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European Journal of Pharmacology 358 (1998) 261–268

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An agonist of adenosine A_3 receptors decreases interleukin-12 and interferon-γ production and prevents lethality in endotoxemic mice

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Received 7 May 1998; revised 3 August 1998; accepted 18 August 1998

Abstract

We have recently observed that the selective adenosine A_3 receptor agonist N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (IB-MECA) augments interleukin-10 and inhibits tumor necrosis factor-α production in endotoxemic mice. In the present study, we extended our investigations into the effect of this compound on the bacterial lipopolysaccharide (endotoxin)-induced inflammatory response in the BALB/c, as well as in the C57BL/6 interleukin-$10^{+/+}$ and the interleukin-10 deficient C57BL/6 interleukin-$10^{0/0}$ mice strains. In the BALB/c mice, i.p. pre-treatment with IB-MECA (0.2 and 0.5 mg/kg) decreased lipopolysaccharide (60 mg/kg i.p.)-induced plasma levels of interleukin-12 (p40 and p70), interferon-γ, and nitrite/nitrate (breakdown products of nitric oxide (NO)). On the other hand, pre-treatment with this compound failed to influence lipopolysaccharide-induced plasma interleukin-1α, interleukin-6, and corticosterone concentrations. Similar to its effect in BALB/c mice, IB-MECA enhanced the release of interleukin-10 in the C57BL/6 interleukin-$10^{+/+}$ mice. Furthermore, IB-MECA inhibited the production of interleukin-12, interferon-γ, and NO in both the C57BL/6 interleukin-$10^{+/+}$ and C57BL/6 interleukin-$10^{0/0}$ mice, suggesting that the inhibition of pro-inflammatory cytokine production by this compound is independent of the increased release of interleukin-10. Finally, pre-treatment with this compound protected mice against lipopolysaccharide (60 mg/kg i.p.)-induced lethality. These results indicate that stimulation of adenosine A_3 receptors has potent anti-inflammatory effects and may represent a potential strategy in the treatment of septic shock and other inflammatory diseases.

Keywords: Lipopolysaccharide; Cytokine; Nitric oxide (NO); Shock; Inflammation; Inflammatory mediator

1. Introduction

The sequential release of pro-inflammatory mediators in response to bacterial, viral, or fungal infections is essential in the fight against the invading microorganisms. However, the overproduction of these mediators may be detrimental for the host leading to multi-organ failure, shock, and finally death. The systemic administration of bacterial lipopolysaccharide (endotoxin), a cell-wall component of Gram-negative bacteria is a prototypic stimulus for the activation of an inflammatory cascade. Early in the course of this cascade appear the monocyte/macrophage derived pro-inflammatory cytokines tumor necrosis factor-α, interleukin-1, and interleukin-12, which play a key role in the pathogenesis of endotoxic shock (Beutler, 1995; Trinchieri, 1995). These cytokines are central to the induction of interferon-γ production by natural killer cells and T lymphocytes (D'Andrea et al., 1993; Heinzel et al., 1994; Wysocka et al., 1995), which is another important intermediate in the development of endotoxin shock (Doherty et al., 1992). Tumor necrosis factor-α, interleukin-1, and interferon-γ trigger the subsequent induction of the macrophage type inducible nitric oxide (NO) synthase (Szabó et al., 1993; Thiemermann et al., 1993; Salkowsky et al., 1997) leading to the overproduction of NO. Formation of this free radical, or the related toxic oxidant product peroxynitrite crucially contribute to the development of hypotension, vascular hyporeactivity, endothelial injury,

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PII: S0014-2999(98)00619-0

and mortality in endotoxin shock (Szabó, 1995). Interleukin-10, which is also released in the early phases of endotoxemia, inhibits the production of tumor necrosis factor-α, interleukin-12, interferon-γ, and NO (Berg et al., 1995; Haskó et al., 1998a), and is protective in both lipopolysaccharide- and staphylococcal enterotoxin B-induced lethality (Berg et al., 1995; Haskó et al., 1998b).

