The aim of this study was to investigate the pyrogenic activity of factor(s) released by rat peritoneal macrophages following a brief stimulation with LPS. The effect of this factor on the number of circulating leukocytes and serum Fe, Cu and Zn levels, was also evaluated. The possibility that the content of Interleukin (II)-1β, IL-6 and tumour necrosis factor (TNF) in the supernatant could explain the observations was investigated. Supernatant produced over a period of 1 h by peritoneal macrophages, following a 30 min incubation with LPS at 37°C, was ultrafiltered through a 10 000 MW cut-off Amicon membrane, sterilized, and concentrated 2.5, 5, 10 and 20 times. The intravenous (i.v.) injection of this supernatant induced a concentration-dependent fever in rats with a maximal response at 2 h. The pyrogenic activity was produced by macrophages elicited with thioglycollate and by resident cells. The supernatants also induced neutrophilia and reduction in Fe and Zn 6 h after the injection. Absence of activity in boiled supernatants, or supernatants from macrophages incubated at 4°C with LPS, indicates that LPS was not responsible for the activity. In vitro treatment with indomethacin (Indo), dexamethasone (Dex), or cycloheximide (Chx) did not modify the release of pyrogenic activity into the supernatant or its effects on the reduction in serum metal levels. Although Chx abolished the production of mediator(s) inducing neutrophilia, and Dex reduced the induction of IL-1β, TNF and IL-6, injection of the highest concentration of these cytokines detected in the supernatants did not induce fever. In vivo treatment with Dex, but not Indo, abolished the fever induced by the supernatant. These results suggest that macrophages contain pre-formed pyrogenic mediator(s), not related to IL-1β, IL-6 or TNF, that acts indirectly and independently of prostaglandin. It also seems likely that the pyrogenic activity is related to the factor responsible for the reduction of serum Fe and Zn levels, but not the neutrophilia.

Key words: Lipopolysaccharide, Macrophages, Pyrogenic factor

Introduction

Exogenous pyrogens such as bacterial endotoxin (lipopolysaccharide, LPS) initiate a complex host response which includes fever, neutrophilia and changes in circulating glucocorticoid, glycoproteins and serum metal levels, collectively called the acute-phase reaction (APR). It is thought that LPS-induced APR is caused by the release of cytokines into circulation by several cell types including macrophages, endothelial cells and fibroblasts. Many of these cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor (TNF), interleukin-8 (IL-8) and macrophage inflammatory protein-1 (MIP-1), generally known as endogenous pyrogens (EP) can induce fever when injected into experimental animals and humans (see Reference 1 for a review). Although all endogenous pyrogens can induce fever the real role of each in the febrile response is not clearly established.1

It has been suggested that IL-1 (largely IL-1β) is a primary mediator of fever since peripheral or central injection of this cytokine induces fever in different species.2-4 In addition, antiserum to IL-1β prevents the increase in body temperature and plasma IL-6 activity induced by intraperitoneal (i.p.) injections of LPS in rats.5 However, there is little data demonstrating an elevation of IL-1β in the plasma of febrile animals and no increase in this cytokine was detected in the cerebrospinal fluid of cats6 or in the hypothalamus of rats7 after peripheral injection of LPS. An increased synthesis of IL-1β in the

A pre-formed pyrogenic factor released by lipopolysaccharide stimulated macrophages

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Recent studies in guinea-pigs\textsuperscript{9} and rats\textsuperscript{2} have shown an increase in intrahypothalamic concentrations of IL-6 and TNF-like activity during LPS-induced fever. In spite of a strong correlation between plasma IL-6 activity and increase in the body temperature in guinea-pigs, there was no correlation between fever and plasma TNF, or between fever and IL-6 or TNF in the hypothalamic perfusate. In rats, the hypothalamic infusion of IL-6, but not TNF, at a rate that would mimic the amount of cytokine activity found in the anterior hypothalamus during LPS-induced fever, was effective in inducing an increase in body temperature.

