis factor. Therefore locally produced kininogen is implicated in pathogenesis of inflammation.

Keywords: Kininogen; Fibroblast; cAMP; Prostaglandin E₂; Tumor necrosis factor; Inflammation; (Mouse)

1. Introduction

Kininogens are endogenous protein substrates for tissue and plasma kallikreins, which by proteolytic cleavage form vasoactive kinin peptides [1]. In addition, kininogens contain domains that function as cysteine proteinase inhibitors and act as cofactors for the contact activation of blood coagulation [1]. Three forms of kininogens have been identified in mammals: high-molecular-weight kininogen (H-kininogen), low-molecular-weight kininogen (L-kininogen) and T-kininogen [2]. We found a kininogen in the ascitic fluid of tumor-bearing mice [3], and subsequently identified it as mouse L-kininogen by analyzing amino acid sequences and the susceptibility to glandular kallikreins [4].

It has been thought for many years that the source of kininogens is restricted to the liver. However, it has been demonstrated that L- or H-kininogen gene is expressed in human kidney or endothelial cells, respectively [5,6]. In the rat, T-kininogen gene is expressed in extrahepatic tissues including the lung, kidney, brain, and heart [7]. These lines of evidence suggest that the local tissues and organs independently synthesize kininogens. However, further evidence supporting this hypothesis has not been produced.

Fibroblasts are ubiquitously distributed in several organs and tissues, and play a critical role in morphogenesis, dictating the structure of the skeleton, and organization of the skin [8]. When connective tissue sustains an injury, fibroblasts migrate to the site from neighboring connective tissue, where they proliferate, synthesize and remodel new matrix to repair the damage [9]. Thus, fibroblasts play an important role in the process of inflammatory reactions and tissue repair. To obtain evidence supporting the exis-
tence of a local kallikrein-kinin system, we studied whether or not mouse fibroblasts produce L-kininogen in vitro. The results demonstrated for the first time that mouse fibroblasts produce L-kininogen in response to dibutyryl cAMP (Bt2cAMP), forskolin, prostaglandin E$_2$ (PGE$_2$) and tumor necrosis factor $\alpha$ (TNF).

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial sources: Bt2cAMP from Boehringer-Mannheim-Yamanouchi; forskolin, PGE$_2$, trypsin (bovine pancreas) and kallikrein (porcine pancreas) from Sigma; horseradish peroxidase-labeled avidin and biotinylated goat anti-rabbit IgG from Bio-Rad; Immobilon-PSQ membrane from Millipore; protein A-agarose from Pierce; bradykinin and T-kinin from Peptide Institute (Osaka, Japan); Na$^{+51}$ (94.3 GBq/μmol) from American Radiolabeled Chemicals; Dulbecco’s modified Eagle’s medium (DMEM) from Nissui (Osaka, Japan); Williams’ medium E from ICN Biomedicals; rabbit anti-mouse fibronectin from Funakoshi (Tokyo, Japan); octadecyl disposable-column from J.T. Baker; recombinant mouse interleukin-6 (IL-6; 1-10$^7$ U/mg) from Genzyme; L929 cells, Balb/3T3 clone A31 (3T3) cells and SV40-transformed Balb/3T3 (SV40-3T3) cells from Dainippon Pharmaceutical, Osaka, Japan. Recombinant human interleukin-1$\alpha$ (IL-1; 2-10$^7$ U/mg) and TNF (3-10$^9$ U/mg) were donated by Dainippon Pharmaceutical (Osaka, Japan). The bradykinin-directed monoclonal antibody to kininogen was a gift from Dr. J. Chao, Department of Pharmacology, Medical University of South Carolina, Charleston, USA. Mouse kallikrein was purified from submandibular glands as described previously [3].