Adenosine is a purine nucleoside that is released from cells in response to metabolic stress (Dubyak and El-Moatassim, 1993) or from the sympathetic nervous system (White and MacDonald, 1990; Sperlagh and Vizi, 1992; Haskó and Szabó, 1998), and occupies adenosine A_1, A_{2A}, A_{2B} and A_3 receptors on target cells. Adenosine or selective agonists of the different adenosine receptors are important regulators of the production of inflammatory mediators in endotoxemia (Rose et al., 1988; Schrier et al., 1990; Le Vraux et al., 1993; Parmely et al., 1993; Bouma et al., 1994; Cronstein, 1994). Recently, a role for the adenosine A_3 receptor in the modulation of immune response has been proposed, since the suppression of tumor necrosis factor-α production (Le Vraux et al., 1993; Haskó et al., 1996), NO formation (Haskó et al., 1996; Moochhala et al., 1996), or the inhibition of major histocompatibility complex-unrestricted cytolytic activity of natural killer cells (Hoskin et al., 1994) by selective A_1 and A_2 receptor agonists was not clearly characteristic of either the A_1 or A_2 subtype. Moreover, in recent studies, using selective agonists of adenosine A_3 receptors, we and others have demonstrated that the stimulation of this receptor subtype potently inhibits tumor necrosis factor-α (Haskó et al., 1996; McWhinney et al., 1996; Sajjadi et al., 1996) and augments interleukin-10 (Haskó et al., 1996) production.

The aim of the present study was to further characterize the effect of adenosine A_3 receptor activation on the course of inflammatory processes. Using N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (IB-MECA), a selective agonist of adenosine A_3 receptors (Gallo-Rodriguez et al., 1994), we investigated whether the stimulation of this receptor subtype modulates interleukin-12, interferon-γ, interleukin-6, interleukin-1α, corticosterone and NO production. Since up-regulation of interleukin-10 production by certain pharmacological agents (Kambayashi et al., 1995; Le Moine et al., 1996; Van der Poll et al., 1996) has been attributed to the suppression of pro-inflammatory cytokines, we have compared the effect of IB-MECA in normal and interleukin-10 deficient endotoxemic mice. Furthermore, we determined whether the activation of adenosine A_3 receptors with IB-MECA prevents lipopolysaccharide-elicited lethality.

2. Materials and methods

2.1. Animals

Male BALB/c mice (20–25 g) were purchased from Charles River Laboratories (Budapest, Hungary). Male C57BL/6 interleukin-$10^{+/+}$ and C57BL/6 interleukin-$10^{0/0}$ mice (7-week old) were obtained from the Jackson Laboratory (Bay Harbor, ME, USA). Animals received food and water ad libitum, and lighting was maintained on a 12-h cycle. The animal experiments were performed with the approval of the Institutional Animal Care and Use Committee.

2.2. Materials

IB-MECA was purchased from Research Biochemicals International (Natick, MA, USA). Lipopolysaccharide from *Escherichia coli* 055:B5 and all other drugs were obtained from Sigma (St. Louis, MO, USA).

2.3. Experimental design for plasma interleukin-12, interferon-γ, interleukin-6, interleukin-10, nitrite / nitrate (breakdown products of NO), and corticosterone measurements

For stimulation of adenosine A_3 adenosine receptors, animals were treated with IB-MECA at a dose of 0.2–0.5 mg/kg. The selection of this dose, at which IB-MECA specifically binds to adenosine A_3 receptors, was based on previous in vivo studies (Von Lubitz et al., 1994; Auchampach et al., 1997; Tracey et al., 1997). Animals were injected i.p. with drug vehicle (physiologic saline containing 2% dimethylsulfoxide) or IB-MECA in a volume of 0.1 ml/10 g body weight. Thirty minutes later, they were challenged with 60 mg/kg of lipopolysaccharide administered i.p. The animals were killed at various time points after lipopolysaccharide treatment. Blood was collected in ice-cold Eppendorf tubes containing heparin and centrifuged for 10 min at 4°C. The plasma was stored at −70°C until assayed.

