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Bradykinin stimulates tumor necrosis factor and interleukin-1 release from macrophages

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Bradykinin and related kinins have been implicated in the initiation and maintenance of inflammation. Cytokines appear to be the primary mediators of many inflammatory diseases. The potential ability of bradykinin to stimulate release of tumor necrosis factor and interleukin-1 from macrophages was examined. Bradykinin stimulated release of both cytokines from P388-D1 and RAW264.7 murine macrophages. Studies with selective agonists and antagonists suggest that cytokine release is mediated by a B_1 kinin receptor.

Bradykinin; Cytokine; Tumor necrosis factor; Interleukin-1; Macrophage; Bradykinin antagonist

1. INTRODUCTION

Multiple mediators appear to play roles in the pathogenesis of chronic inflammation. Macrophages and lymphocytes interact to release a variety of inflammatory cytokines, including tumor necrosis factor (TNF) and interleukin-1 (IL-1) which induce proliferation of connective tissue and stimulate eicosanoid and proteolytic enzyme release [1].

Bradykinin, unlike many other biologically active peptides, is synthesized at the site of its action by plasma or tissue kallikrein, acting on several circulating kininogen substrates [2]. It is rapidly degraded at its site of action by kininases, principally by circulating kininase I, which yields desArg^{9}bradykinin [3], and in some tissues by kininase II, which cleaves the 2 carboxy-terminal residues [3]. Several lines of evidence suggest a role for bradykinin and related kinins in the generation and maintenance of chronic inflammation. All components of the kinin system: kininogens, kallikrein, and kinins, have been reported from inflammatory effusions [3].

In at least some instances, certain kininogen substrates are among the acute phase proteins induced by inflammatory cytokines [4]. Kinins reproduce several inflammatory processes also induced by cytokines including increased capillary permeability, stimulation of eicosanoid synthesis and induction of connective tissue proliferation [3]. We now report that bradykinin and desArg^9-bradykinin stimulate macrophages to release TNF and IL-1.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

SV-T_2 fibroblasts (ACTCC CCL 163.1), 3T3-L1 adipocytes (ATCC CCL 92.1), P388-D1 macrophages (ATCC TIB 63) and RAW264.7 macrophages (ATCC TIB 71) obtained from the American Type Culture Collection were maintained as previously described [5-8].

2.2. *Stimulation protocol*

Macrophages were washed once with serum-free medium. Then serum-free medium containing the kinin analog was added, and incubation continued for 24 h. Supernatants were collected, centrifuged to remove any cells, and calf serum was added to 10% final concentration to degrade bradykinin [9]. High-pressure liquid chromatography was used to confirm that all bradykinin was degraded [9].

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2.3. *Kinin analogs*

Both IL-1 [5] and TNF [10] stimulate prostaglandin E_2 (PGE) synthesis in SV-T_2 fibroblasts. Dose responses for various kinin agonists and antagonists on fibroblast PGE synthesis were determined by radioimmunoassay of PGE in fibroblast supernatants as described [5]. Fibroblasts were incubated with serum-repleted macrophage supernatants for 24 h.

2.4. *IL-1 activity*

IL-1 activity was measured as the ability to stimulate [^{3}H]thymidine incorporation in thymocytes [11]. Activity in the cell supernatants was quantitated by comparing the ability of serial dilutions of supernatant to stimulate [^{3}H]thymidine incorporation with standard concentrations of recombinant IL-1.

2.5. *TNF activity*

TNF completely inhibits lipoprotein lipase activity in 3T3-L1 cells [12]. Activity in the cell supernatants was quantitated by comparing the ability of serial dilutions of supernatant to inhibit lipoprotein lipase with recombinant murine TNF.

2.6. *Labeling of macrophage proteins*

Macrophage culture media were supplemented with [^{14}C]valine or [^{35}S]methionine, then kinins added for 24 h. Media were collected, filtered through 0.2 μM membranes to remove cells, and concentrated by ultrafiltration through 10 kDa cut-off membranes (Amicon, Danvers, MA). The concentrated supernatants were subjected to 12% polyacrylamide gel electrophoresis in SDS [13], silver stained (BioRad), dried and subjected to autoradiography.

