Pergamon

Biochemical Pharmacology, Vol. 50, No. 11, pp. 1851–1857, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)-02078-O

ADENOSINE MODULATION OF TUMOR NECROSIS FACTOR-α-INDUCED NEUTROPHIL ACTIVATION

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(*Received* 31 *March* 1995; *accepted* 27 *June* 1995)

Abstract—We hypothesized that adenosine, known to be released from inflammatory sites, could lessen the potentially damaging activity of neutrophils (PMN) primed by tumor necrosis factor-α (TNFα) at sites of infection. We investigated the effect of adenosine on PMN primed with cell-free medium from mononuclear leukocytes (MNL) that had been treated with lipopolysaccharide (LPS) yielding a conditioned medium rich in TNFα and on PMN primed with recombinant human TNFα (rhTNFα). LPS (10 ng/mL) minimally primed PMN, but LPS-MNL-conditioned medium increased PMN chemiluminescence in response to f-Met-Leu-Phe (fMLP) 1242% compared with unprimed PMN. LPS-MNL-conditioned medium contained adenosine (~30 nM). Converting the adenosine in the LPS-MNL-conditioned medium to inosine with adenosine deaminase (ADA) or blocking adenosine binding to PMN with the adenosine receptor antagonist 1,3-dipropyl-8-(phenyl-*p*-acrylate)-xanthine (BW A1433U) resulted in a near doubling of chemiluminescence. The LPS-MNL-conditioned medium contained TNFα (836 pg/mL; ~1 U/mL). Recombinant human TNFα (1 U/mL) primed PMN for a 1033% increase in chemiluminescence. Added adenosine decreased rhTNFα-primed PMN chemiluminescence (IC_{50} ~100 nM), and adenosine (100 nM) decreased both superoxide and myeloperoxidase release from rhTNFα-primed fMLP-stimulated PMN. The activity of adenosine was counteracted by ADA and BW A1433U, and the modulating effect of adenosine was on the primed response rather than on priming *per se*. Thus, physiological concentrations of adenosine reduce the effects of recombinant human TNFα and native human TNFα (released from LPS-treated MNL) on PMN activity. Endogenous adenosine may preclude or minimize damage to infected tissue by damping the TNFα-primed PMN oxidative response.

Key words: neutrophil; adenosine; tumor necrosis factor-α; superoxide; chemiluminescence; degranulation

The purine adenosine is a ubiquitous endogenous product that is released from injured tissues [1–4] and platelets [5, 6] as a result of the breakdown of purine nucleotides. Endogenous levels of adenosine are 4–5 times higher in the blood of septic patients compared with that of non-septic patients [7]. This secreted adenosine has the potential to diminish the production of deleterious oxidative metabolites by blocking PMN activation [4]. Adenosine, when bound to A_2 receptors on the PMN plasma membrane, inhibits the PMN oxidative burst in response to several stimuli including microbial chemotactic peptides [8, 9].

LPS§ from Gram-negative bacteria induce MNL and PMN to release cytokines including TNFα [10]. Both TNFα and LPS can prime PMN for an enhanced release of oxidative metabolites and non-oxidative products in response to a second stimulus [11]. While superoxide generation and granule enzyme release are necessary to control infection, PMN-induced tissue damage is thought to contribute to such conditions as septic shock and the acute respiratory distress syndrome.

We hypothesized that the adenosine released from inflammatory sites decreases tissue damage by lessening the oxidative burst of TNFα-primed PMN. We now report that physiological concentrations of adenosine (either spontaneously released from PMN and MNL or added to the PMN) decrease the enhanced PMN oxidative burst and degranulation primed by LPS-activated MNL conditioned medium and rhTNFα. Hence, adenosine may act as an immunomodulator in infected patients and thus lessen tissue damage associated with infection.