2.4. Cytokine assays

Cytokine levels in plasma were determined by ELISA kits that are specific against murine cytokines. Plasma levels of interleukin-12 (p40 and p70), interferon-γ, interleukin-6, interleukin-1α, and interleukin-10 were measured using ELISA kits purchased from Genzyme (Boston, MA, USA). Plasma levels of macrophage inflammatory protein-1α were measured using ELISA kits purchased from R&D Systems (Minneapolis, MN, USA). Plates were read at 450 nm by a Spectramax 250 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Detection limits were >5 pg/ml for interleukin-12, >5 pg/ml for interferon-γ, >15 pg/ml for interleukin-6, >15 pg/ml for interleukin-1α, >1.5 pg/ml for macrophage inflammatory protein 1α, and >0.15 pg/ml for interleukin-10. Assays were performed as described previously (Haskó et al., 1998a,b) and according to the manufacturer's instructions. IB-MECA did not interfere with any of the assays used.

2.5. Measurement of plasma nitrite / nitrate concentrations

For determination of total nitrite/nitrate concentrations in plasma samples, nitrate was first converted to nitrite by incubation with 60 mU nitrate reductase and 25 μM NADPH for 180 min. Nitrite was then measured by adding Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamide in 5% phosphoric acid) to plasma samples diluted (1:10) in phosphate buffered saline (Németh et al., 1997). The optical density at 550 nm (OD_{550}) was measured using the Spectramax 250 microplate reader. Nitrite/nitrate concentrations were calculated by comparison with OD_{550} of standard solutions of sodium nitrite and sodium nitrate. The measurements of nitrite/nitrate were performed using reagents free of nitrite and nitrate: no basal or background nitrite or nitrate levels were detected.

2.6. Corticosterone radioimmunoassay

Plasma corticosterone was determined in the plasma as previously described (Haskó et al., 1995). The interference of plasma transcortin was eliminated by inactivated transcortin at low pH. The sensitivity of the assay was 0.1 pmol/tube and intra and interassay variations were 6.4 and 23.8%, respectively.

2.7. Lethality studies

BALB/c mice were injected i.p. with drug vehicle or IB-MECA (as described above), followed by an i.p. lipopolysaccharide challenge (60 mg/kg) 30 min later. Survival was recorded 24, 48, 72, 96 h, and 2 weeks after the lipopolysaccharide injection.

2.8. Statistical evaluation

Values in the figures and text are expressed as mean ± S.E.M. of n observations. Statistical analysis of the data was performed by One-way analysis of variance followed by Dunnett's test, as appropriate. Survival differences were evaluated with the chi-square test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of IB-MECA on lipopolysaccharide-induced plasma interleukin-12 (p40 and p70), interferon-γ, interleukin-6, interleukin-1α, nitrite / nitrate, and corticosterone concentrations in BALB / c mice

Intraperitoneal injection of lipopolysaccharide (60 mg/kg) to BALB/c mice induced the appearance of plasma interleukin-12 p70 levels, which reproducibly peaked at 4 h (in the pg/ml range). As shown in Fig. 1a, i.p. pre-treatment of the mice with either 0.2 or 0.5 mg/kg IB-MECA 30 min before lipopolysaccharide abolished the plasma interleukin-12 p70 response, as determined at 4 h post-lipopolysaccharide. Previous studies demonstrated that the production of the p40 subunit exceeds the production of p70 by 40- to 500-fold (Wysocka et al., 1995; Jansen et al., 1996). In agreement with these findings, we detected high levels (in the ng/ml range) of interleukin-12 p40 at 2, 4, and 8 h after lipopolysaccharide injection (Fig. 1b). Pre-treatment of the animals with IB-MECA 30 min before the lipopolysaccharide challenge caused a significant reduction of plasma interleukin-12 p40 levels at all time-points tested (Fig. 1b).