2.2. Cell culture

Fibroblasts were prepared from the meninges and the embryos of ICR mice as followed. The meninges were isolated from the hemispheres of 1-day-old newborn mouse under a light microscope, incubated with 0.05% trypsin/0.02% EDTA for 2 min at 37°C, then triturated using a pasteur pipette. The suspended cells were washed twice with DMEM containing 5% fetal bovine serum (FBS), then filtered through a stainless steel net (mesh size of 150 μm). Cells in the filtrate were seeded in 90-mm culture dishes with DMEM supplemented with 5% FBS and 50 μg/ml of gentamycin (growth medium) and cultured at 37°C in humidified atmosphere of 95% air and 5% CO$_2$. Embryonic fibroblast cultures were initiated from 16–18-day-old decapitated and degutted embryos. The embryonic skin was washed with phosphate-buffered saline, pH 7.5, minced with scissors, then digested twice by stirring gently in 0.05% trypsin/0.02% EDTA for 5 min each. After washing and filtration, the cells were cultured as described above. After reaching confluence, cells were passaged by incubation with trypsin, and reseeded onto 90-mm culture dishes. After reaching confluence again, the medium was replaced with FBS-free DMEM. These cultures contained mainly fibronectin-positive cells (>95%), when tested with rabbit antibody directed against mouse fibronectin.

The mouse fibroblasts cell lines, L929, 3T3 and SV40-3T3 cells, were maintained in the growth medium as described above. Experiments were carried out at confluence in FBS-free DMEM.

Parenchymal hepatocytes were isolated from male ICR mice, weighing 35–40 g, by perfusing the liver with collagenase in situ, essentially as described by Seglen [10]. The cells (2·10$^7$/well) were seeded in 24-well culture plates in 1 ml of Williams’ medium E supplemented with 5% FBS, 10 nM insulin, 10 nM dexamethasone and 50 μg/ml of gentamycin, and cultured at 37°C in 95% air and 5% CO$_2$. The medium was replaced after the first 2 h of culture to remove unattached cells. After 24 h, cells were used for study.

2.3. Radioimmunoassay of mouse kininogen

Kininogen was determined by a radioimmunoassay using $^{125}$I-L-kininogen and rabbit anti-L-kininogen antibody, as described [3]. This assay detected 0.2 ng of L-kininogen. The intra-assay precision, as represented by the coefficient of variation of duplicates ranging throughout the standard curve, was ±4.2%, and the inter-assay reproducibility was 5.3%. Cell protein levels were measured using the BCA protein Assay Reagent (Pierce).

2.4. Western blots of kininogen

Mouse meningeal or SV40-3T3 fibroblasts were cultured for 5 or 2 days, respectively, in FBS-free DMEM containing 1 mM Bt2cAMP or a combination of 1 mM Bt2cAMP and 1 μM dexamethasone, respectively, and the conditioned medium was harvested, dialyzed against distilled water, then lyophilized. The lyophilized sample, together the conditioned medium of mouse hepatocytes, mouse serum and the ascitic fluid of Sarcoma 180 tumor-bearing mice, were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% gel according to the method of Laemmli [11]. The resolved proteins were electrophoretically transferred to Immobilon-PSQ membrane [12], which was then blocked with 10% skim milk followed by incubation with rabbit anti-mouse L-kininogen or bradykinin-directed kininogen monoclonal antibody. Rabbit IgG or mouse IgG on the membrane was detected by means of chemiluminescent protein detection system (Immuno-Lite II, Bio-Rad) using goat anti-rabbit or
2.5. Assay and identification of kinin liberated from the conditioned medium of fibroblasts

SV40-3T3 cells were cultured in FBS-free DMEM supplemented with 1 mM Bt2cAMP and 1 μM dexamethasone for 2 days. The conditioned medium was dialyzed against distilled water and lyophilized. The lyophilized sample was dissolved in 0.1 M Tris-HCl, pH 8.0, containing 2 mM o-phenanthroline, and an aliquot (1.8 μg) was incubated with kininogenases, such as bovine pancreas trypsin, porcine pancreas kallikrein, and mouse submandibular gland kallikrein, at 37°C for 90 min. The reaction was terminated by heating in boiling water for 2 min, and the liberated kinin was determined by means of the bradykinin radioimmunoassay using 125I-Tyr-kallidin and rabbit anti-bradykinin antibody, as described previously [3].