MATERIALS AND METHODS

Materials

HBSS was purchased from Whittaker M.A. Bioproducts (Walkersville, MD), HSA from Immuno-U.S. (Rochester, MN), heparin from Elkins-Sinn Inc. (Cherry Hill, NJ), LPS (extracted from *Escherichia coli* K235) from List Biochemical Laboratories (Campbell, CA), and ADA from Boehringer Mannheim (Indianapolis, IN). Cytochrome *c* (type VI from horse heart), catalase, SOD (from bovine liver), xanthine, xanthine oxidase, polymyxin B, purified human leukocyte MPO, *o*-dianisidine, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), and adenosine were purchased from the Sigma

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§ Abbreviations: ADA, adenosine deaminase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AU, arbitrary relative chemiluminescence units; BW A1433U, 1,3-dipropyl-8-(phenyl-*p*-acrylate)-xanthine; fMLP, f-Met-Leu-Phe; HBSS, Hanks' balanced salt solution; HSA, human serum albumin; LPS, lipopolysaccharide(s); MNL, mononuclear leukocyte(s); MPO, myeloperoxidase; PMN, neutrophil(s); rhTNFα, recombinant human tumor necrosis factor-alpha; SOD, superoxide dismutase; and TNFα, tumor necrosis factor-alpha.

Chemical Co. (St. Louis, MO). Ficoll–Hypaque was purchased from ICN Biomedicals (Aurora, OH), Accurate Chemical and Scientific Corp. (Westbury, NY) and Cardinal Associates (Sante Fe, NM).

BW A1433U (was a gift from the Burroughs Wellcome Co. (Research Triangle Park, NC), and rhTNFα (sp. act. ~600 pg/U) was a gift from the Dianippon Pharmaceutical Co. Ltd. (Osaka, Japan). Monoclonal IgG1 anti-human TNFα (cA2) was supplied by Centocor (Malvern, PA).

PMN preparation

Purified PMN (~98% PMN; >95% viable as determined by trypan blue exclusion) containing <50 pg/mL of LPS (as determined by the *Limulus* amebocyte lysate assay) were obtained from normal, heparinized (10 U/mL) venous human blood by a one-step Ficoll–Hypaque separation procedure [12]. The PMN were washed three times with HBSS. Residual erythrocytes were removed by hypotonic lysis. PMN experiments were conducted in HBSS containing 0.1% HSA unless otherwise stated.

Mixed MNL preparation

MNL (~15–20% monocytes and 80–85% lymphocytes) were obtained from the Ficoll–Hypaque separation (see "PMN preparation" above). The MNL were washed three times with HBSS and resuspended in HBSS containing 0.1% HSA.

Cell-free MNL conditioned medium

MNL (2×10^6/mL) were incubated with LPS (10 ng/mL) for 60 min. The MNL were subsequently removed by centrifugation, and the supernatants were recovered.

Assay for TNFα production

Release of TNFα from LPS-stimulated MNL was determined by incubating MNL (2×10^6/mL) for 60 min (HBSS-0.1% HSA; 37°; 5% CO_2) with or without LPS (10 ng/mL). After a 60-min incubation, the leukocytes were removed by centrifugation (2000 *g* for 10 min), and TNFα released into the surrounding medium was assayed by ELISA with kits purchased from Cistron Biotechnology (Pine Brook, NJ).

Assay for adenosine release into the medium

PMN, MNL, and PMN incubated with MNL were assayed for adenosine release by incubating PMN (1×10^6/mL) and MNL (2×10^6/mL) for 60 min at 37° with or without LPS (10 ng/mL). After the incubation, the leukocytes were removed by centrifugation (2000 *g* for 10 min), and the supernatants were frozen. The samples were assayed for adenosine by radioimmunoassay as described by Linden *et al.* [13].

Luminol-enhanced chemiluminescence

PMN were incubated with or without LPS (10 ng/mL), unstimulated MNL conditioned medium, and LPS-stimulated MNL conditioned medium, rhTNFα (1 U/mL), ADA (1–5 U/mL), BW A1433U (10 μM), and adenosine (1–10000 nM) at 37° and stimulated with fMLP (100 nM) in a Chronolog Photometer. Peak chemiluminescence was reported in AU compared with unstimulated chemiluminescence, which was assigned a value of zero.