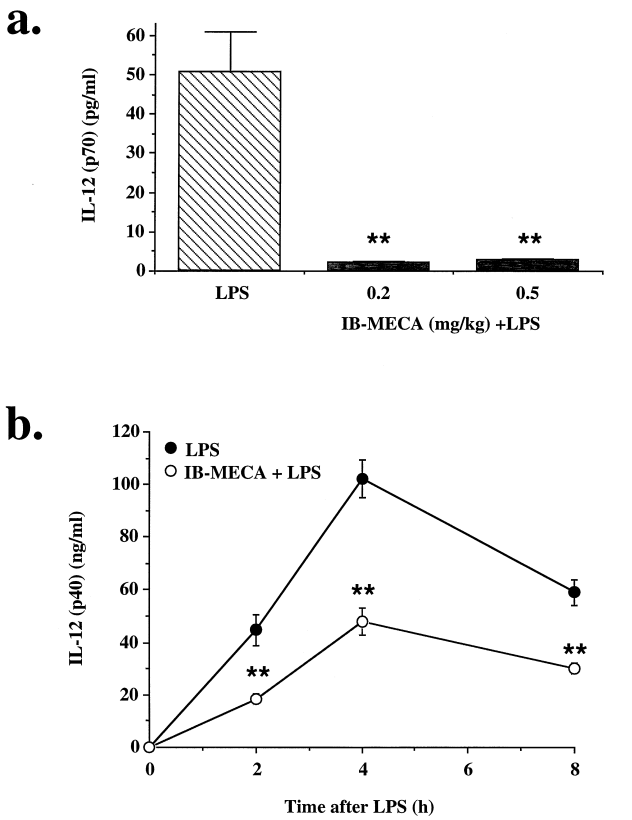

Fig. 1. IB-MECA suppresses the production of interleukin-12 in endotoxemic mice. (a) Effect of i.p. pre-treatment of the mice with either 0.2 or 0.5 mg/kg IB-MECA 30 min before lipopolysaccharide (60 mg/kg i.p.) on the plasma interleukin-12 p70 response, as determined at 4 h post-lipopolysaccharide. (b) Effect of i.p. pre-treatment of the mice with 0.5 mg/kg IB-MECA 30 min before lipopolysaccharide (60 mg/kg i.p.) on the plasma interleukin-12 p40 response, as determined at 2, 4 and 8 h post-lipopolysaccharide. $n = 7–9$ mice; $** P < 0.01$ indicates significant inhibition by IB-MECA treatment.

Lipopolysaccharide (60 mg/kg i.p.) induced plasma levels of interferon-γ, which peaked at 8 h and were still detectable at 24 h post-lipopolysaccharide. Pre-treatment with IB-MECA (0.5 mg/kg) significantly suppressed plasma concentrations of this cytokine at the 4 h and at the 8 h time-points (Fig. 2a). Lipopolysaccharide (60 mg/kg i.p.)-induced plasma levels of interleukin-6, however, were

