Clin Exp Immunol 1995; 99:461–466

# Anti-inflammatory activity of salmeterol: down-regulation of cytokine production

L. SEKUT, B. R. CHAMPION, K. PAGE*, J. A. MENIUS JR† & K. M. CONNOLLY‡ *Departments of Cell Physiology, †Research Computing and ‡Pharmacology, Glaxo Research Institute, Glaxo Inc., Research Triangle Park, NC, USA, and *Cellular Sciences Division, Glaxo Research and Development, Glaxo Inc., Greenford, UK*

*(Accepted for publication 31 October 1994)*

## SUMMARY

Elevation of intracellular cAMP levels has been shown previously to inhibit cytokine secretion by various cell types *in vitro*. Since salmeterol is a $\beta_2$-agonist which activates adenylate cyclase, its ability to inhibit cytokine production was evaluated. Though salmeterol, and the related drug albuterol, did not inhibit IL-1$\beta$ production *in vitro*, both drugs did inhibit tumour necrosis factor-alpha (TNF-$\alpha$) secretion by lipopolysaccharide (LPS)-activated THP-1 cells with similar $IC_{50}$s of approximately 0·1 $\mu$M. This inhibition was effectively reversed by the $\beta_2$-antagonist oxprenolol, indicating that the inhibition was mediated through the $\beta_2$-adrenergic receptor. A strikingly different reactivity profile was seen with T cells. Salmeterol was able to inhibit the activation of both mouse and human T cells, as measured by proliferation and IL-2 secretion in response to anti-CD3 antibody, whereas albuterol was completely inactive in these assays. This T cell inhibition by salmeterol was about 10-fold less potent than that for TNF-$\alpha$ production, and was not reversed by a $\beta_2$-antagonist, indicating that a different mechanism was involved in the effect of salmeterol on T cells. Paralleling the TNF-$\alpha$ inhibitory activity *in vitro*, oral dosing of salmeterol and albuterol inhibited LPS-induced increase in murine serum TNF level *in vivo*, with $ED_{50}$s of approximately 0·1 mg/kg. This inhibition could be abrogated by dosing orally with the $\beta$-blocker propranolol. The long-acting pharmacological profile of salmeterol was apparent in that it maintained its efficacy for 3 h, while albuterol had a much shorter duration of action. Salmeterol also had some protective effects in the galactosamine/LPS model of endotoxic shock, which is dependent upon TNF-$\alpha$ production. Though salmeterol inhibited serum TNF-$\alpha$ levels by up to 94% in this assay, it protected less than 50% of the animals from the lethal effects of the LPS/galactosamine mixture. This observation suggests that functional levels of TNF-$\alpha$ localized in tissues may not be accurately reflected by serum levels.

**Keywords** salmeterol IL-2 $\beta_2$-agonist TNF-$\alpha$

## INTRODUCTION

Selective regulation of cytokine production appears to be an important element in many of the pathological manifestations of inflammation. In particular, the inhibition of TNF-$\alpha$ and IL-1 production appears to be particularly important, since inhibition of these cytokines can play an effective role in the treatment of septic shock and other inflammatory conditions, such as rheumatoid arthritis [1–4]. One mechanism for reducing secretion of many cytokines produced by monocyte/macrophages [5–9] or T cells [10] is elevation of intracellular cAMP levels. Moreover, agents which increase intracellular cAMP concentrations not only reduce cytokine secretion, they can also inhibit the release of hydrolytic enzymes and other proinflammatory mediators [11–14].

Correspondence: Dr Les Sekut, Glaxo Research Institute, Five Moore Drive, Research Triangle Park, NC 27709, USA.

Increasing intracellular cAMP levels can be achieved in a number of ways, including phosphodiesterase (PDE) inhibition and stimulation of prostaglandin and $\beta_2$-adrenergic receptors [7–10]. PDE-IV inhibitors, which slow cAMP degradation by inhibiting type IV phosphodiesterases, are potent inhibitors of cytokine production [15,16] and are effective in lipopolysaccharide (LPS)-induced endotoxic shock models (Sekut *et al*, manuscript submitted). It has also been demonstrated that agents which directly activate adenylate cyclase, such as prostaglandin $E_2$ ($PGE_2$) or forskolin, elevate cAMP levels and inhibit IL-1$\beta$ and TNF-$\alpha$ production *in vitro* [7]. Since $\beta_2$-agonists also elevate intracellular cAMP levels, they could be potentially useful anti-inflammatory agents if the unacceptable

side-effects accompanying systemic administration could be overcome by localizing or targeting the $\beta_2$ agonist. Isoproterenol, a non-specific $\beta$-agonist, has been shown to inhibit TNF-$\alpha$ production by THP-1 cells, acting at the transcriptional level, and to affect post-transcriptional regulation of IL-1 [10,17]. The $\beta_2$-agonist albuterol has also been reported to inhibit TNF-$\alpha$ production by human monocyte cell lines [10]. We have now examined the activity of salmeterol, the newest long-acting $\beta_2$-agonist, in both *in vitro* and *in vivo* models of cytokine production. The data indicate that salmeterol is also a potent inhibitor of TNF-$\alpha$ production *in vitro* and *in vivo*, and, at a 10-fold higher concentration, also has inhibitory effects on T cell activation which are not shared by albuterol and are not due to the $\beta_2$-agonist properties of this drug.