Data from many studies suggest that the first cytokine to increase in plasma after LPS injection in different animal species is TNF\textsuperscript{9-11} but its role in fever is still unresolved. Intravenous infusions of TNF in humans, or bolus injection of this cytokine in rats and other species, result in fever.\textsuperscript{10,12-14} Furthermore, pre-treatment of rabbits with antisera to TNF inhibits LPS-induced fever.\textsuperscript{15} However, recent studies attribute to TNF the role of an endogenous cryogen, rather than an endogenous pyrogen, since antisera to TNF has been shown to increase LPS-induced fever in rats.\textsuperscript{16-19}

Interleukin-8 is a cytokine released by a wide variety of cell types upon exposure to inflammatory stimuli or endogenous mediators such as IL-1 and TNF\textsuperscript{20-22} and has been shown to induce fever after central administration in rats. Unlike the response to other cytokines, the febrile response induced by IL-8 is not dependent on prostaglandin synthesis.\textsuperscript{23,24}

Macrophages represent one of the main sources of known cytokines\textsuperscript{25} and have also been shown to release a chemotactic factor that specifically induces neutrophil migration.\textsuperscript{26} This chemotactic substance, macrophage-released neutrophil chemotactic factor (MNCF), is released by rat peritoneal macrophages following a brief\textit{ in vitro} stimulation factor with heterologous serum, LPS, TNF\textsubscript{a} or IL-1\textbeta.\textsuperscript{27-30} This chemotactic factor is distinguishable from other known chemotactic agents/or cytokines\textsuperscript{31} and acts directly.\textsuperscript{32}

Based on the ability of macrophages to release a neutrophil chemotact substance different from the known cytokines, and because of the uncertain role of known cytokines in the genesis of fever, the purpose of this study was to investigate whether this factor could induce fever and other APR components such as changes in circulating white blood cells and the concentration of serum metals. In addition, the concentration of IL-1\textbeta, TNF and IL-6 released by macrophages during a brief incubation with LPS was determined as well as the effect of\textit{ in vitro} treatment with steroidal and non-steroidal anti-inflammatory drugs and protein synthesis blockers on the release and action of the factor(s).

**Material and Methods**

**Animals**: Male Wistar rats weighing 180–200 g were housed at 24 ± 1°C with 12 h light/dark cycle, with lights on at 0600, and given water and food \textit{ad libitum}.

**Preparation of the supernatant from LPS-stimulated macrophage monolayers**: The supernatant was prepared using a process similar to that described previously by Cunha and Ferreira.\textsuperscript{29} Briefly, 4 days before the harvest of peritoneal macrophages, rats received an intraperitoneal injection of 10 ml of 3% thioglycollate. To evaluate possible effects of thioglycollate, an equal number of macrophages from non-injected animals were used in some experiments. Peritoneal cells were harvested, using 10 ml of RPMI 1640 medium (pH 7.4) containing 5 U/ml heparin, and incubated in culture dishes for 1 h at 37°C. To discard the non-adherent cells, the monolayers were washed three times with phosphate buffered saline (PBS). Adherent cells in two or three plates of each treatment were scraped using a rubber policeman, resuspended in media, diluted in Turk's stain, and counted in a Neubauer chamber. The differential cell count was performed as described previously by Souza and Ferreira\textsuperscript{27} and cellular viability was tested using 1% eosin Y.\textsuperscript{28} The monolayers consisted of 95% macrophages, resulting in a total of 0.6–1.0 × 10\textsuperscript{7} viable cells/plate. The cells were incubated with LPS (10 g/μg of lipopolysaccharide \textit{E. coli} 0111:B4/μl of RPMI) for 30 min at 37°C. The monolayers were then washed three times with PBS and incubated with 5 ml of medium without LPS, for 1 h at 37°C. To evaluate the effect of the incubation temperature on the release of substances, some experiments were conducted at 4°C after adherence of the cells. The supernatant was ultracentrifuged with an Amicon YM 10 membrane, the retained portion was resuspended in the saline for intravenous injections or lyophilized and stored at −20°C for cytokine assays. To discount the possibility of contamination with LPS, supernatant (10 times concentrated) and LPS were boiled for 30 min. The supernatants were sterilized with a 0.22 μm (Millipore Corporation, Bedford) membrane prior to intravenous (i.v.) injections.

**Treatment of macrophage monolayers with dexamethasone, cyclobeximide and indomethacin**: Macrophage monolayers were incubated with 3 or 9 μg/ml dexamethasone (Dex), 3 and 9 μg/ml cyclobeximide (Chx) or 0.5 and 2 μg/ml indomethacin (Indo) for 60, 30 or 15 min respectively, before LPS addition. During and after LPS...
stimulation, the monolayers were incubated with RPMI medium containing the same concentration of the drugs. Control supernatants had the drugs added at the end of the incubation period. The preparation of the supernatant for injection was carried out as described above.