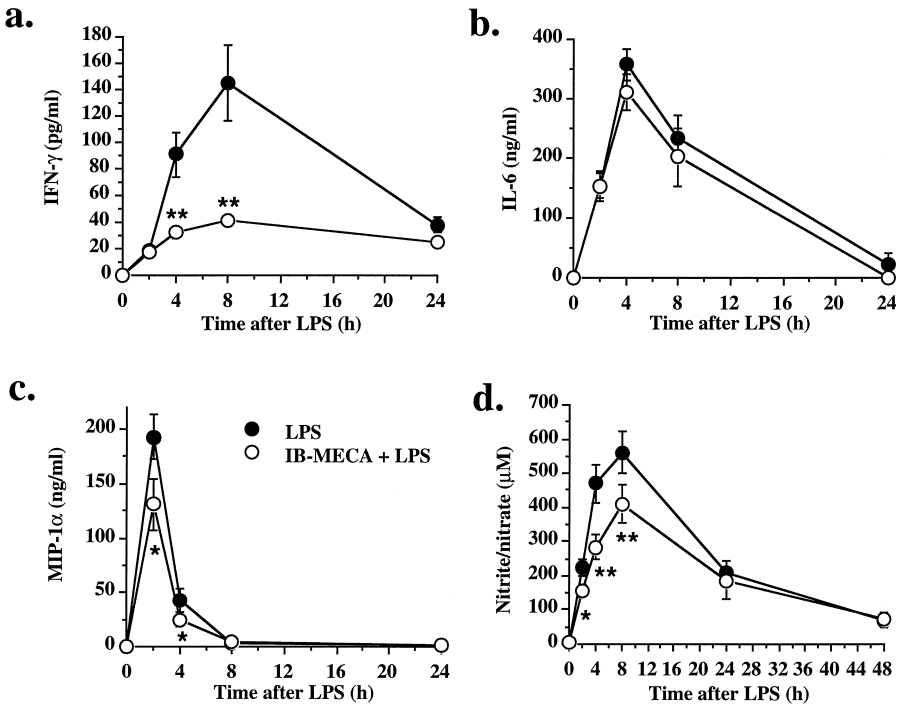

Fig. 2. Effect of IB-MECA on the production of various inflammatory mediators in endotoxemic mice. Mice were pre-treated with 0.5 mg/kg IB-MECA 30 min before lipopolysaccharide (60 mg/kg i.p.), and plasma interferon-γ (a), interleukin-6 (b), nitrite/nitrate (d) and macrophage inflammatory protein 1-α (c) levels were determined at various time points (2–48 h). $n = 7–9$ mice; $* P < 0.05$ and $* * P < 0.01$ indicate significant inhibition by IB-MECA treatment.

unaffected by IB-MECA treatment (Fig. 2b). I.p. injection of lipopolysaccharide (60 mg/kg) gave rise to an elevation of plasma interleukin-1α concentrations, reaching 98.72 ± 40.25 pg/ml at 4 h. However, IB-MECA pre-treatment failed to alter plasma levels of this cytokine, which were 65.43 ± 13.9 pg/ml and 105.28 ± 38.88 pg/ml in the case of 0.2 and 0.5 mg/kg of IB-MECA, respectively. I.p. injection of lipopolysaccharide induced plasma nitrite/nitrate levels peaking at 24 h after lipopolysaccharide. Intraperitoneal pre-treatment with IB-MECA (0.5 mg/kg) caused a modest reduction of plasma nitrite/nitrate at 2, 4 and 8 h and of macrophage inflammatory protein-1α at 2 and 4 h after lipopolysaccharide, but not at later time points (Fig. 2c). Finally, plasma corticosterone levels were not significantly altered by IB-MECA pre-treatment as measured 4 h after lipopolysaccharide injection. The respective values were 1870 ± 118, 2045 ± 162 and 1829 ± 146 pmol/ml in animals pre-treated with vehicle, 0.2 mg/kg of IB-MECA and 0.5 mg/kg IB-MECA.

3.2. Effect of IB-MECA on lipopolysaccharide-induced plasma interleukin-12, interferon-γ, and nitrite/nitrate concentrations in C57BL/6 interleukin-10$^{+/+}$ and C57BL/6 interleukin-10$^{-/-}$ mice

We recently demonstrated that stimulation of adenosine A_3 receptors by IB-MECA causes a potentiation of lipopolysaccharide-induced plasma interleukin-10 levels (Haskó et al., 1996). Endogenous production of interleukin-10 is crucial in the prevention of overproduction of pro-inflammatory mediators in endotoxemia: in interleukin-10 deficient mice, lipopolysaccharide injection induces markedly higher interleukin-12, interferon-γ, and NO production when compared to wild-type animals (Berg et al., 1995; Haskó et al., 1998a). We therefore tested the possibility that the decreased production of the pro-inflammatory mediators by IB-MECA is due to the increase in interleukin-10 release. First, we confirmed that our interleukin-10 deficient C57BL/6 interleukin-10$^{-/-}$ mice did not produce detectable levels of interleukin-10 upon lipopolysaccharide stimulation (60 mg/kg i.p.; Fig. 3a). We then treated C57BL/6 interleukin-10$^{+/+}$ and C57BL/6 interleukin-10$^{-/-}$ mice with IB-MECA (0.5 mg/kg i.p.) 30 min before the administration of 60 mg/kg of lipopolysaccharide and determined interleukin-10, interleukin-12 p70, interferon-γ, and nitrite/nitrate levels 4 h after the lipopolysaccharide challenge. Fig. 3a shows that IB-MECA pre-treatment augmented plasma interleukin-10 in the C57BL/6 interleukin-10$^{+/+}$ mice, which confirms our similar finding in BALB/mice (Haskó et al., 1996). In addition, the A_3 agonist decreased plasma interleukin-12 p70 (Fig. 3b), interferon-γ (Fig. 3c), and nitrite/nitrate (Fig. 3d) levels in the C57BL/6 interleukin-10$^{+/+}$ mice in a similar manner as in BALB/c mice. Although plasma