## MATERIALS AND METHODS

*Animals*

Female inbred C57Bl/6 and C3H/hen mice (approximately 22 g each) were obtained from Charles River Labs Inc. (Raleigh, NC). Both strains gave a similar pattern of serum TNF-$\alpha$ activity in response to LPS injection, although C57Bl/6 mice were somewhat more sensitive to LPS in combination with galactosamine, while C3H/hen mice were more sensitive to LPS alone.

*Compounds*

Salmeterol hydroxynaphthoate (US patent number 4992 474) was synthesized by Glaxo Inc. Albuterol (US patent number 3705 233), propranolol and oxprenolol were obtained from Sigma Chemical Co (St Louis, MO). For oral dosing, compounds were suspended at the appropriate concentration in 0·1% methyl cellulose and ground in a homogenizer, and 0·5 ml administered to each mouse by oral gavage. For *in vitro* studies, compounds were dissolved in DMSO and diluted to 0·1% in culture medium. LPS (Serotype 0111:B4; Sigma) was prepared and stored as frozen stocks in saline at 5 mg/ml.

*Cytokine secretion* in vitro

The THP-1 cell line, a human monocytic cell line, was obtained from ATCC (Rockville, MD). Supernatants taken from cells cultured for 1 week in antibiotic-free media tested negative for mycoplasma infection (Lineburger Cancer Centre, University of North Carolina). The cells were routinely cultured in 75-cm$^2$ tissue culture flasks (Costar, Cambridge, MA) in RPMI1640 (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS; Hyclone Labs, Logan, UT), 2 mm L-glutamine (Gibco) and 50 $\mu$g/ml gentamicin sulphate (Sigma). THP-1 cells were incubated in 96-well plates at $5 \times 10^5$ cells/well with various concentrations of compound plus 10 $\mu$g/ml LPS in RPMI medium. Supernatants were removed 4 h later for TNF-$\alpha$ analysis and 24 h later for IL-1$\beta$ analysis. Commercially available ELISA kits (Genzyme, Cambridge, MA) were used to measure cytokines in culture supernatants.

*T cell activation studies*

Human splenic mononuclear cells, prepared by Ficoll–Hypaque density centrifugation, and stored in frozen aliquots before use, were cultured in 96-well flat-bottomed plates at $2 \times 10^5$ cells/well in DMEM containing 10% FBS in the presence of OKT3 anti-CD3 MoAb (20 ng/ml). Proliferation of the cells was assessed at 72 h by measuring the incorporation of $^3$H-methyl thymidine during the last 18 h of culture. Parallel experiments were done with CBA mouse spleen cell suspensions, in this case stimulated with purified hamster monoclonal anti-mouse CD3 (1452C11) at a 1:1000 dilution. For IL-2 secretion studies, experiments were set up in the same way, with 100 $\mu$l culture supernatants taken after 24 h. IL-2 in these supernatants was measured in a standard CTLL proliferation assay, and results for compounds were expressed as a percentage inhibition of proliferation induced by supernatants activated in the absence of compound. This was always submaximal when compared with the response to recombinant IL-2.

*Serum TNF* in vivo *following sublethal LPS injection*

To induce elevation of serum TNF-$\alpha$ levels, LPS (5 $\mu$g/mouse) was injected intraperitoneally into C3H mice which had been fasted overnight. Mice were dosed orally with compounds 60 min before LPS injection, or as indicated in Results. Exactly 90 min after LPS injection, previously determined to be the peak for TNF-$\alpha$ levels in this system [18], mice were bled from the heart. The serum from the clotted blood was collected the following day and serum TNF-$\alpha$ levels were determined by ELISA as above.

*LPS/galactosamine model of endotoxic shock*

C57Bl/6 mice were injected intraperitoneally with 0·5 ml of a mixture of LPS (0·01 or 0·1 $\mu$g/mouse) and galactosamine (600 mg/kg; Aldrich Chem. Co., Milwaukee, WI). Serum was obtained from one group of mice ($n = 4$) 90 min after injection and analysed for TNF-$\alpha$ levels as described above. Survival was assessed after 24 h on the remaining mice ($n = 6$).