**Temperature measurements:** Rectal temperature was measured by inserting a thermistor probe (Y.S.I. nr. 402) 3 cm into the rectum. The animals were picked up gently and held manually during the temperature measurements. This procedure was performed at least twice on the day before the experiment to minimize stress-induced temperature changes secondary to handling. On the day of the experiment, the basal temperature of each animal was determined four times, at 30 min intervals, before any injection. The experiments were carried out between 0800 and 1700 h at 28 ± 1 °C, which is considered the thermoneutral zone for rats.33

**Leukocyte counts and serum metal assays:** Two, 6 and 24 h after the i.v. injection of pyrogenic stimuli, animals were anesthetized with pentobarbitone sodium (40 mg/kg, i.p.) and blood samples were taken from the abdominal aorta for haematological determination. Total and differential cell counts were performed and the results are reported as the number of neutrophils per ml of blood.27 Before the serum metal assays, serum was prepared by adding the same volume of trichloroacetic acid 20% and heating at 90°C for 15 min. The mixture was centrifuged at 1000g by 15 min and the Fe, Zn and Cu levels were measured in the supernatant using atomic absorption spectrophotometry.

**Experimental protocols:** The rectal temperature of the rats was measured at 30 min intervals for 2 h before, and up to 6 h after i.v. injections of pyrogens. LPS (0.5 μg/kg), murine recombinant IL-1β (2.5 μg/kg) or supernatants were administered intravenously in the penial venous sinus in a volume of 0.2 ml. The effects of steroidal and non-steroidal anti-inflammatory drugs on temperature responses to i.v. injections of the supernatant, LPS and IL-1β were also investigated. Indo (2 mg/kg, i.p.) and Dex (0.5 mg/kg s.c.) were given 30 min and 1 h, respectively, before the i.v. injection of supernatants. Control animals were treated with the appropriate vehicle only. The pyrogenic stimuli were injected at 1100 h.

**Cytokine assays:** Cytokine bioactivities were measured in supernatants from Dex-treated macrophages and control supernatants. For these studies, the supernatant was lyophilized and resuspended in culture medium. IL-1 was assayed as described previously, using the D10(N4)M (D10) cell line.34 Activity was determined by comparison to the National Institute of Biological Standards and Controls (NIBSC) interim standard for IL-1β (lot. 86.552, 1 U = 10 pg).

IL-6 was assayed using the B9 hybridoma as described previously by Holt et al.35 For the assay, B9 cells were used 3–4 days after the last subculture. Bioactivity was determined by comparison to a recombinant IL-6 preparation provided by Dr L. A. Aarden.37

TNF was assayed using L929 fibroblasts, using a protocol adapted from Matthews and Neale.39 The cells were maintained in DMEM supplemented with 5% FCS and 2 mM glutamine. Cells isolated with 0.25% trypsin, were seeded into microplates wells at 1 × 10^4 cells/wells, and cultured overnight with 200 pg/ml actinomycin D for 1 h before addition of samples or controls. The TNF concentration was determined by comparison to the NIBSC standard for TNFα (lot. 88/552). Cellular activity in all assays was determined by monitoring metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its formazan product.40

**Materials:** Lipopolysaccharide from E. coli (O111:B4), cycloheximide, glutamine, trypsin and MTT were purchased from Sigma Chemicals and Co., USA; thioglycollate from Difco Laboratories, USA; RPMI from Gibco Laboratories, USA; heparin from Roche, Brazil; yellow eosin from Merck; dexamethasone (Decadronal) from Prodome, Brazil; and DMEM from Northumbria Biologicals. Indomethacin was a gift from Merck, Sharp & Dohme, Brazil. Recombinant murine cytokines used to induce fever were IL-1β (lot. No. BN091), IL-6 (lot. No. BEIN3.19) and TNFα (lot. No. CS184), all from R & D Systems, Inc., Minneapolis.

**Statistical analysis:** Febrile responses were assessed by measuring the increases in rectal temperature and are expressed as the difference between rectal temperature before and at specified times after injection of the pyrogenic stimuli. All data are expressed as the mean ± S.E.M. and were analysed using one-way analysis of variance, followed by Tukey's test, and a level of significance at p < 0.05.