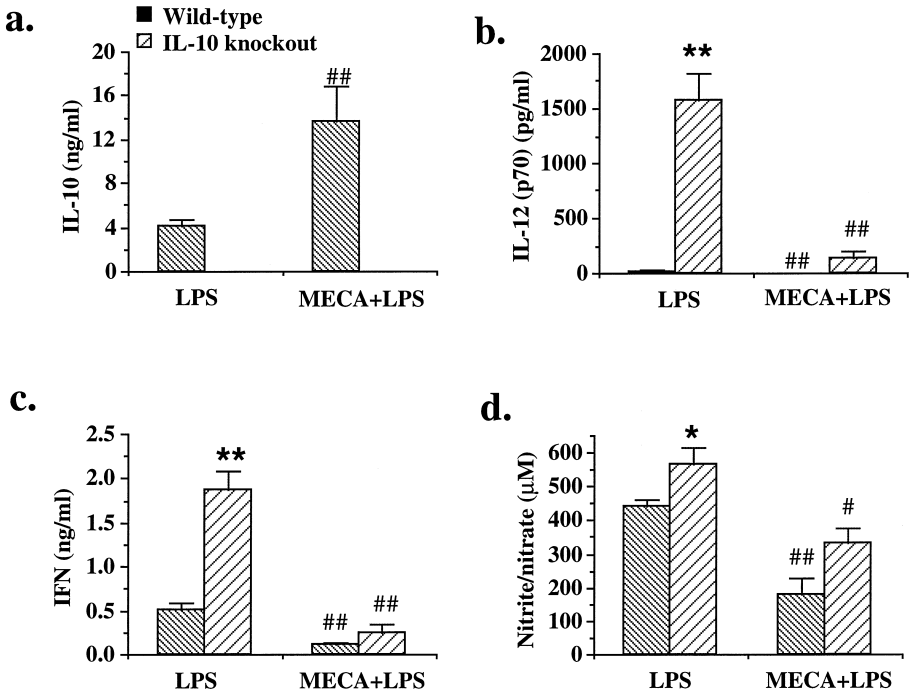

Fig. 3. The anti-inflammatory effect of IB-MECA in lipopolysaccharide-treated mice is independent of increased interleukin-10 levels. Interleukin-10 deficient C57BL/6 interleukin-$10^{-/-}$ mice or corresponding wild-type animals (C57BL/6 interleukin-$10^{+/+}$) were pre-treated with IB-MECA (0.5 mg/kg i.p.) 30 min before the administration of 60 mg/kg of lipopolysaccharide, and plasma interleukin-10 (a), interleukin-12 p70 (b), IFN-γ (c), and nitrite/nitrate levels (d) were determined at 4 h after the lipopolysaccharide challenge. $n = 7$–9 mice; $*P < 0.05$ and $**P < 0.01$ indicate significantly higher lipopolysaccharide-induced cytokine levels in the interleukin-10 deficient C57BL/6 interleukin-$10^{-/-}$ mice, when compared to the C57BL/6 interleukin-$10^{+/+}$ animals; $\#P < 0.05$ and $\#\#P < 0.01$ indicate significant effect of IB-MECA treatment.

concentrations of interleukin-12 p70, interferon-γ, and nitrite/nitrate were significantly higher in the C57BL/6 interleukin-$10^{-/-}$ mice than in their wild type counterparts, IB-MECA maintained its ability to suppress the production of these mediators in these interleukin-10 deficient animals (Fig. 3b–d). Interleukin-12 p70, interferon-γ and nitrite/nitrate were reduced by 0.5 mg/kg IB-MECA by $93 \pm 14\%$, $75 \pm 12\%$ and $54 \pm 9\%$ in the C57BL/6 interleukin-$10^{+/+}$ mice, whereas the percent reductions amounted to $90 \pm 12\%$, $80 \pm 10\%$, and $44 \pm 7\%$ in the C57BL/6 interleukin-$10^{-/-}$ animals, respectively.