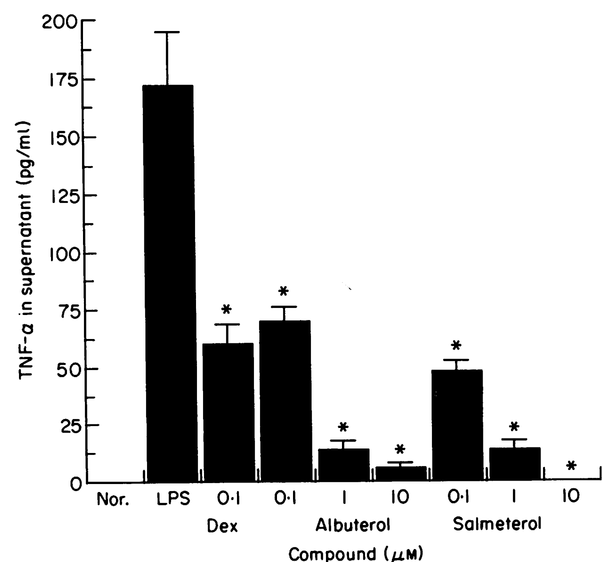


**Fig. 1.** Effect of dexamethasone $\beta_2$-agonists on tumour necrosis factor (TNF) secretion from lipopolysaccharide (LPS)-induced THP-1 cells. THP-1 cells were incubated with the test compounds just before addition of 10 $\mu$g/ml LPS. Cell supernatants were removed 4 h later, and TNF-$\alpha$ measured using a commercially available ELISA kit. * $P < 0.05$ compared with LPS control. Nor, Normal; Dex, dexamethasone.

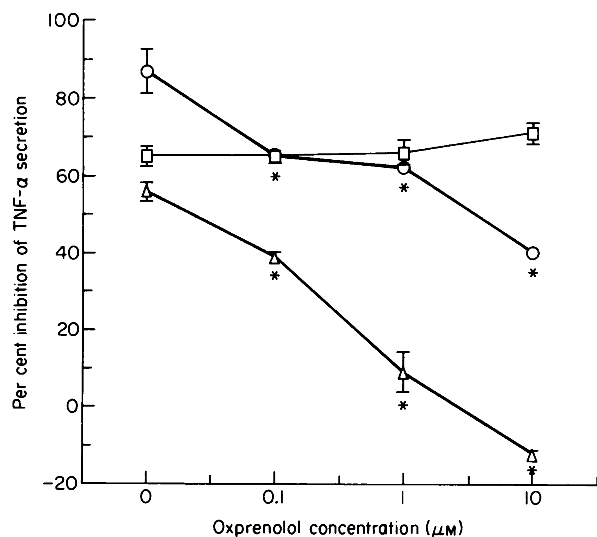


**Fig. 2.** *In vitro* abrogation of tumour necrosis factor-alpha (TNF-$\alpha$) inhibition by salmeterol using the $\beta$-receptor antagonist oxprenolol. Conditions were identical to those in Fig. 1, except that oxprenolol was added to some cultures immediately before salmeterol addition. *$P < 0{\cdot}05$ compared with zero control. ○ Salmeterol 1 μM; △, salmeterol 0·1 μM; □, dexamethasone 1 μM.

### *Statistical analysis*

Differences in serum TNF-$\alpha$ levels between dose groups were determined using analysis of variance, with specific dose group comparisons made using simultaneous 95% confidence limits. Differences in survival rates between dose groups were determined using Fisher's exact test.

## RESULTS

### *Effect of salmeterol on cytokine secretion* in vitro

Salmeterol and albuterol were incubated with THP-1 cells, a human monocyte cell line, to determine whether the drugs inhibited LPS-induced secretion of IL-1$\beta$ and TNF-$\alpha$. Though neither compound inhibited IL-1$\beta$ (data not shown), both drugs reduced TNF-$\alpha$ in the cell supernatant in a dose-dependent fashion (Fig. 1). Salmeterol and albuterol possessed similar potency, inhibiting TNF-$\alpha$ by 72% and 59%, respectively, at 0·1 μM. The *in vitro* profile of salmeterol inhibition was not statistically different from that of albuterol. Dexamethasone (Dex), used as a positive standard at 0·1 μM, reduced TNF-$\alpha$ levels by approximately 65%. Normal untreated control supernatant (Nor) possessed no detectable TNF-$\alpha$. This pattern of inhibition was similar to that obtained using human peripheral blood cells (data not shown). The inhibition of TNF-$\alpha$ production by salmeterol, especially at the low concentration, could be attributed to its $\beta_2$-agonist function, since inhibition was reversed by the $\beta$-blocker oxprenolol (Fig. 2). Oxprenolol itself had no effect on TNF-$\alpha$ production.