Superoxide release

PMN, isolated as above, were incubated with or without rhTNFα (1 U/mL) at 37° for 30 min. Cytochrome *c* (120 μM), catalase (0.062 mg/mL), and fMLP (100 nM) were added, and the samples were incubated for 10 min more at 37°. SOD (200 U/mL) was added to matched samples. The samples were then iced and centrifuged (800 *g* for 10 min). The optical density of the supernatants was read at 550 nm against matched SOD samples and the nanomoles of SOD-inhibitable superoxide released in 10 min were calculated using an extinction coefficient of 2.11×10^4 cm^2/mmol [14].

We assayed by methods adapted from Goldstein *et al.* [15] for the possibility that the xanthine compound BW A1433U is a substrate for xanthine oxidase and thus could induce superoxide release. Briefly, BW A1433U (10 μM) was incubated in 1 mL of HBSS containing 0.1% HSA, cytochrome *c* (5 μM), and xanthine oxidase (3 mU/mL) with or without SOD (200 U/mL) for 10 min at 37°. The optical density of the solutions was read at 550 nm and the nanomoles of SOD-inhibitable superoxide released in 10 min were determined as described above. As a positive control, the production of superoxide when xanthine oxidase was incubated with xanthine (100 μM) was determined.

MPO release

MPO was measured by a method adapted from Klebanoff [16]. The samples from the chemiluminescence assay were iced, and the cells were removed by centrifugation. MPO was assayed by incubating 0.4-mL samples of cell-free supernatant with 0.6 mL of fresh dye (0.5 mL of H_2O_2 [5 mM] + 0.1 mL of *o*-dianisidine [20 mg/mL]). The samples were incubated for 20 min at room temperature and then iced. The OD was read at 460 nm against a water blank. The micrograms of MPO were calculated from a standard curve determined using purified human leukocyte MPO.

Statistics

Significance ($P < 0.05$) was determined by a paired Student's *t*-test.

RESULTS

Chemiluminescence of PMN primed with LPS-stimulated MNL-conditioned medium

In the following assays, luminol-enhanced chemiluminescence was used as a measure of PMN oxidative activity. LPS (10 ng/mL) primed PMN for a very small increase in chemiluminescence in response to fMLP (Figs. 1A, 1B and 2) ($P = 0.047$ compared with fMLP-stimulated chemiluminescence). Unstimulated MNL conditioned medium did not induce priming of PMN for increased oxidative activity in response to fMLP (Figs. 1C and 2). In contrast, cell-free LPS-stimulated conditioned medium from MNL primed PMN for a 12-fold increase in oxidative activity in response to fMLP (Figs. 1D and 2) ($P = 0.001$). Thus, LPS induced the MNL to release factor(s) into the medium, which primes PMN for increased oxidative activity in response to fMLP.

To determine the role of LPS carryover in the present experiment, we utilized polymyxin B as an LPS-inactivator. Polymyxin B treatment resulted in a loss of the conditioned medium's ability to prime PMN if added

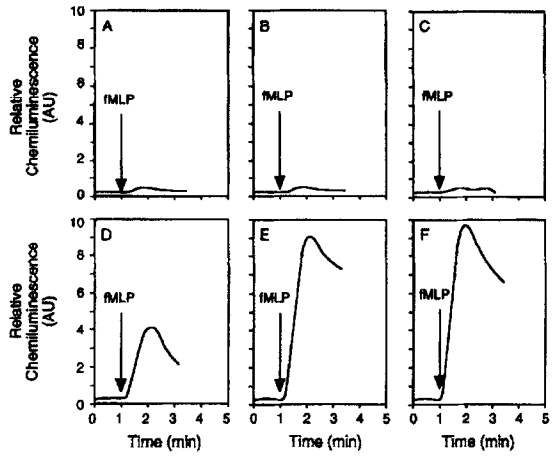

Fig. 1. Chemiluminescence traces showing ADA and BW A1433U enhancement of LPS-MNL-conditioned medium-primed fMLP-stimulated PMN oxidative activity. PMN (1×10^6) were incubated at 37° for 30 min with or without LPS (10 ng/mL), MNL-conditioned medium, LPS-MNL-conditioned medium, and ADA (5 U/mL) or BW A1433U (10 μM) and then stimulated with fMLP (100 nM; indicated by the arrow). Representative traces from six separate experiments are shown. (A) medium control; (B) LPS-primed PMN; (C) PMN primed with MNL-conditioned medium; (D) PMN primed with LPS-MNL-conditioned medium; (E) PMN primed with LPS-MNL-conditioned medium in the presence of ADA; and (F) PMN primed with LPS-MNL-conditioned medium in the presence of BW A1433U.