2.7. *Materials*

Bradykinin, desArg^{9}bradykinin was obtained from Nova Pharmaceutical Corporation. Recombinant human IL-1β, recombinant murine TNF, and anti-murine TNF were obtained from Genzyme, Boston, MA.

2.8 *Statistical analyses*

Experiments were run in multi-well plates. Comparison were thus made using Student's *t*-test for paired observations considering the 3 triplicate wells for each manipulation in a single plate as one observation.

3. RESULTS

3.1. *Kinins stimulate cytokine release from macrophages*

When 24-h macrophage supernatants were serum-repleted and added to SV-T_2 cells, PGE synthesis was stimulated. For example, in 5 experiments, control PGE synthesis was 1.1 ± 0.5 ng/ml. Addition of P388-D1 supernatant caused PGE synthesis to increase to 11.2 ± 3.6 ng/ml, and when bradykinin (10 μM) had been added to the macrophages, PGE synthesis further increased to 31.0 ± 17.7 ng/ml. If macrophages had been incubated with bradykinin-free media, then media collected and bradykinin plus serum added to degrade it, the degradation products of bradykinin did not affect PGE synthesis. The increased PGE synthesis in the absence of bradykinin represents cytokine released basally in these cells [7] since anti-TNF serum blocked basal and bradykinin-stimulated activity (see below).

Bradykinin receptors are divided into 2 main subtypes, B_1 and B_2 [14]. In additional experiments (table 1), it was found that the B_1-selective agonist desArg^{9}bradykinin stimulated cytokine release as well as bradykinin, and the mixed B_1/B_2 antagonist DArg[Hyp^{3}DPhe^7]bradykinin [2] and the B_1-selective antagonist desArg^9[Leu^8]bradykinin [14] blocked kinin-induced cytokine release. A similar pattern was observed when another macrophage line, RAW264.7, was used (data not shown).

3.2. *Kinins stimulate release of 17 kDa proteins selectively*

When bradykinin was added to macrophages in the presence of [^{14}C]valine or [^{35}S]methionine and supernatants collected, it was found that the kinin rather selectively caused release into the media of protein(s) of about 17 kDa (fig.1).

3.3. *Kinins stimulate TNF release*

Both TNF and IL-1 inhibit lipoprotein lipase in 3T3-L1 cells [12], but IL-1 is able to inhibit about

Table 1

Kinins release cytokine activity from P388-D1 macrophage

Treatment	PGE (% of control)
Bradykinin (1 μM)	130 ± 50[a]
Bradykinin (100 μM)	187 ± 32[b]
Bradykinin (100 μM) + DArg[Hyp^{3}DPhe^7]bradykinin (1 mM)	89 ± 18[a]
Bradykinin (100 μM) + desArg^9[Leu^8]bradykinin (100 μM)	138 ± 13[c]
desArg^{9}bradykinin (1 μM)	118 ± 36[a]
desArg^{9}bradykinin (100 μM)	223 ± 70[b]
desArg^{9}bradykinin (100 μM) + desArg^9[Leu^8]bradykinin (100 μM)	147 ± 27[c]

[a] Not significantly different from control
[b] $P<0.05$ compared to control
[c] $P<0.05$ compared to agonist
Mean ± SE for 3 experiments. Neither DArg[Hyp^{3}DPhe^7]-bradykinin nor desArg^9[Leu^8]bradykinin had any effect by themselves at concentrations up to 1 mM