We also evaluated the effect of salmeterol on activation of murine and human lymphocytes. As shown in Fig. 3, salmeterol inhibited proliferation of anti-CD3-activated mouse (Fig. 3a) and human (Fig. 3b) splenic T cells. Salmeterol also inhibited IL-2 production from activated mouse spleen cells (Fig. 3c). This effect was about 5–10-fold less potent than the inhibition of TNF-$\alpha$ production in THP-1 cells, and was not reversed in the presence of a $\beta$-blocker (data not shown). The related, but short-acting $\beta_2$-agonist albuterol was completely inactive in these assays. Together, these results suggest that, unlike inhibition of TNF-$\alpha$, inhibition of T cell activation is not related to the $\beta_2$-receptor binding activity of salmeterol.

### *Effect of salmeterol on LPS-induced serum TNF levels*

Since the $\beta_2$-agonists were active against TNF-$\alpha$ production *in vitro*, they were tested *in vivo*, for inhibition of LPS-induced murine serum TNF-$\alpha$ production. In preliminary studies not shown, mice were pretreated orally with salmeterol or albuterol 30 min before LPS injection and blood taken 90 min later. In this initial study, both salmeterol and albuterol significantly ($P < 0{\cdot}05$) reduced LPS-induced serum TNF levels, with $ED_{50}$ values of approximately 0·1 mg/kg (data not shown). Since salmeterol is a long-acting $\beta_2$-agonist, the time between

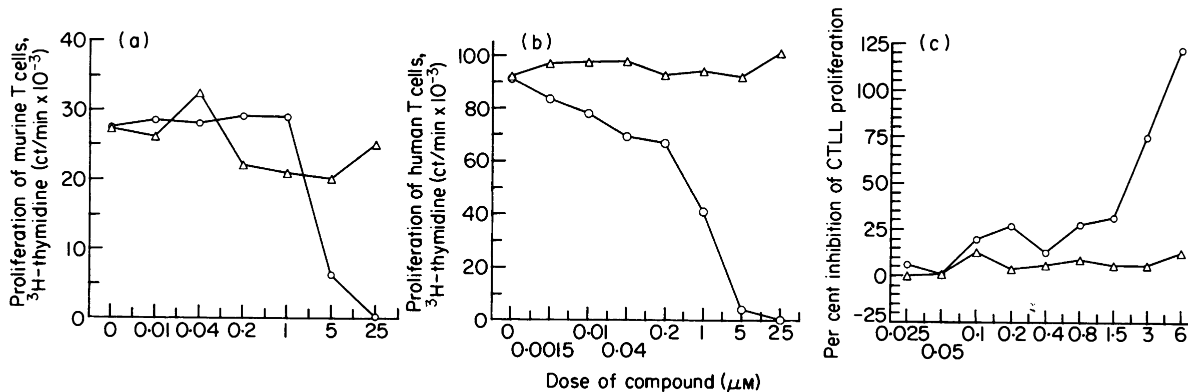


**Fig. 3.** Inhibition of T cell activation by salmeterol but not albuterol. Mouse (a) or human (b) spleen cells were activated with anti-CD3 antibodies in the presence or absence of salmeterol (○) or albuterol (△) at the concentration shown. Proliferation of splenic T cells was assessed after 3 days culture by the incorporation of tritiated thymidine. (c) Twenty-four hour culture supernatants from mice spleen cells activated with anti-CD3 antibodies in the presence of compounds, were assessed for IL-2 activity by their ability to support the proliferation of murine CTLL cells. Results are represented as percentage inhibition of the CTLL proliferation response. Each point represents the mean of three replicates with s.d < 10%.

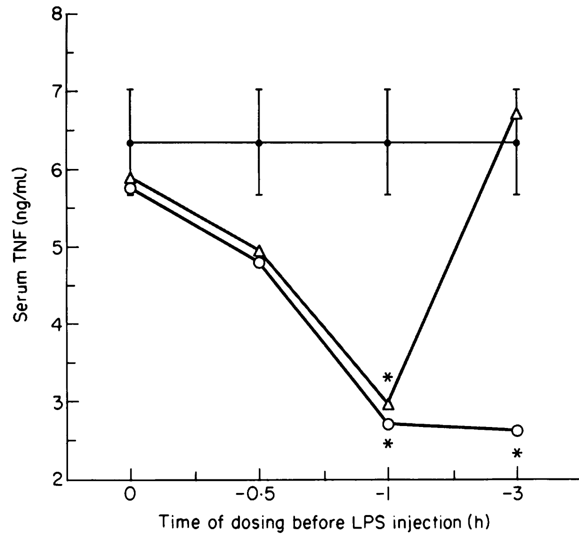


**Fig. 4.** Duration of action of $\beta$-agonists in mice injected with a sublethal dose of lipopolysaccharide (LPS). Groups of six C3H mice were dosed orally at various intervals before LPS injection (5 $\mu$g/mouse). Ninety minutes after injection, animals were bled and serum tumour necrosis factor-alpha (TNF-$\alpha$) level assessed using a commercially available murine TNF-$\alpha$ ELISA kit. Each point represents the mean of six samples ± s.e.m. * $P < 0·05$ compared with zero control. ●, LPS 5 $\mu$g/mouse; ○, salmeterol 0·1 mg/kg; △, albuterol 0·1 mg/kg.