**Results**

Intravenous injection of supernatant from thioglycollate-elicited macrophages stimulated in vitro with LPS induced a concentration dependent fever as illustrated in Fig. 1A. The i.v. injection of 2.5-fold concentrated supernatant did not change the rectal temperature of the animals, while 5-, 10- and 20-fold concentrated supernatant induced a considerable febrile response which peaked at 2 h. Both 10- and 20-fold concentrated supernatant, induce a maximal response, although the response to the higher concentration declined more rapidly. The 20-fold concentrated supernatant was used in the remaining experiments. Induction of the macrophages
with thioglycollate was not essential to the release of pyrogenic factor(s) since supernatant from LPS-stimulated resident macrophages induced fever with similar time-course and magnitude (Fig. 1B) when compared to the response induced by supernatant from LPS-stimulated macrophages previously elicited by thioglycollate. Injection of the medium, supernatant from unstimulated macrophages or saline, did not change the body temperature of the rats (Fig. 1C). LPS contamination was not responsible for the supernatant-induced fever since supernatant from LPS-stimulated macrophages cultured at 4°C did not induce fever. Furthermore, as shown in Table 1, no change in body temperature was observed after i.v. injection of supernatant boiled for 30 min. In vitro treatment of macrophages with 3 or 9 μg/ml Dex, 3 or 9 μg/ml Chx or 0.5 or 2.0 μg/ml Indomethacin did not modify the pyrogenic activity of the supernatant (Fig. 2).

Supernatant from LPS-stimulated macrophages, harvested from thioglycollate-elicited peritoneal cavities, induced a significant neutrophilia and reduction of the serum Fe level 2 and 6 h after i.v. injection, while reduction of serum Zn levels was evident only at 6 h. On the other hand, LPS caused neutrophilia 2 and 6 h after i.v. injection, while reduction of Fe and Zn levels was observed only at 6 h. In both cases the observed neutrophilia and reduction of serum metal levels were more marked at 6 h (Table 1). Neither the supernatant nor LPS induced any change of the serum Cu level. Boiling diminished the neutrophilia, abolished the reduction of the Zn level, but did not modify the reduction of the Fe level induced by the supernatant (Table 1).

Table 1. Changes in body temperature, circulating neutrophils, serum Fe and Zn levels induced by saline, LPS, non-boiled and boiled supernatants from LPS-stimulated macrophages in rats

| Pyrogenic stimulus | Time (h) | Temperature change (°C) | Neutrophils (cells/ml.10⁶) | Fe (μg/100ml) | Zn (μg/100ml) |
|-------------------|---------|------------------------|---------------------------|--------------|--------------|
| Saline (1 ml/kg)  | 2       | 0.02 ± 0.10            | 1.66 ± 0.18               | 240.0 ± 23.7 | 348.4 ± 20.2 |
|                   | 6       | 0.00 ± 0.10            | 1.17 ± 0.14               | 133.3 ± 17.5 | 267.3 ± 11.5 |
| LPS (0.5 μg/kg)   | 2       | 1.14 ± 0.15            | 5.75 ± 0.37               | 223.8 ± 14.7 | 329.5 ± 11.9 |
|                   | 6       | 0.13 ± 0.21            | 6.84 ± 0.77               | 19.4 ± 10.4  | 147.0 ± 23.1 |
| Non-boiled supernatant (10×) | 2       | 1.02 ± 0.06*           | 3.13 ± 0.39               | 106.5 ± 7.8  | 311.7 ± 10.3 |
|                   | 6       | -0.08 ± 0.05           | 9.10 ± 1.18               | 78.9 ± 14.1  | 202.1 ± 14.1 |
| Boiled supernatant (10×) | 2       | -0.10 ± 0.06*          | ND                        | ND           | ND           |
|                   | 6       | -0.43 ± 0.07           | 3.51 ± 0.44               | 16.7 ± 3.6   | 267.0 ± 16.0 |