prior to the formation of the conditioned medium but not after conditioned medium formation, and LPS added to the PMN after the formation of the conditioned medium did not prime the PMN (data not shown). Thus, the priming in these experiments was from a cell-produced mediator rather than from LPS itself.

LPS (10 ng/mL) increased the amount of TNFα released from MNL (2×10^6/mL) in 60 min from less than

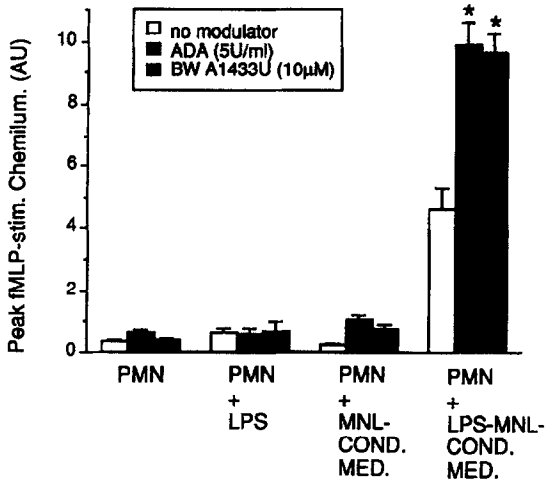

Fig. 2. ADA and BW A1433U enhancement of the PMN-primed response to LPS-MNL-conditioned medium, as measured by chemiluminescence. The samples were prepared as described in Fig. 1. Values are means ± SEM of 6 separate experiments. Key: (*) $P = 0.001$ compared with PMN primed with no modulator.

20 pg/mL to 836 ± 92 pg/mL ($P < 0.001$). A monoclonal antibody to human TNFα (3 μg/mL; cA2) completely neutralized PMN chemiluminescence primed by rhTNFα (1 U/mL; 600 pg/U) and decreased chemiluminescence primed by LPS-MNL-conditioned medium 55%. Thus, TNFα is a major priming factor in the LPS-MNL-conditioned medium, and there may be another, as yet undefined, priming factor(s) in the LPS-MNL-conditioned medium.

Release of adenosine by PMN and MNL

We measured the concentration of adenosine in the medium surrounding the PMN and MNL, which has been cultured as in the other assays described. We determined that both PMN and MNL released adenosine to the surrounding medium. PMN (1×10^6/mL) alone released 5.5 ± 1.4 nM adenosine into the medium in 1 hr, and MNL (2×10^6/mL) released much more adenosine (30.6 ± 11.4 nM). When mixed, PMN (1×10^6/mL) plus MNL (2×10^6/mL) released 37.0 ± 8.7 nM adenosine. LPS (10 ng/mL) had virtually no effect on adenosine release from PMN or MNL (data not shown).

Modulation of the LPS-MNL-conditioned medium-primed PMN response by endogenous adenosine

We designed the following experiments to explore the role of adenosine released from leukocytes in the modulation of primed PMN activity. ADA (1 U/mL) converts adenosine to inosine (an inactive product) at a rate of 1 μmol/min at 25° [17]. The xanthine BW A1433U is a non-specific PMN adenosine receptor antagonist [18, 19]. We observed that BW A1433U was not a substrate for xanthine oxidase, and, therefore, was not acting by this means (data not shown). An excess of ADA (5 U/mL) and BW A1433U (10 μM) had little effect on unprimed PMN activity (Fig. 2). Similarly, PMN primed with LPS in the presence of ADA or BW A1433U experienced no higher chemiluminescence than did PMN primed with LPS alone (Fig. 2). In addition, ADA and BW A1433U had little effect on the already low MNL-conditioned medium-primed (no LPS) PMN chemiluminescence (Fig. 2). However, adding ADA to PMN plus LPS-MNL-conditioned medium caused chemiluminescence to nearly double ($P = 0.001$ compared with PMN plus LPS-MNL-conditioned medium without ADA) (Figs. 1E and 2). Similarly, adenosine receptor blockage with BW A1433U significantly increased peak chemiluminescence upon stimulation with fMLP ($P = 0.001$ compared with PMN plus LPS-MNL-conditioned medium without BW A1433U) (Figs. 1F and 2). Thus, either destruction of adenosine with ADA or preventing adenosine binding to receptors on the PMN surface with BW A1433U counteracts the effect of adenosine present in the LPS-MNL-conditioned medium.