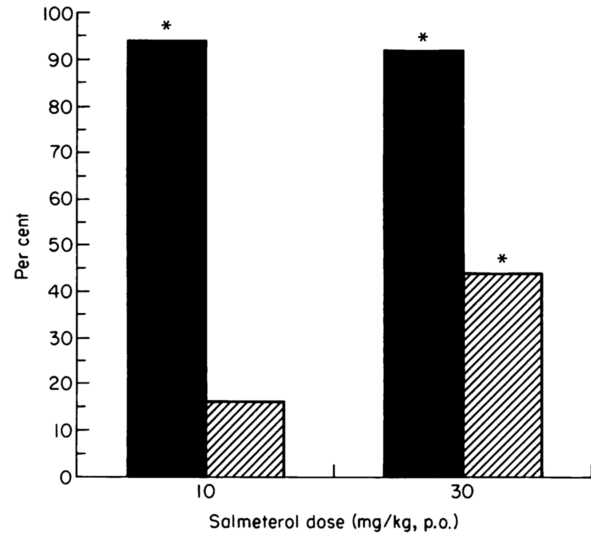


**Fig. 6.** Effect of salmeterol on survival rate and serum tumour necrosis factor-alpha (TNF-$\alpha$) levels in the lipopolysaccharide (LPS)/galactosamine endotoxic shock model. Four mice were bled 90 min after LPS/galactosamine injection while the remaining six were observed for 24 h so that the survival rate could be measured. For additional details see Materials and Methods. * $P < 0·05$ compared with untreated LPS/galactosamine controls. ■, Inhibition of serum TNF; ▨, survival.

compound dosing and LPS injection was lengthened (Fig. 4). For both albuterol and salmeterol, maximal inhibition was reached when drugs were administered 1 h before LPS injection. However, when LPS injection was delayed for 3 h, albuterol had no inhibition, whereas salmeterol was still maximally active. Salmeterol did not inhibit if dosed 6 h before LPS, and TNF levels rose to those of the untreated LPS control (data not shown).

We wished to determine if the *in vivo* inhibition of TNF-$\alpha$ by salmeterol was dependent upon its $\beta$-agonist properties as shown by its inhibition of TNF *in vitro*. Serum TNF levels were measured in LPS-injected mice dosed with salmeterol with and without the $\beta$-blocker propranolol (Fig. 5). Salmeterol was given p.o. 60 min before the LPS injection. Propranolol (10 mg/kg, p.o.) was given twice: 30 min before and after dosing with salmeterol. As shown in Fig. 5, salmeterol alone inhibited serum TNF-$\alpha$ in a dose-dependent manner. However, propranolol abolished the salmeterol-induced inhibition ($P < 0·05$). The elevation of serum TNF-$\alpha$ levels in mice receiving propranolol and a high dose of salmeterol could be due in part to the fact that propranolol alone potentiated serum TNF-$\alpha$ levels approximately 20%.

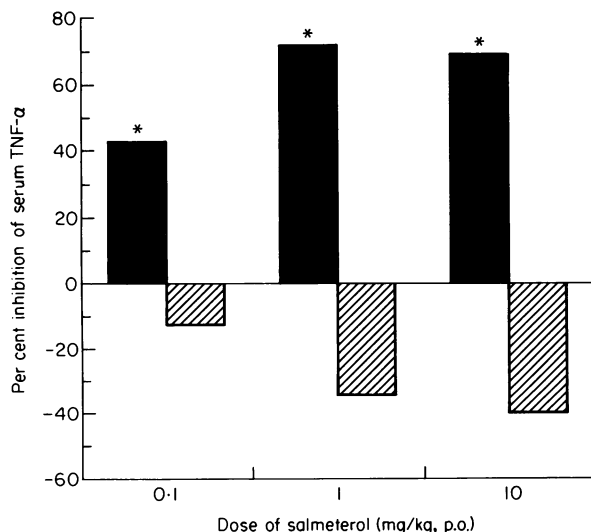


**Fig. 5.** Ability of propranolol to block salmeterol-induced reduction of serum tumour necrosis factor-alpha (TNF-$\alpha$) in lipopolysaccharide (LPS)-treated mice. Mice were dosed orally with salmeterol 60 min before LPS injection. Mice were given two doses of propranolol (10 mg/kg, p.o.), 30 min before salmeterol dosing and 30 min after salmeterol dosing. For additional details see Fig. 4. * Significant inhibition $P < 0·05$ compared with untreated LPS controls. ■, Salmeterol alone; □, salmeterol plus propanolol (10 mg/kg, p.o.).