The lyophilized sample described above was heated in boiling water for 3 min to inactivate kinin-destroying enzymes, then incubated with trypsin (200 μg/ml) in 50 mM Tris-HCl, pH 8.0, for 2 h. The reaction was terminated by adding trifluoroacetic acid (TFA) at a final concentration of 30%, and the mixture was applied to an octadecyl extraction-column (3-ml bed volume), primed with methanol followed by 1% TFA. The column was washed 3 times with 5 ml of 20 mM Tris-HCl in 0.1 M NaCl (pH 8.0), then eluted with 90% methanol containing 0.1% TFA. The eluate was evaporated, and the residue was dissolved in 40 mM triethylamine-formate, pH 4.0, then analyzed by reverse-phase high-performance liquid chromatography (HPLC) (model 880-PU; Japan Spectroscopic), using an octadecyl column (Biofine RPC-SC18B), equilibrated with a mixture of 40 mM triethylamine-formate (pH 4.0) and acetonitrile (81/19) [2]. Kinin was isocratically eluted with the same solvent at a flow rate of 0.8 ml/min, and 1-min fractions were collected. After evaporating the solvent from each fraction, the kinin levels were determined by the kininogen radioimmunoassay. A mixture containing 0.5 μg each of standard bradykinin and T-kinin (ile-ser-bradykinin) was analyzed by HPLC under the same conditions.

2.6. Purification and N-terminal amino acid sequence analysis of kininogen from conditioned medium of mouse fibroblasts

Meningeal fibroblasts were cultured in FBS-free DMEM containing 1 mM Bt2cAMP for 5 days, and the conditioned medium (500 ml) was dialyzed against distilled water, then lyophilized. The lyophilisate sample was dissolved in 5 ml of 20 mM Tris-HCl in 0.1 M NaCl (pH 8.0), then centrifuged at 3000 x g for 5 min. The supernatant was applied to a column (2-ml bed volume) of rabbit anti-mouse L-kininogen IgG cross-linked with protein A-agarose [13]. The column was washed with 50 ml of 20 mM Tris-HCl in 0.1 M NaCl (pH 8.0) and eluted with 0.1 M glycine-HCl (pH 2.5). Fractions containing kininogen were pooled, dialyzed against distilled water, then lyophilized. The lyophilisate sample was resolved by SDS-PAGE, and proteins were transferred to Immobilon-PSQ membrane as described above, then stained by Coomassie brilliant blue. Membrane strip of the protein band was subjected to N-terminal sequence analysis.

The N-terminal sequence of purified protein was determined by automated Edman degradation on a pulsed-liquid sequencer (Model PPSQ-10, Shimazu, Kyoto, Japan) connected on line to an HPLC apparatus for PTH-amino acid identification.

2.7. Statistical analysis

Results are presented as mean ± S.D. The statistical significance of the difference between means was determined by Student's t-test.

3. Results

3.1. Mouse fibroblasts secreted kininogen-like material

Kininogen radioimmunoassay using anti-mouse L-kininogen antibody revealed that the conditioned media of mouse meningeal, embryonic, L929 and SV40-3T3 fibroblasts contained kininogen-like immunoreactive material (ir-kininogen). The conditioned medium of meningeal, embryonic or L929 fibroblasts cultured for 5 days contained 35.2, 9.2 or 6.9 ng/mg cell protein (n = 3), respectively, and that of SV40-3T3 cells after 2 days contained 117 ng/mg (n = 3). We could not detect ir-kininogen in the conditioned medium of 3T3 cells, the parent line of the SV40-3T3 cells, within the detection limit of the L-kininogen radioimmunoassay.

3.2. Effects of Bt2cAMP, forskolin and dexamethasone on the secretion of ir-kininogen by mouse fibroblasts

The ir-kininogen secreted by meningeal or L929 fibroblasts was stimulated by 1 mM Bt2cAMP, and by forskolin (10 μM), an activator of adenylate cyclase, for at least 5 days (Fig. 1), in a dose-dependent manner (data not shown). Bt2cAMP also stimulated the secretion of ir-kininogen by embryonic fibroblasts (data not shown).

When the conditioned medium of Bt2cAMP-stimulated meningeal or L929 fibroblasts was concentrated by lyophilization and assayed by kininogen radioimmunoassay, the displacement of 125I-L-kininogen binding to antibody by various concentrations of lyophilized sample paralleled that by standard L-kininogen (data not shown).

Dexamethasone (1 μM) alone did not influence the basal secretion of ir-L-kininogen by meningeal and L929 anti-mouse IgG, respectively, according to the instruction manual provided with the kit.
fibroblasts, whereas it inhibited the Bt2cAMP-induced secretion of ir-kininogen by 70% in meningeal fibroblasts or by 86% in L929 cells (Fig. 1). In contrast, ir-kininogen secretion by SV40-3T3 cells was not affected by Bt2cAMP alone, but was markedly stimulated by the addition of both 1 mM Bt2cAMP and 1 μM dexamethasone (Fig. 2).