Macrophages from thioglycollate-elicited peritoneal cavities were stimulated in vitro with LPS for 30 min. The supernatants were ultrafiltered with an Amicon YM-10 membrane and resuspended in saline. Non-boiled and boiled (30 min boiling period) supernatant was sterilized with a 0.22 μm membrane and injected i.v. in a volume of 0.2 ml/rat. Values represent the mean ± S.E.M. of each parameter observed in 6-13 animals. ND = Not done; *significantly different from saline; **significantly different from non-boiled supernatant, p < 0.05.
The neutrophilia, but not the reduction of serum metal levels, induced by the supernatant was significantly reduced when the macrophages were incubated in the presence of Chx (Fig. 3). The treatment of the macrophages with Dex or Indo did not significantly affect the neutrophilia induced by the supernatant 6 h after the injection (control supernatant, 6.47 ± 1.05; Dex, 9 µg/ml, 4.05 ± 0.72; Indo, 2 µg/ml, 8.45 ± 1.17 neutrophils × 10^6/ml). When rats were pretreated with Dex (0.5 mg/kg, s.c.) 1 h before the harvesting of the peritoneal macrophages and all further steps of the supernatant preparation were conducted in presence of 9 µg/ml Dex, the magnitude of fever induced by the resulting supernatant was similar to that induced by supernatant from macrophages obtained from untreated animals (Fig. 4). Treatment of rats with Dex (0.5 µg/kg, s.c.) inhibited febrile responses to i.v. injection of LPS, IL-1β and supernatant (Fig. 5). In contrast, Indo (2 mg/kg, i.p.) significantly reduced the febrile response to i.v. injections of LPS and IL-1β, but did not modify the response induced by the supernatant (Fig. 6). The treatment of animals with Dex and Indo did not affect the neutrophilia or the reduction of serum Fe and Zn levels induced by LPS or supernatant.

The IL-1β, IL-6 and TNF bioactivities were measured in supernatants. Values varied quite widely...
between different supernatants and the highest values are shown in Table 2. Unlike the pyrogenic activity Dex inhibited the release of these cytokines. The injection of the mixture of murine recombinant IL-1β, TNFα and IL-6 at the highest concentrations found in the supernatants induced a maximal increase in body temperature of 0.12 ± 0.04°C, 30 min after injection, which was not significantly different to saline treated controls (0.13 ± 0.03°C).

**Discussion**

This study shows that macrophages briefly stimulated with LPS release a factor that induces an increase in rectal temperature, neutrophilia and reduction in serum Fe and Zn levels, mimicking important components of APR.

It is unlikely that these activities represent a nonspecific response due to contamination of the supernatant with LPS since when the metabolic activity of the macrophages was reduced by cooling to 4°C, they did not release pyrogenic factor(s), and boiling the supernatant abolished the fever and Zn reduction and reduced the neutrophilia. The release of the pyrogenic factor(s) was not related to previous thioglycollate stimulation, since supernatant from LPS-stimulated resident macrophages also induced fever.

The treatment of the cells, or rats acting as the source of macrophages, with Dex did not modify the pyrogenic activity, although production of IL-1, IL-6 and TNF was inhibited. Since glucocorticoids suppress the cytokine or LPS-stimulated production of IL-1, TNF, IL-6, IL-8 and MNCF these findings from in vitro Dex treatment distinguish the pyrogenic factor from other known pyrogenic or chemotactic cytokines. In contrast, treatment of rats with Dex (0.5 mg/kg) inhibits fever induced by the supernatant or IL-1β.

The failure of Chx to inhibit the release of the factor that induces fever and reduction of the serum metal levels supports the hypothesis that this factor is not produced following synthesis of cytokines, though it did abolish the release of the factor that induced neutrophilia. Moore et al. and Nordlund et al. showed that Chx inhibits the production of EP by rabbit granulocytes or human monocytes stimulated with LPS or Staphylococcus albus. However, this inhibition was related to activity observed 12–16 h after stimulation. Therefore, it is plausible that macrophages release pre-formed factor. Similar findings have been recently obtained by Won and
Table 2. Maximal concentration of IL-1β, IL-6 and TNFα in supernatants from LPS-stimulated macrophages treated or not with Dex.

| Treatment | IL-1β (ng/ml) | IL-6 (ng/ml) | TNFα (ng/ml) |
|-----------|---------------|--------------|--------------|
| None      | 6.8 ± 0.45    | 10.17 ± 0.00 | 12.5 ± 0.23  |
| Dex       | 2.3 ± 0.19′   | 0.29 ± 0.2′  | 2.3 ± 0.52′  |