Modulation of the LPS-MNL-conditioned medium-primed PMN response by exogenous adenosine

The following experiment was designed to examine modulation of the primed PMN response by exogenously added adenosine. Concentrations of exogenously added adenosine as low as 100 nM further reduced the already muted LPS-MNL-conditioned medium-primed PMN chemiluminescence response ($P = 0.009$ compared with PMN without adenosine). There was a concentra-

tion–response effect as 1 μM adenosine halved PMN chemiluminescence ($P = 0.002$) upon stimulation with fMLP, and 10 μM adenosine decreased activity by two-thirds resulting in activity close to unprimed chemiluminescence ($P < 0.001$) (Fig. 3).

Modulation of rhTNFα-primed PMN by adenosine

We performed the following experiments to better determine the role of adenosine in modulating TNFα-primed PMN chemiluminescence. Exogenously added adenosine as low as 10 nM lowered rhTNFα (1 U/mL)-primed PMN chemiluminescence, and adenosine (30 nM) decreased chemiluminescence comparable to endogenous adenosine in LPS-MNL-conditioned medium. Adenosine (1 μM) decreased peak chemiluminescence to levels comparable to unprimed PMN (Fig. 4). Similar trends were noted among assays measuring luminol-enhanced chemiluminescence, superoxide production, and MPO release.

Chemiluminescence. Recombinant human TNFα priming (30 min at 37°) increased PMN chemiluminescence 14-fold in response to fMLP from 0.6 ± 0.3 to 8.2 ± 1.4 AU ($P = 0.002$). With the addition of 100 nM adenosine, peak TNF-primed PMN chemiluminescence was decreased by 65% ($P < 0.001$). ADA (5 U/mL) and BW A1433U (10 μM) counteracted the modulatory effect of adenosine on TNF-primed PMN chemiluminescence. ADA increased TNF-primed PMN chemiluminescence in the presence of 100 nM adenosine by 70% compared with PMN with adenosine and no ADA ($P = 0.011$). BW A1443U increased chemiluminescence from 2.6 ± 0.3 AU with 100 nM adenosine to 6.8 ± 0.6 AU with adenosine and BW A1433U ($P = 0.005$) (Fig. 5A).

Superoxide release. PMN primed with rhTNFα increased their superoxide release more than 11-fold compared with unprimed PMN in response to fMLP ($P < 0.001$). Adenosine (100 nM) decreased this output from

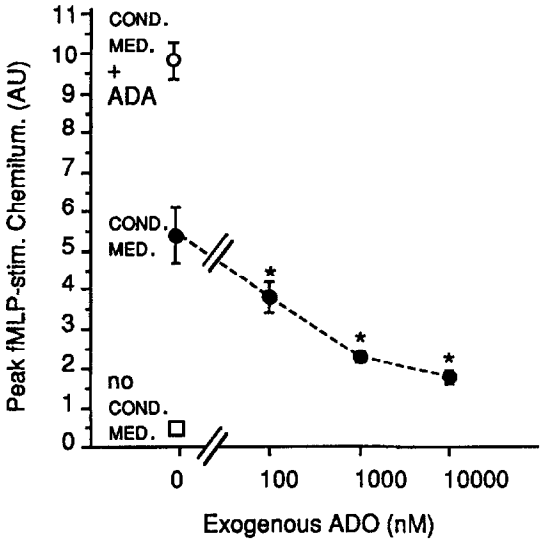

Fig. 3. Concentration–response of exogenous adenosine (ADO) on the LPS-MNL-conditioned medium-primed response of PMN as measured by fMLP-stimulated chemiluminescence. Values are means ± SEM of 5–18 experiments. Key: (*) $P < 0.05$ compared with PMN primed with LPS-MNL-conditioned medium without adenosine.