3.3. Western blots of ir-kininogen secreted by mouse fibroblasts

To characterize ir-kininogen, meningeal or SV40-3T3 fibroblasts were cultured for 5 or 2 days in the presence of Bt2cAMP or a combination of Bt2cAMP and dexamethasone, respectively, and the conditioned media were lyophilized. These samples, together with purified mouse L-kininogen, ascitic fluid of Sarcoma 180 tumor-bearing mouse, mouse serum and conditioned medium of mouse hepatocytes in primary culture, were resolved by SDS-PAGE, and the immunoreactivity was localized following Western blotting using rabbit anti-L-kininogen antibody. At least two major bands with molecular weights of about 80,000 and 67,000 were detected in ascitic fluid, serum and conditioned medium of hepatocytes, and the mobility of the band with a molecular mass of 67,000 corresponded to that of purified L-kininogen (Fig. 3). The conditioned medium of meningeal or SV40-3T3 fibroblasts showed virtually a single band, with a molecular weight that was slightly higher than that of purified L-kininogen and which corresponded to an immunoreactive band with a molecular weight of 80,000 in ascitic fluid, serum or conditioned medium of hepatocytes (Fig. 3).

When the immunoreactivity on the blot was localized by the bradykinin-directed monoclonal antibody for kininogens, an immunoreactive band with a molecular weight of 80,000 was found in the conditioned medium of either meningeal or SV40-3T3 fibroblasts, as well as in that of mouse hepatocytes (Fig. 3). There was also a faint band corresponding to a molecular weight of 67,000 in the conditioned medium of hepatocytes.

3.4. N-terminal amino acid sequence of ir-kininogen purified from the conditioned medium of mouse fibroblasts

N-terminal amino acid sequence (30-residues) of ir-kininogen, which was purified from the conditioned medium of meningeal fibroblasts by an immunoaffinity chromatography followed by SDS-PAGE, was EEAQEID-CNDEAVFOAVDFSLLKFQNVKS that corresponded to N-terminal sequence of mouse L-kininogen purified from ascitic fluid [4].

3.5. Kinin liberation by trypsin and kallikreins from the conditioned medium of SV40-3T3 cells

Mouse L-kininogen liberates bradykinin upon exposure to trypsin or mouse submandibular gland kallikrein, whereas it is relatively unsusceptible to tissue kallikreins of other species, such as porcine pancreatic and rat urinary kallikreins [3,4]. To further characterize ir-kininogen, the conditioned medium of SV40-3T3 cells was lyophilized, then incubated with trypsin, porcine pancreatic kallikrein and mouse submandibular gland kallikrein. As shown in Fig. 4, the conditioned medium liberated a kinin, that was determined by bradykinin radioimmunoassay, upon incubation with trypsin and mouse submandibular gland
Fig. 3. Western blots of ir-kininogen secreted by mouse fibroblasts. Meningeal or SV40-3T3 fibroblasts were cultured with 1 mM Bt2cAMP for 5 days or with a combination of 1 mM Bt2cAMP and 1 μM dexamethasone for 2 days, respectively. The conditioned medium was concentrated, and aliquots (100 ng ir-kininogen of meningeal fibroblasts and 25 ng ir-kininogen of SV40-3T3 cells) were separated by SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. Purified mouse L-kininogen, ascitic fluid of Sarcoma 180 tumor-bearing mouse, mouse serum, and conditioned medium of mouse hepatocytes in primary culture were also resolved (100 ng kininogen/lane). The blot was incubated with anti-L-kininogen antibody (panel A) or bradykinin-directed anti-kininogen monoclonal antibody (panel B) followed by detection with Bio-Rad Immun-Lite I. (A) Lane 1, L-kininogen; lane 2, ascitic fluid; lane 3, serum; lane 4, conditioned medium of mouse hepatocytes; lane 5, conditioned medium of SV40-3T3 cells; lane 6, conditioned medium of meningeal fibroblasts. (B) Lane 1, L-kininogen; lane 2, ascitic fluid; lane 3, conditioned medium of mouse hepatocytes; lane 4, conditioned medium of SV40-3T3 cells; lane 5, conditioned medium of meningeal fibroblasts.