3.3. IB-MECA pre-treatment protects mice against lethal endotoxin shock

BALB/c mice were pre-treated with IB-MECA (0.5 mg/kg) or drug vehicle 30 min before the i.p. administration of 60 mg/kg lipopolysaccharide. Fig. 4 shows that IB-MECA pre-treatment significantly decreased mortality as compared to the drug vehicle group as evaluated at 96 h after the lipopolysaccharide challenge ($P < 0.005$). No further changes in survival were observed during an additional 10 days. The improvement in survival is consistent with the effects of IB-MECA on cytokine and NO production in endotoxemia (see above). While interleukin-10 protects against endotoxin induced mortality (Berg et al., 1995), the pro-inflammatory cytokines tumor necrosis factor-α, interferon-γ and NO produced by inducible NO synthase contribute to lipopolysaccharide-induced mortality (Doherty et al., 1992; Beutler, 1995; Szabó, 1995). The modulation by IB-MECA of the production of all the above inflammatory mediators may contribute to the beneficial effect of this agent.

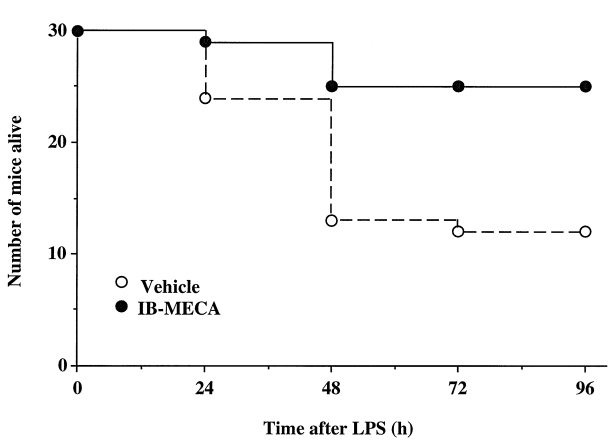

Fig. 4. IB-MECA improves survival of mice subjected to a lethal dose of lipopolysaccharide. BALB/c mice were pre-treated with drug vehicle or 0.5 mg/kg IB-MECA 30 min before the injection of 60 mg/kg of i.p. lipopolysaccharide. Survival was recorded at 24, 48, 72 and 96 h after the lipopolysaccharide injection. Results from the summary of two different experiments are shown. $n = 30$ animals in each group. IB-MECA improved survival rate at 24–96 h ($P < 0.05$).

4. Discussion

Recently, there has been a growing interest in determining the role of adenosine A_3 receptors in the modulation of immune/inflammatory processes. In a recent study using specific adenosine A_1, A_2 and A_3 receptor agonists and antagonists, it was demonstrated that inhibition of tumor necrosis factor-α production by lipopolysaccharide-stimulated U937 (human monocyte) cells was mainly an adenosine A_3 receptor-mediated process (Sajjadi et al., 1996). Similarly, adenosine receptor agonists, in a dose-dependent manner characteristic of the adenosine A_3 receptor, blocked endotoxin induction of the tumor necrosis factor-α gene and protein expression in the murine J774.1 macrophage cell line (McWhinney et al., 1996). We reported that stimulation of adenosine A_3 receptors by the selective adenosine A_3 receptor agonist IB-MECA decreased plasma tumor necrosis factor-α and increased interleukin-10 in lipopolysaccharide-treated mice (Haskó et al., 1996).

In the present study, we demonstrated for the first time that selective stimulation of adenosine A_3 receptors suppresses the production of the pro-inflammatory mediators interleukin-12, interferon-γ, and NO. Consistent with the pathogenic role of these mediators and tumor necrosis factor-α in endotoxic shock (Doherty et al., 1992; Szabó, 1995; Wysocka et al., 1995), IB-MECA pre-treatment protected lipopolysaccharide-injected mice from shock-induced death. Also, the up-regulation of interleukin-10 release may have contributed to the beneficial effect of this drug, as interleukin-10 is protective in lipopolysaccharide-induced shock (Berg et al., 1995). Although interleukin-10 inhibits the formation of interleukin-12, interferon-γ and NO in lipopolysaccharide-treated mice (Berg et al., 1995; Haskó et al., 1998a), the inhibition of these mediators by IB-MECA is not dependent on the augmentation of interleukin-10 production, as this compound continued to inhibit the production of pro-inflammatory mediators in interleukin-10 deficient mice. On the other hand, it is plausible that the suppression of interferon-γ production is secondary to the decrease in tumor necrosis factor-α and/or interleukin-12 release, since both these cytokines are required for interferon-γ production during endotoxemia (Heinzel et al., 1994; Wysocka et al., 1995). Furthermore, as the induction of inducible NO synthase and therefore the production of NO are regulated by both tumor necrosis factor-α and interferon-γ under systemic inflammatory conditions (Szabó et al., 1993; Thiemermann et al., 1993; Salkowsky et al., 1997), the possibility exists that the down-regulation of these two cytokines may have contributed to the reduction in plasma NO levels by IB-MECA. At this point, it should be noted that the beneficial effect of IB-MECA was only evident, when animals were pre-treated by this drug, and not when IB-MECA was injected 2 h after LPS (not shown). This suggests that the mechanism of action of IB-MECA is related to the prevention of induction of the inflammatory response, however, when inflammatory cells are already activated, the drug is no longer capable to suppress the inflammatory process.

