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Modulation of haem oxygenase-1 expression by nitric oxide and leukotrienes in zymosan-activated macrophages

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1 Phagocytosis of unopsonized zymosan by RAW 264.7 macrophages upregulated protein expression of haem oxygenase-1 (HO-1), inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2) in a time- and concentration-dependent manner.

2 In the presence of zymosan, exogenous prostaglandin E_2 (PGE_2) did not exert significant effects on the expression of these three enzymes. In contrast, exogenous leukotriene B_4 (LTB_4) and LTC_4 in the nanomolar range inhibited HO-1 and iNOS expression, as well as nitrite accumulation.

3 The COX inhibitors indomethacin and NS398 weakly inhibited HO-1 expression but had no effect on iNOS and COX-2 expression or nitrite. In contrast, the 5-lipoxygenase (5-LO) inhibitor ZM 230,487 significantly decreased HO-1, iNOS and nitrite, which were not affected by zileuton. Dexamethasone showed an inhibitory effect on HO-1 expression induced by zymosan.

4 ZM 230,487 but not zileuton, inhibited the shift due to nuclear factor-κB (NF-κB), whereas they did not modify activator protein-1 (AP-1) binding. Our results suggest that inhibition of NF-κB binding could mediate the effects of ZM 230,487 on the modulation of HO-1 and iNOS protein expression.

5 NOS inhibition by L-N^G-nitroarginine methyl ester (L-NAME) or 1400 W abolished nitrite production and strongly reduced HO-1 expression. These results show an induction of HO-1 protein expression by zymosan phagocytosis in macrophages, with a positive modulatory role for endogenous NO and a negative regulation by exogenous LTs, likely dependent on the reduction of iNOS expression and NO production.
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Keywords: Haem oxygenase-1; nitric oxide; inducible nitric oxide synthase; RAW 264.7 macrophages; cyclo-oxygenase-2; leukotrienes; 5-lipoxygenase

Abbreviations: AP-1, Activator protein-1; CO, carbon monoxide; COX, cyclo-oxygenase; ECL, enhanced chemiluminescence; EMSA, electrophoretic mobility shift assay; F.U., fluorescence units; HO, haem oxygenase; iNOS, inducible nitric oxide synthase; LT, leukotriene; L-NAME, L-N^G-nitroarginine methyl ester; 5-LO, 5-lipoxygenase; MTT, 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, nuclear factor-κB; NO, nitric oxide; PG, prostaglandin

Introduction

Macrophages play a key role in the immune defence system against microorganisms or tumour cells since they participate in the processing of antigens and presentation to lymphocytes, as well as in the phagocytosis and killing of microbes. In this process, high amounts of nitric oxide (NO) and reactive oxygen intermediates are generated contributing to intracellular destructive mechanisms. In addition, activated macrophages secrete degradative enzymes and produce cytokines and eicosanoids (MacMicking *et al*., 1997).

Zymosan, a yeast cell wall derivative composed of alfa-mannan and beta-glucan, is a particulate ligand for different receptors leading to cellular activation and production of inflammatory mediators. Phagocytosis of unopsonized zymosan by macrophages is mediated at least in part through the mannose receptor (Linehan *et al*., 1999), which is involved in the recognition and phagocytosis of unopsonized microorganisms, such as bacteria, fungi, and protozoa through interactions with polysaccharide components of cell walls (Ofek *et al*., 1995).

Excessive mediator generation can lead to the spread of cytotoxicity to the host tissues, resulting in detrimental effects. Nevertheless, macrophages possess regulatory pathways where protective mechanisms can operate to control proinflammatory responses and thus limit the destructive potential. Haem oxygenase (HO) is a rate-limiting enzyme in haem catabolism, leading to the formation of biliverdin, which is reduced to bilirubin, carbon monoxide (CO) and iron. Inducible (HO-1) and constitutive (HO-2, HO-3) isozymes have been identified in many cell types (Abraham *et al*., 1988; McCoubrey *et al*., 1997).

There is a large body of evidence suggesting that induction of HO-1 is a protective cellular response against environmental and oxidative stress, although the precise mechanisms remain unclear. Overexpression of HO-1 prevents tumour necrosis factor-α-induced apoptosis in murine fibroblasts, an effect possibly mediated *via* CO (Petrache *et al*., 2000). In addition, cells from HO-1 knockout mice are less resistant to

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the cytotoxicity induced by oxidative stress (Poss & Tonegawa, 1997b) and these findings have been confirmed *in vivo* in mice (Poss & Tonegawa, 1997a) as well as in human HO-1 deficiency (Yachie *et al*., 1999). Nevertheless, exaggerated HO-1 expression may not be beneficial due to iron accumulation (Suttner & Dennery, 1999) and reduction in haem availability for haem-dependent proteins (Deramaudt *et al*., 1999).

The aim of this work was to assess if HO-1 participates in the response to zymosan in the macrophage and characterize the mediators involved. We have shown that zymosan induces HO-1 protein expression in a time- and concentration-dependent manner in RAW 264.7 macrophages. This induction appears to be mediated by NO and regulated by exogenous products of the 5-lipoxygenase (5-LO) pathway.