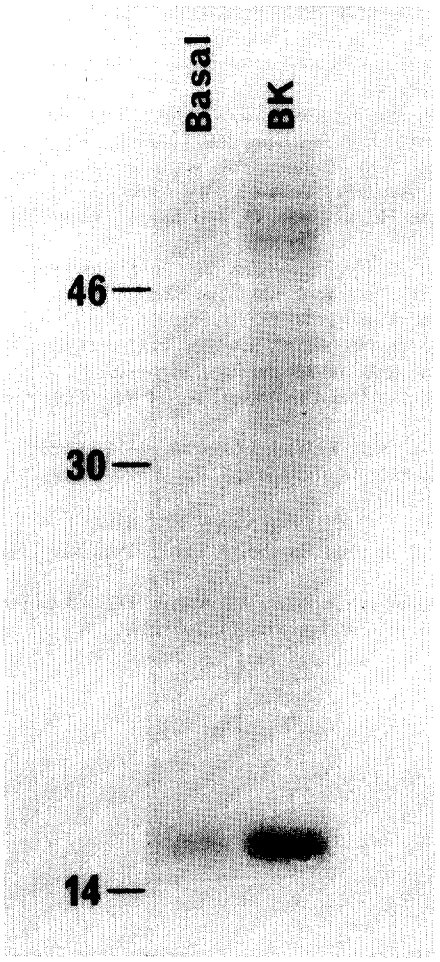

Fig.1. Bradykinin stimulates release of 17 kDa protein(s) from P388-D1 macrophages. Cells were labeled with [^{35}S]methionine for 24 h in the presence (BK) or absence (control) of 10 μM bradykinin. The proteins released into the supernatant were processed (section 2) and subjected to polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol. Gels were dried and subjected to autoradiography. Values on the left indicate migration of marker proteins (kDa).

50% of the activity while TNF is able to completely inhibit activity [12]. We confirmed this finding using recombinant TNF and IL-1 (data not shown). P388-D1 macrophage supernatant completely inhibited lipoprotein lipase activity. Quantitation of TNF release using serial dilutions of macrophage supernatant revealed that bradykinin increased TNF release 6-fold (table 2A). That the cytokine released was primarily TNF is supported by the observation that anti-TNF largely neutralized by

Table 2

Kinins stimulate release of TNF and IL-1 from macrophages

Treatment	TNF (pmol/well)	IL-1 (pmol/well)
A. Kinin-stimulated cytokine concentrations		
Control	0.5 ± 0.1	0.034 ± 0.012
Bradykinin (10 μM)	2.6 ± 0.15	0.076 ± 0.015
desArg^{9}bradykinin (10 μM)	–	0.082 ± 0.022
		Lipoprotein lipase (nmol/min per well)
B. Anti-TNF neutralizes macrophage cytokine activity		
Control		4.30
P388-D1 media		2.05
P388-D1 media + 1:500 anti-TNF		1.98
P388-D1 media + 1:125 anti-TNF		2.59
P388-D1 media + 1:62.5 anti-TNF		3.64

In all experiments P388-D1 macrophages were grown in 24-well plates. $n = 2$–5 for A; a representative experiment (among 3 experiments) is shown for B. Macrophage media was incubated with assay cells for 3 days (IL-1) or 24 h (TNF)

the ability of the macrophage supernatant to inhibit lipoprotein lipase (table 2B). In control experiments the antibody was found to neutralize authentic TNF without affecting authentic IL-1 (data not shown).

3.4. *Kinins stimulate IL-1 release*

Using a thymocyte activation assay, bradykinin was found to stimulate release of IL-1 activity (table 2A). In this assay, we found that desArg^9-bradykinin added directly to the thymocyte assay could itself stimulate thymidine incorporation to about 20% the level of bradykinin- or desArg^9-bradykinin-incubated macrophage supernatant, confirming an earlier finding [15]. This activity was subtracted from the values in table 2A.

4. DISCUSSION

The present study demonstrates that kinins stimulate cytokine release from macrophages. Analog studies suggest that a B_1 kinin receptor mediates the effect. The stimulation was rather selective in that by electrophoresis, proteins of 17 kDa were increased predominantly, the molecular mass of both TNF and IL-1. The release of TNF was stimulated to a far greater extent than bradykinin (table 2A).

Bradykinin is generated at inflammatory sites in-

cluding rheumatoid joints [2]. Bradykinin acts on B_1 and B_2 receptors, being much more potent at B_2 receptors [14]. A major metabolic degradation product, desArg^{9}bradykinin is a selective agonist for the B_1 receptor [14]. Thus, the presence of B_1 receptors on macrophages that stimulate cytokine release, suggests that kinins may be significant stimulators of TNF and IL-1 release in inflammatory lesions. Further work will be required to test this hypothesis in vivo.

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