### *Activity of salmeterol in LPS/galactosamine-induced endotoxic shock*

Since salmeterol was such a potent inhibitor of serum TNF-$\alpha$ production, it was evaluated for its ability to protect mice from lethality in the LPS/galactosamine model of endotoxic shock (Fig. 6). At 10 and 30 mg/kg, salmeterol reduced serum TNF levels 94% and 92%, respectively, in mice injected with galactosamine plus 0·1 $\mu$g LPS. However, it protected less than half the animals from lethality, even at the high 30 mg/kg dose. In a second experiment, when a 10-fold lower injection of LPS (0·01 $\mu$g/mouse) was administered, salmeterol at 10 mg/kg and 30 mg/kg still protected only 50% and 66% of mice from death by endotoxic shock, while still inhibiting serum TNF-$\alpha$ by greater than 90%.

## DISCUSSION

Cytokine interactions form a complex web which may up-regulate or down-regulate the response to infection or injury to the host. Since cytokines have both pro- and anti-inflammatory activities, it would be desirable to be able to selectively regulate cytokine production in the treatment of inflammatory diseases [1,3,19,20]. *In vitro* experiments have shown that cytokine production can be inhibited by elevation of intracellular cAMP, and suggest that this may be a very effective way of down-regulating the response of the cells to inflamagens such as endotoxin.

$\beta_2$-adrenergic receptors are 7-transmembrane proteins which are G-protein coupled to adenylate cyclase. Thus, binding of agonists triggers the production of cAMP. We have shown here that the $\beta_2$-agonists albuterol and salmeterol inhibited LPS-induced TNF-$\alpha$ production by THP-1 cells, but had no effect on IL-1$\beta$ production. This inhibition of TNF-$\alpha$ production was a potent effect ($IC_{50}$ values approximately 100 nM) and was clearly mediated through the $\beta_2$-receptor since it was reversed by a $\beta_2$-antagonist. The selective effect of $\beta_2$-agonists on TNF-$\alpha$ production contrasts with that of non-selective $\beta$-agonists, which inhibit both TNF-$\alpha$ and IL-1. This suggests that different intracellular cAMP compartments and/or trafficking events may be involved.

Since increases in intracellular cAMP concentrations have been shown to inhibit T cell activation we examined the effects of salmeterol and albuterol on anti-CD3-stimulated activation of mouse and human T cells. Salmeterol (but not albuterol) inhibited T cell activation, albeit at an approximately 10-fold higher concentration than that at which it inhibited TNF. Whether this *in vitro* inhibitory effect of salmeterol on T cells will translate into *in vivo* immunosuppressive activity remains to be investigated.

An inhibitory effect of salmeterol and albuterol on TNF-$\alpha$ production was also shown *in vivo*. Serum TNF-$\alpha$ levels in mice injected with a sublethal concentration of LPS (5 $\mu$g/mouse) were inhibited by orally administered compounds, with $ED_{50}$ values of 0·1 mg/kg. Our data from the serum TNF-$\alpha$ model confirmed the long-acting nature of salmeterol as reported by Johnson [21] and Ball *et al.* [22]. Our results showed that salmeterol had a longer duration of action than albuterol and was efficacious even when given 3 h before LPS injection, at a time when albuterol was inactive. Despite salmeterol's potency in the reduction of serum TNF-$\alpha$, this compound, used alone, offered only partial protection in the LPS/galactosamine model of endotoxic shock.

The use of $\beta_2$-agonists in the control of inflammatory responses has also been evaluated by other investigators [10,23–27]. One group has reported inhibition of TNF-$\alpha$ secretion in mast cells following *in vitro* incubation with $\beta_2$-agonists [28]. Others have demonstrated the anti-inflammatory activity of salmeterol against neutrophils [29] or T cells [30]. In some cases $\beta$-agonists appear to inhibit at the transcriptional level, as Dimitry *et al.* have reported inhibition of IL-5 transcription in lung fragments treated with isoproterenol [31]. Sever *et al.* on the other hand, demonstrated that adrenaline and isoproterenol blocked TNF-$\alpha$ production in human blood cells at the post-transcriptional level [10]. If $\beta$-agonists inhibit cytokines at a relatively late (post-transcriptional) stage in cytokine secretion, then they may be useful in the later stages of acute inflammation, at a time when earlier acting transcriptional inhibitors are not very useful. They may also complement or synergize with the actions of other anti-inflammatories, such as PDE inhibitors or steroids [32]. In addition, $\beta_2$-agonists rapidly inhibit LPS-induced TNF-$\alpha$ release *in vitro* as opposed to the delayed action of steroids, which showed maximal effectiveness when present before LPS stimulation [33]. This property of steroids represents a major disadvantage when using them as therapy late in acute, life-threatening forms of inflammation. Thus, despite their high potency against cytokines, steroids have not improved the survival rate of patients with sepsis [34–36].