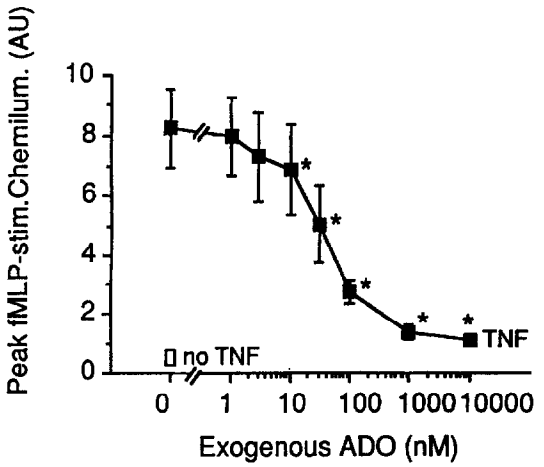

Fig. 4. Concentration–response of exogenous adenosine (ADO) on rhTNFα-primed fMLP-stimulated PMN chemiluminescence. PMN (1×10^6/mL) were incubated at 37° for 30 min with rhTNFα (1 U/mL; 600 pg/mL) and then stimulated with fMLP (100 nM). Values are means ± SEM of 5–10 separate experiments. Key: (*) $P < 0.05$ compared with rhTNFα-primed PMN without adenosine.

22.4 ± 0.8 to 15.66 ± 1.0 nmol superoxide ($P < 0.001$). However, ADA (5 U/mL) and BW A1433U (10 μM) counteracted the inhibitory effect of adenosine ($P = 0.001$) (Fig. 5B).

MPO. Recombinant human TNFα doubled PMN MPO release to fMLP stimulation ($P < 0.001$). With the addition of adenosine (100 nM), MPO release decreased 20% ($P < 0.001$). ADA and BW A1433U offset the modulatory effect of adenosine and increased PMN MPO release ($P = 0.012$ and $P = 0.005$, respectively) (Fig. 5C).

Decreased rhTNFα-primed chemiluminescence by adenosine inhibition of the PMN response to the second stimulus

In the absence of added exogenous adenosine, ADA (5 U/mL) had little effect on either unprimed fMLP-stimulated PMN chemiluminescence or on rhTNFα-primed fMLP-stimulated activity. In contrast, ADA completely counteracted adenosine (100 nM) diminution of rhTNFα-primed fMLP-stimulated PMN chemiluminescence. ADA had a comparable effect if it was added prior to priming or after PMN priming with rhTNFα (Fig. 6). These data indicate that adenosine acts on fMLP stimulation rather than on priming *per se*.

DISCUSSION

Damage to host tissues in infection is enhanced by inflammatory cytokines, such as TNFα [10], and activated neutrophils. Adenosine is a natural "antiinflammatory" molecule produced by damaged tissues. Adenosine, through binding to adenosine A_2 receptors, decreases superoxide anion release from PMN activated by several stimuli including the chemoattractants C_{5a} and fMLP, adherence to biological surfaces, and ingestion of bacteria [5, 20–29]. Our data now indicate that the very small amounts of adenosine released from leukocytes in culture are sufficient to halve the much enhanced oxidative burst of PMN primed by physiological concen-