Fig. 4. Kinin liberation from ir-kininogen by incubation with trypsin and kallikreins. SV40-3T3 cells were cultured for 2 days with 1 mM Bt2cAMP and 1 μM dexamethasone, then the conditioned medium was lyophilized. The lyophilized material containing 1.8 μg of ir-kininogen was incubated with trypsin and kallikreins at pH 8.0 for 90 min, and the amount of kinin generated was determined by a bradykinin radioimmunoassay. The values represent the means of two determinations.

3.6. Effects of PGE₂, TNF, IL-1 and IL-6 on the secretion of ir-kininogen by meningeal and L929 fibroblasts

The fact that both Bt2cAMP and forskolin stimulated the secretion of ir-kininogen by mouse fibroblasts suggested that elevated intracellular cAMP levels triggered ir-kininogen production in fibroblasts. To confirm this notion, we studied the effect of PGE₂, which stimulates cAMP synthesis in fibroblasts [14], on the secretion of ir-kininogen by fibroblasts. As shown in Fig. 5, 10 μM PGE₂ did not affect the secretion of ir-kininogen by meningeal and L929 fibroblasts within 24 h, but significantly stimulated it after 24 h. A lower concentration (0.1 μM) of PGE₂ also stimulated L929 cells.

Fig. 5. The effect of PGE₂ on the secretion of ir-kininogen by mouse meningeal and L929 fibroblasts. Fibroblasts were cultured for 3 days under basal conditions (○) or in the presence of PGE₂, at 0.1 μM (●) and 10 μM (▲). The levels of ir-kininogen in the conditioned medium was determined by kininogen radioimmunoassay. The results are expressed as the means ± S.D. of ir-kininogen/mg cell protein of 4 wells. Statistical analyses in comparison to unstimulated culture: * P < 0.05, ** P < 0.01, *** P < 0.001.
Since cytokines such as TNF and IL-1 increase PGE\(_2\) synthesis in mouse fibroblasts, which in turn stimulates cAMP synthesis [15,16], we studied the effect of these cytokines on the secretion of ir-kininogen by fibroblasts. The secretion of ir-kininogen by meningeal fibroblasts was stimulated by TNF (5–500 U/ml), but not by IL-1 or IL-6 at a concentration of 500 U/ml (Fig. 6).

3.7. Effects of BT2cAMP, PGE\(_2\), TNF and IL-6 on the secretion of kininogen by mouse hepatocytes

Mouse hepatocytes secreted 1.03 ± 0.18 \(\mu\)g kininogen/mg cell protein after 2 days in culture (mean ± S.D. of 4 wells), as measured by kininogen radioimmunoassay, and the secretion was not altered by BT2cAMP (1 mM), PGE\(_2\) (10 \(\mu\)M), TNF (500 U/ml), IL-1 (500 U/ml) and IL-6 (500 U/ml) (data not shown).

4. Discussion

Mouse L-kininogen was first isolated as a cysteine proteinase inhibitor from the ascitic fluid of the Sarcoma 180 tumor-bearing mouse and subsequently characterized as L-kininogen [3,4]. It has a highly homologous structure to other mammalian L-kininogens and releases bradykinin upon exposure to trypsin and mouse submandibular gland kallikrein, but is relatively unsusceptible to kallikreins of other species, which probably due to a unique -Met-Ala-Arg-bradykinin sequence [3,4]. We investigated two primary cultures (meningeal and embryonic) and three cell lines (L929, 3T3 and SV40-transformed 3T3) of mouse fibroblasts to determine whether fibroblasts produce kininogen. Except for 3T3 cells, all fibroblasts secreted ir-kininogen during culture, though the levels were very low as compared to that by mouse hepatocytes. Western blotting using anti-L-kininogen antibody revealed that the molecular weight of ir-kininogen secreted by mouse meningeal or SV40-3T3 fibroblasts was slightly higher than that of L-kininogen. A similar protein was also present in ascitic fluid, serum and conditioned medium of cultured hepatocytes. A monoclonal antibody against bradykinin moiety of kininogens recognized the same molecule in the conditioned medium of fibroblasts, as well as in that of cultured hepatocytes, indicating that the ir-kininogen contains bradykinin sequence. This was confirmed by findings that trypsin and mouse submandibular gland kallikrein liberated kinin from fibroblast-conditioned medium, but that porcine kallikrein had little effect. Furthermore, N-terminal amino acid sequence (30 residues) in kininogen secreted from meningeal fibroblasts was the same in mouse L-kininogen. Thus, mouse fibroblasts secrete a kininogen that is indistinguishable from L-kininogen with respect to immunoreactivity, susceptibility to tissue kallikreins and N-terminal structure. However, we cannot exclude a possibility that a polyclonal antibody to mouse L-kininogen used in this study recognized H-kininogen as well as L-kininogen, since a heavy chain at the amino-terminus of kininogen, a large portion of kininogen structure, is identical in L- and H-kininogens [1]. Therefore, further study is required to determine the relationship between the 80 kDa-kininogen derived from fibroblasts and L- or H-kininogen in plasma, especially with regard to their structures of light-chains.