In the current study we investigated the effect of $\beta_2$-agonists on TNF-$\alpha$ inhibition *in vitro* and in animal models of inflammation. Though this study focused mainly on anti-inflammatory activity in animal models of acute inflammation, work done by Seo & Saeki [25] demonstrated the combined effectiveness of albuterol and aminophylline in adjuvant arthritis, a chronic model of inflammation. The authors further reported that aminophylline (a PDE inhibitor) alone, possessed only weak anti-inflammatory activity, possibly due to the fact that cells from arthritic rats treated with aminophylline alone did not have significantly elevated levels of intracellular cAMP [25]. It is possible that the use of $\beta_2$-agonists, in combination with PDE inhibitors, might serve to boost cAMP level and provide a more potent inhibition of cytokine production. In addition, combination therapy may lower the therapeutic dose, thereby minimizing side-effects.

In apparent contrast to Seo & Saeki's work [25], Coderre *et al.* reported that catecholamines exacerbated arthritis by acting on presynaptic $\beta_2$-adrenoceptors [26,27]. However, in these experiments compounds were continously infused over a 28-day period, which is known to cause desensitization of $\beta$-receptors [37–39]. In addition, the activity of the catecholamines on the $\alpha$-adrenergic receptors might have caused a proinflammatory reaction, since Spengler *et al.* [40] have demonstrated that stimulation of $\alpha$-adrenergic receptors increased TNF production *in vitro*.

Finally, it is possible that specific $\beta_2$-agonists like salmeterol, in conjunction with MoAbs, soluble cytokine receptors or traditional steroids, might be useful in a multi-step approach towards the treatment of septic shock syndrome or other serious diseases in which cytokines play a pathogenic role, like acute episodes of lupus or flairs associated with rheumatoid arthritis.

## ACKNOWLEDGMENTS

The authors wish to thank Dr Michael K. James, Dr Iain D. Dukes and Dr Malcolm Johnson for their critical review of the manuscript.

## REFERENCES

1 Molly RG, Mannick JA, Rodrick ML. Cytokines, sepsis and immunomodulation. Br J Surg 1993; **80**:289–97.
2 Pfeffer K, Matsuyama T, Kunding TM *et al.* Mice deficient for the 55 kD tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. Cell (USA) 1993; **73**:457–67.
3 Jäättelä M. Biology of disease; biologic activites and mechanisms of action of tumor necrosis factor-$\alpha$/cachectin. Lab Invest 1991; **64**:724–42.

4 Brennan FM, Maini RN, Feldmann M. TNFα—a pivotal role in rheumatoid arthritis? Br J Rheumatol 1992; **31**:293–8.

5 Kammer GM. The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. Immunol Today 1988; **9**:222–9.

6 Bourne HR, Lichtenstein LM, Melmon KL *et al.* Modulation of inflammation and immunity by cAMP. Science 1974; **184**:19–28.

7 Kunkel SL, Wiggins RC, Chensue SW, Larrick J. Regulation of macrophage tumor necrosis factor production by prostaglandin $E_2$. Biochem Biophys Res Commun 1986; **137**:404–10.

8 Hart PH, Whitty GA, Piccoli DS, Hamilton JA. Control by IFN-gamma and $PGE_2$ and TNFα and IL-1 production by human monocytes. Immunol 1989; **66**:376–83.

9 Strieter RM, Remick DG, Ward RN *et al.* Cellular and molecular regulation of tumor necrosis factor-alpha production by pentoxifyline. Biochem Biophys Res Commun 1988; **155**:1230–6.

10 Sever A, Rapson NT, Hunter C A, Liew FY. Regulation of tumor necrosis factor production by adrenaline and β-adrenergic agonists. J Immunol 1992; **148**:3441–5.

11 Dobbins DE, Soika CY, Premen AI *et al.* Blockade of histamine and bradykinin-induced increases in lymph flow, protein concentration, and protein transport by terbutaline *in vivo*. Microcirc Res 1982; **2**:127–50.

12 Joyner WL, Svensjo E, Arfors KE. Simultaneous measurements of macromolecular leakage and arteriolar blood flow as altered by $PGE_1$ and $beta_2$-stimulation in the hamster cheek pouch. Microcirc Res 1979; **18**:301–10.

13 Henney CS, Bourne HR, Lichtenstein LM. The role of cyclic 3′,5′-adenosine monophosphate in the specific cytolytic activity of lymphocytes. J Immunol 1972; **108**:1526–34.

14 Goodman Gilman A, Rall TW, Nies AS, Taylor P. Goodman and Gilman's the pharmacological basis of therapeutics, 8th edn. New York: McGraw-Hill, Inc., 1990:187–220.

15 Semler JH, Wachtel H, Endres S. The specfic type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor-α production by human mononuclear cells. Int J Immunopharmacol 1993; **15**:409–13.

16 Molnar-Kimber KL, Yonno L, Heaslip RJ *et al.* Differential regulation of TNF-α and IL-1β production from endotoxin stimulated human mocytes by phosphodiesterase inhibitors. Mediator Infl 1992; **1**:411–17.