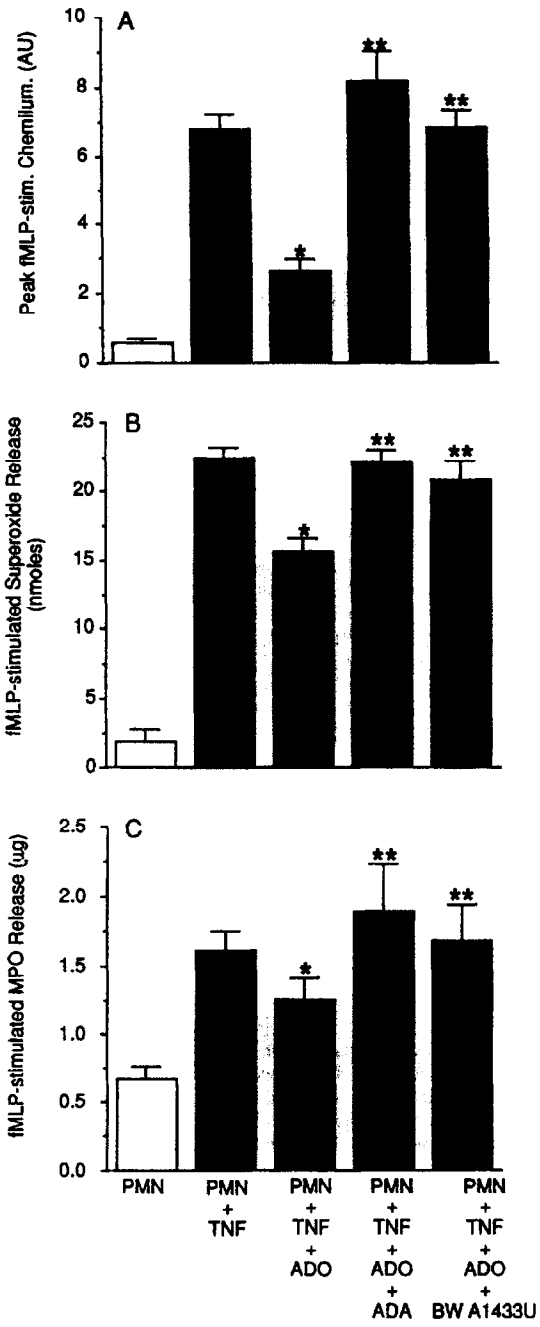

Fig. 5. Adenosine degradation with ADA and adenosine receptor blockage with BW A1433U counteraction of adenosine-modulated rhTNFα-primed fMLP-stimulated PMN activity. PMN (1×10^6/mL) were incubated for 30 min at 37° with or without rhTNFα (1 U/mL; 600 pg/mL), adenosine (100 nM), and ADA (5 U/mL) or BW A1433U (10 μM), and then stimulated with fMLP (100 nM). In each graph, the control unprimed PMN response to fMLP is indicated by the unfilled bar. Values are means ± SEM of 5–20 experiments. ADO = adenosine. Key: (*) $P < 0.05$, decreased activity compared with rhTNFα and without ADO; and (**) $P < 0.05$, increased activity compared with ADO and rhTNFα and without ADA and BW A1433U.

trations of TNFα. Thus, tissue concentrations of adenosine have the potential to modulate significantly cytokine-augmented PMN activity and decrease the inflammatory response in infection.

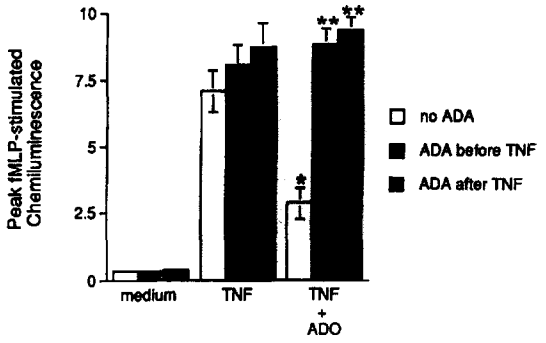

Fig. 6. Adenosine decrease of the primed PMN response to fMLP. PMN (1×10^6/mL) were incubated for 30 min at 37° with or without rhTNFα (1 U/mL; 600 pg/mL), adenosine (ADO) (100 nM), and ADA (5 U/mL; added either before or after priming with rhTNFα), and then stimulated with fMLP (100 nM). Values are means ± SEM (3 separate experiments). Key: (*) $P < 0.05$, decreased chemiluminescence compared with rhTNFα and without ADO; and (**) $P < 0.05$, increased chemiluminescence compared with ADO and rhTNFα and without ADA.