The kininogen production by fibroblasts, except for SV40-3T3 cells, was markedly stimulated by BT2cAMP and forskolin, suggesting that the elevation of intracellular cAMP triggers an induction of kininogen synthesis in fibroblasts. BT2cAMP-induced kininogen production was inhibited by a low concentration of dexamethasone. In contrast, kininogen production by SV40-3T3 cells was not altered by BT2cAMP alone but was stimulated with a combination of BT2cAMP and dexamethasone. Why the responsiveness of SV40-3T3 cells to dexamethasone differs from that of other fibroblasts is unknown, but it is possible that a signal transduction pathway in the cAMP-dependent regulation of ir-kininogen expression is altered in the transformed cells.

We found that PGE\(_2\) and TNF stimulated kininogen production by mouse fibroblasts. Since PGE\(_2\) potently stimulates intracellular cAMP levels [14], it is likely that the effect is mediated by cAMP. Burch et al. have reported that TNF stimulates PGE\(_2\) synthesis in mouse fibroblasts, which in turn, stimulates cAMP accumulation [15], suggesting that the effect of TNF on kininogen production is secondary to PGE\(_2\) release. However, IL-1, which also elevates intracellular cAMP levels in fibroblasts via stimulating PGE\(_2\) synthesis [16], did not influence kininogen production. The precise mechanisms by which TNF stimulates kininogen production in fibroblasts remain to be determined.

PGE\(_2\) and TNF are produced at sites of inflammation and play important roles in its pathogenesis [17,18]. These
inflammatory mediators stimulated kininogen production by fibroblasts, suggesting that kininogen production is enhanced in the connective tissues during inflammation, therefore implying the involvement of kininogen in the generation and maintenance of such conditions. The fact that neither these inflammatory mediators nor cAMP-elevating agents influenced kininogen production by mouse hepatocytes may support this hypothesis. Kininogens are endogenous substrates for plasma and tissue kallikreins by which kinins are released. Kinins are important mediators of inflammation, producing vasodilatation, increased vascular permeability, leukotaxis and pain [19]. Tissue kallikreins are found in salivary, pancreas, pituitary and mucous/serous secreting glands [20,21]. A kallikrein-like enzyme has also been identified in basophils and mast cells [22]. Figueroa et al. have demonstrated that tissue kallikrein is present in the intracellular granules of human neutrophils that release kallidin from human L-kininogen [23]. Their findings suggested that neutrophils which accumulate at sites of inflammatory reactions, release tissue kallikrein by degranulation or secretion. This in turn, generates kinins from L-kininogen. These lines of evidence suggest that PGE₂ or TNF released from fibroblasts themselves, as well as from neutrophils, macrophages and lymphocytes [17,18] at the site of inflammation, triggers the induction of kininogen production by fibroblasts, thereby releasing kinins by the action of kallikreins that derive from the circulation or are produced at local tissues. Like interactions involving cells in inflammation, there seems to be complex interactions between the generation of kinins and other inflammatory mediators. PGE₂ and TNF may enhance kinin generation via the stimulation of kininogen production, then the released kinin stimulates PGE₂ release from fibroblasts and other cells [19] and TNF release from macrophages [24]. Such amplification by positive feedback loops may be an important mechanism in maintaining chronic inflammation. This may be true of rheumatoid arthritis where prostaglandins, cytokines and kinins are present [18,25,26]. Further studies are required to confirm this hypothesis.

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