17 Hetier E, Ayala J, Bousseau A, Prochiantz A. Modulation of interleukin-1 and tumor necrosis factor expression by β-adrenergic agonists in mouse ameboid microglial cells. Exp Brain Res 1991; **86**:407–13.

18 Sekut L, Menius JA Jr, Brackeen MF *et al.* Evaluation of the significance of elevated levels of systemic and localized TNF in different animal models of inflammation. J Lab Clin Med 1994; **124**:813–20.

19 Barton BE, Jackson JV. Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model. Infect Immun 1993; **61**:1496–9.

20 Bellini F, Bruni A. Role of serum phospholipase A1 in the phosphatidylserine-induced T cell inhibition. FEBS Lett 1993; **316**:1–4.

21 Johnson M. Salmeterol: a novel drug for the treatment of asthma. Agents Actions Suppl 1991; **34**:79–95.

22 Ball DI, Brittain RT, Coleman RA *et al.* Salmeterol, a novel, long-acting beta 2-adrenoceptor agonist: characterization of pharmacological activity *in vitro* and *in vivo*. Br J Pharmacol 1991; **104**:665–71.

23 Goto M, Griffin AJ, Chiemmongkoltip P, Ohya N. Beta-adrenergic drug therapy in newborn canine endotoxic shock. Circ Shock 1990; **32**:123–32.

24 Tanniere-Zeller M, Rochette L, Bralet J. PAF-acether-induced mortality in mice: protection by benzodiazepines. Drugs Exp Clin Res 1989; **15**:553–8.

25 Seo S, Saeki K. Inhibition of adjuvant arthritis by salbutamol and aminophylline. Eur J Pharmac 1990; **34**:521–3.

26 Coderre TJ, Basbaum AI, Dallman MF *et al.* Epinephrine exacerbates arthritis by an action at presynaptic $\beta_2$-adrenoceptors. Neurosci 1990; **34**:521–3.

27 Coderre TJ, Chan AK, Helms C *et al.* Increasing sympathetic nerve terminal-dependent plasma extravasation correlates with decreased arthritic joint injury in rats. Neurosci 1991; **40**:185–9.

28 Elyse Y, Bissonnette E, Befus AD. Anti-inflammatory effects of $\beta_2$-adrenergic agonists in TNFα activity of human mast cell. Am J Respir Crit Care Med 1994; **149**:A805.

29 Hyers TM, Tricorni SM. Sameterol shows anti-inflammatory effects on human neutrophils *in vitro*. Am J Respir Care Med 1994; **149**:A804.

30 Oddera S, Silvestri M, Quaglia R *et al.* The allergen-induced proliferative response of blood T-lymphocytes is inhbited *in vitro* by salmeterol via a non-$\beta_2$ aderoreceptor pathway. Eur Respir J 1993; **6**:308s.

31 Dimitry EA, Jaffe JS, Post TJ *et al.* β-adrenergic agonist-induced attenuation of interleukin-5 mRNA and protein from anti-IgE challenged human lung fragments. Am J Respir Care Med 1994; **149**:A804.

32 Oddera S, Silvestri M, Lantera S *et al.* Combined effects of salmeterol and dexamethasone on the downregulation of allergen-induced T-cell proliferation and GM-CSF release by blood mononuclear cells of atopic individuals. Am J Respir Care Med 1994; **149**:A805.

33 Waage A, Bakke O. Glucocorticoids suppress the production of tumor necrosis factor by lipopolysaccharide-stimulated human monocytes. Immunol 1988; **63**:299–302.

34 Marks JD, Marks CB, Luce JM *et al.* Plasma tumor necrosis factor in patients with septic shock. Mortality rate, incidence of adult respiratory distress syndrome, and effects of methylprednisolone administration. Am Rev Respir Dis 1990; **141**:94–7.

35 Sprung CL, Caralis PV, Marcial EH *et al.* The effects of high-dose corticosteroids in patients with septic shock. A prospective, controled study. N Engl J Med 1984; **311**:1137–43.

36 The Veterans Administration Systemic Sepsis Cooperative Study Group. Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. N Engl J Med 1987; **317**:659–665.

37 Sibley DR, Lefkowitz RJ. Molecular mechanisms of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 1985; **317**:124–9.

38 Hausdorff WP, Caron MG, Lefkowitz RJ. Turning off the signal: desensitization of β-adrenergic receptor function. FASEB J 1990; **4**:2881–9.

39 Jones SB, Romano FD. Myocardial beta adrenergic receptor coupling to adenylate cyclase during developing septic shock. Circ Shock 1990; **30**:51–61.

40 Spengler RN, Chensue SW, Giacherio DA *et al.* Endogenous norepinephrine regulates tumor necrosis factor-α production from macrophages *in vitro*. J Immunol 1994; **152**:3024–31.