The major effect of adenosine is to decrease the PMN TNF-primed oxidative response to fMLP. In contrast to our studies, Stewart and Harris [30] failed to observe a significant effect of adenosine on TNFα-enhanced PMN oxidative activity. However, their experiments did not show marked TNF priming. They found a 2-fold increase, and we like others (e.g. [31]) found a 12-fold increase. The difference in PMN activity may be due to differences in PMN isolation procedures [32, 33].

Adenosine is produced by the degradation of intracellular adenine nucleotides and is released especially by dead and injured tissues [2, 3]. Blood plasma concentrations of adenosine are normally between 30- and 100-nM and rise to approximately 400–600 nM in sepsis [7].

Generally, our value for the concentration of adenosine released from PMN in culture was lower than data reported by others [34–36]. It is difficult to compare the differences in observed adenosine concentrations surrounding cultured neutrophils because of the great variation in PMN separation and culturing procedures. One major difference in our assays is the intentional omission of agents that affect either endogenous adenosine uptake or adenosine conversion to inosine by ADA. This was done because we were interested in comparing these data to our functional assays that did not contain these substances.

In our studies, a luminol-enhanced chemiluminescence assay was used to measure the PMN oxidative burst. Luminol-enhanced chemiluminescence is dependent upon both the production of superoxide anion and the release of MPO from PMN granules. We observed that the modulating effect of adenosine on rhTNFα-primed activity was on both superoxide production as measured by cytochrome *c* reduction and on degranulation as measured by MPO release. The greater effect of adenosine on PMN "oxidative activity" as measured by luminol-enhanced chemiluminescence compared with cytochrome *c* reduction is probably a reflection of the double effect of adenosine on both cellular activities required for luminol-enhanced chemiluminescence (i.e. MPO mobilization and PMN superoxide release).

There are variable reports on adenosine modulation of PMN degranulation. Some observed very little effect of

adenosine on degranulation [6, 22, 37, 38], and others have observed a significant effect [21, 39]. Richter [39] observed that fMLP-stimulated PMN lactoferrin release was inhibited by the adenosine A_2 receptor agonist 2-chloroadenosine only in the absence of cytochalasin B. All our measurements of chemiluminescence, superoxide release, and MPO release were done in the absence of cytochalasins.

Adenosine not only blocks TNFα-enhanced PMN oxidative activity and degranulation, but at micromolar concentrations it also decreases macrophage production of TNFα stimulated by LPS [40, 41], and counteracts the adverse effects of TNFα on PMN motility and chemotaxis [42]. In light of the antiinflammatory activity of adenosine, recent research has been directed at increasing endogenous adenosine concentrations at sites of inflammation. This can be accomplished with methotrexate treatment, which results in AICAR accumulation from the inhibition of the enzyme AICAR transformylase. AICAR in the cell increases adenosine release from stressed cells, and thus increases the amount of adenosine at sites of tissue injury [43]. Another method of increasing endogenous adenosine levels at sites of injury is to inhibit the major adenosine-metabolizing enzyme, adenosine kinase [44, 45].

Micromolar concentrations of adenosine are not toxic to PMN as evidenced by the observation that these concentrations of adenosine do not adversely affect PMN motility and chemotaxis [42]. Adenosine may decrease microbicidal activity by decreasing PMN oxidative activity and degranulation. Our previous studies show that only very high (non-physiological) concentrations of adenosine (>1 mM) reduce the ability of PMN to kill *Staphylococcus aureus* [27].

Our data indicate that endogenous adenosine (nM) has the potential to be an important modulator of neutrophil-mediated injury by modulating primed-PMN function. The recent data using compounds that can alter adenosine concentrations at sites of inflammation [43, 45] demonstrate that this might be what is happening *in vivo*. Adenosine probably acts by a combination of effects including counteracting TNFα-inhibited PMN migration, decreasing TNFα production, and lessening the inflammatory effects of TNFα on PMN function.

Acknowledgement—This study was supported, in part, by Public Health Service Grant RO1AI09504 from the National Institutes of Health.

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