Macrophages were incubated with Dex (9 µg/ml) before, during and after LPS stimulation. The supernatants were ultrafiltered in YM-10 Amicon membranes, lyophilized and stored at −20°C until the day of the assay, when they were resuspended in culture medium to give a 10-fold concentrated solution. Values represent the mean ± S.E.M. (4–6 wells) cytokine concentration in the sample with the highest level of cytokine. Values significantly different from untreated cells, p < 0.001.
ies, using ultrafiltration in YM 30 membranes, have demonstrated that the MW of our pyrogenic factor is above 30 kDa. All the pyrogenic cytokines released by macrophages possess a molecular weight smaller than 30 kDa.

Kluger has stressed that although many cytokines induce fever when injected into experimental animals or humans, the precise contribution of any EP in naturally occurring fever has not yet been completely determined. This concept, together with evidence for a pre-formed pyrogen factor described here, allow us to hypothesize that this factor may represent a trigger for fever induction by promoting the release of another pyrogenic mediator which acts by a prostaglandin independent mechanism. A possible candidate is CRF, explaining the effectiveness of glucocorticoids and the partial effectiveness of LPS in rat-IL-1β or LPS. These findings could explain the inability of antipyretic drugs to abrogate the fever response observed in a number of clinical situations. Furthermore, the present study emphasizes the importance of macrophages as alarm cells because of their ability to react to foreign substances in situ. Furthermore, the present study emphasizes the importance of macrophages as alarm cells because of their ability to react to foreign substances in situ. Furthermore, the present study emphasizes the importance of macrophages as alarm cells because of their ability to react to foreign substances in situ. Furthermore, the present study emphasizes the importance of macrophages as alarm cells because of their ability to react to foreign substances in situ.
46. Woo SJ, Lin MT. Endogenous pyrogen formation by human blood monocytes stimulated by polyribosinuric acid.polyriboctydilic acid. *Experientia* 1973; 49: 157-159.

47. Knudsen PJ, Dinarello CA, Strom TB. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin-1 activity by intracellular cyclic adenosine monophosphate. *J Immunol* 1990; 135: 3189-3194.

48. Hart PH, Whitty GA, Piccoli LS, Hamilton JA. Control by IFN-γ and PGE2 of TNF-α and IL-1 production by human monocytes. *Immunol* 1989; 66: 376-383.

49. Tocco-Bradley R, Moldawer LL, Jones CT, Gerson B, Blackburn GL, Brizard RR. The biological activity in vivo of recombinant human interleukin-1 in the rat. *Proc Soc Exp Biol Med* 1989; 192: 263-271.

50. Rothwell N. Mechanisms of the pyrogenic actions of cytokines. *Bar Cytokine Net* 1990; 1: 211-213.

51. Ibboty DC, Grimble RF. Temperature and metabolic change in rats after various doses of tumour necrosis factor α. *J Physiol* 1999; 430: 307-380.

52. Suttlies J, Giri JO, Miel SB. IL-1 secretion by macrophages. Enhancement of IL-1 secretion and processing by calcium ionophores. *J Immunol* 1990; 144: 175-182.

53. Beusher HU, Güнтер C, Röllinghoff M. IL-1β is secreted by activated murine macrophages as biologically inactive precursor. *J Immunol* 1990; 144: 2179-2183.

54. Mifiano FJ, Sancibrian M, Vincino M, et al. Macrophage inflammatory protein-1: unique action on hypothalamus to evoke fever. *Brain Res Bull* 1990; 26: 849-852.

55. Ferreira SI. Are macrophages the body's "alarm cells"? *Agents and Actions* 1980; 10: 239-250.

56. Souza GEP, Cunha FQ, Mello R, Ferreira SI. Neutrophil migration induced by inflammatory stimuli is reduced by macrophage depletion. *Agents and Actions* 1988; 24: 377-380.

ACKNOWLEDGEMENTS. The authors gratefully acknowledge Márcio M. Coelho (from this department) for helpful discussions and review of the manuscript, and Saskia Brouwer for technical assistance. The Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Estado de São Paulo is thanked for financial support (Proc. 500077/90 and 92/2012-8, respectively) and a fellowship to AR Zampronio and MCC Melo.

Received 13 April 1994; accepted in revised form 8 June 1994