A further interesting finding of the present study is that the suppression of pro-inflammatory mediator production by IB-MECA is not a general phenomenon, as interleukin-1 production was not down-regulated by this compound. This can also explain the discrepancy that IB-MECA reduces the production of interferon-γ and NO less than the production of interleukin-12 (Fig. 2). Since interleukin-1 is an important costimulatory molecule in the induction of both interferon-γ (D'Andrea et al., 1993) and NO (Szabó et al., 1993), it is possible that although interleukin-12 production was strongly diminished by IB-MECA, the unaffected release of interleukin-1 could still evoke high enough interferon-γ and NO concentrations resulting in a less impressive effect of this compound on the levels of these mediators as opposed to that on interleukin-12.

The mechanisms whereby the stimulation of adenosine A_3 receptors exerts its anti-inflammatory effects is incompletely understood. In this respect, it is of note that several cell types could be involved in the in vivo effects of IB-MECA. Adenosine A_3 receptors have been demonstrated to be present on several immune cell types, including cells of the monocyte/macrophage lineage (McWhinney et al., 1996; Sajjadi et al., 1996), mast cells (Beaven et al., 1994) and eosinophils (Kohno et al., 1996). Based on the observations that with the exception of interferon-γ, monocytes/macrophages are the most important sources of the above inflammatory mediators in endotoxemia (Beutler, 1995; Barsig et al., 1995; Szabó, 1995; Wysocka et al., 1995), and it has been shown that tumor necrosis factor-α (McWhinney et al., 1996; Sajjadi et al., 1996), interleukin-12 (Szabó et al., 1998), and NO production is subject to suppression by adenosine A_3 receptor activation in vitro in monocytes/macrophages, it is likely that this cell type is involved, at least in the decreased production of tumor necrosis factor-α, interleukin-12 and NO by IB-MECA. In addition to its direct effect on macrophages, indirect mode(s) of actions may also have contributed to the observed immunosuppressive effects: such effects may include a stimulation of the release of mast cell mediators (Beaven et al., 1994) or altering regional blood flow (Von Lubitz et al., 1994).

Concerning the intracellular pathways of adenosine A_3 receptor stimulation, the activation of phosphatidyl-inositol metabolism (Beaven et al., 1994), inhibition of adenylyl cyclase (Zhou et al., 1992), and an interference with AP-1 activation (Sajjadi et al., 1996) should be considered. Although in human eosinophils activation of the adenosine A_3 receptor caused an increase in intracellular calcium levels, we failed to detect such an effect in RAW 264.7 macrophages (unpublished observations).

In summary, we provided evidence that stimulation of adenosine A_3 receptors has multiple anti-inflammatory effects by down-regulating the production of pro-in-

flammatory mediators interleukin-12, interferon-γ and NO. Since it has been shown that the anti-inflammatory properties of drugs such as methotrexate (Cronstein et al., 1991), sulfasalazine (Gadangi et al., 1996) and an adenosine kinase inhibitor (Cronstein et al., 1995) are related to their ability to release adenosine, our findings may have clinical importance. Moreover, based on our observation that the adenosine A_3 receptor agonist IB-MECA protected mice from lipopolysaccharide-induced lethality, we suggest that the potential use of this agent for the experimental therapy of septic shock should be considered.

Acknowledgements

The expert technical assistance of Mr. P. Hake, Mr. M. O'Connor, Mrs. E. Tóth and Miss J. Benkô, is gratefully acknowledged. This work was supported by a grant from the Hungarian Medical Research Council to G.H. (ETT 200/96).

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