Methods

Cell culture

RAW 264.7 murine macrophage cell line (American Type Culture Collection, Manassas, VA, U.S.A.) were maintained in DMEM medium supplemented with 10% foetal bovine serum, 2 mM L-glutamine and penicillin/streptomycin. Cells were plated onto 24-well tissue culture dishes (7.5×10^5 cells/well) and allow to grow until confluence. Suspensions of zymosan A from *Saccharomyces cerevisiae* in saline were autoclaved prior to use. After incubation with zymosan and/or drugs at the indicated times, cell supernatants were collected to measure prostaglandin E_2 (PGE_2) by radioimmunoassay (Moroney *et al*., 1988) and nitrite levels by the fluorimetric assay of Misko *et al*. (1993). Degranulation was assessed by the method of Barrett & Heath (1979) using 4-methylumbelliferyl-β-D-glucuronide as substrate and results were expressed as fluorescence units (F.U.). In parallel, cell pellets were scraped and leukotriene C_4 (LTC_4) was measured by radioimmunoassay (Moroney *et al*., 1988). Cell viability was assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethyltiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) to formazan. After appropriate stimulation times, cells were incubated with MTT (200 μg ml^{-1}) for 60 min. The medium was then removed and cells were solubilized in dimethylsulphoxide (100 μl) to quantitate formazan at 550 nm (Gross *et al*., 1991).

Western blot analysis

After incubation, RAW 264.7 macrophages were lysed in 100 μl of buffer (1% Triton X-100, 1% deoxicholic acid, 20 mM NaCl and 25 mM Tris, pH 7.4) and centrifuged at 4°C for 5 min at $10{,}000 \times g$. The protein content was determined by the Bradford method using bovine serum albumin as standard. Cell lysate (40 μg of protein) was mixed with Laemmli sample buffer under reducing conditions. Samples were size-separated in 12.5% SDS–PAGE and transferred onto polyvinylidene difluoride membranes (Amersham Pharmacia Biotech Europe GmbH, Barcelona, Spain), which were blocked in phosphate buffer saline (0.02 M, pH 7.0)-Tween 20 (0.1%) containing 3% fat-free dry milk. Membranes were incubated with specific antibodies: polyclonal antibody against cyclo-oxygenase-2 (COX-2) (1/1000, Cayman Chemical, Ann Arbor, MI, U.S.A.), polyclonal antibody against inducible NO synthase (iNOS) (1/1000, Cayman Chemical, Ann Arbor, MI, U.S.A.), and anti-HO-1 monoclonal antibody (1/2000, Stressgen, Victoria, Canada). Blots were washed and incubated with peroxidase-conjugated goat anti-rabbit IgG (1/20,000, Dako, Gloustrup, Denmark) or peroxidase-conjugated goat anti-mouse IgG (1/20,000, Sigma Chemical Co., St Louis, MO, U.S.A.). The immunoreactive bands were visualized by an enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech Europe GmbH, Barcelona, Spain). Band intensity was quantitated using computer-assisted densitometry.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as described (López-Collazo *et al*., 1998). Protein was determined by the DC Bio-Rad protein reagent (Richmond, CA, U.S.A.). Double-stranded oligonucleotides containing either the consensus nuclear factor-κB (NF-κB) or activator protein-1 (AP-1) sequence (Promega Corp., Madison, WI, U.S.A.) were end-labelled using T4 polynucleotide kinase (Amersham Pharmacia Biotech Europe GmbH, Barcelona, Spain) and [γ-^{32}P]-ATP, followed by purification using G-25 microcolumns (Amersham Pharmacia Biotech Europe GmbH, Barcelona, Spain). Incubations were performed on ice with 6 μg of nuclear extract, 100,000 c.p.m. of labelled probe, 2 μg poly(dI-dC), 5% v/v glycerol, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM NaCl and 10 mM Tris-HCl buffer (pH 8.0) for 15 min. Complexes were analysed by non-denaturating 6% polyacrylamide gel electrophoresis in 0.5 × Tris-borate buffer followed by autoradiography of the dried gel.

Materials

Z-Leu-Leu-Leu-CHO (MG-132) was purchased from Biomol Res. Labs. Inc. (Plymouth Meeting, PA, U.S.A.). Culture reagents were from Life Technologies Inc. (Barcelona, Spain) and [γ-^{32}P]-ATP (3000 Ci mmol^{-1}) from NEN Life Science Products Inc. (Boston, MA, U.S.A.). [5,6,8,11,12,14,15(n)-^{3}H] PGE_2 and [5,6,8,9,11,12,14,15(n)-^{3}H] LTC_4 were from Amersham Iberica (Madrid, Spain). 6-[[3-fluoro-5-(4-methoxy-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)phenoxy]methyl]-1-ethylquinol-2-one (ZM 230,487) and zileuton were kind gifts from Zeneca Pharmaceuticals (Macclesfield, Cheshire, U.K.) and Abbott Laboratories (Abbott Park, Illinois, U.S.A.), respectively. N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS398) and N-[[3-(aminoethyl)phenyl]methyl]-ethanimidamide, dihydrochloride (1400 W) were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.) and the rest of reagents were from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Statistical analysis

Results are presented as mean ± s.e.mean of n experiments. Data were analysed by two-way ANOVA followed by Dunnett's t-test for multiple comparisons.

Results

Zymosan induces HO-1 expression in RAW 264.7 macrophages

Induction of HO-1 by zymosan treatment of RAW 264.7 macrophages was accompanied by induction of enzymes relevant to the inflammatory response, such as COX-2 and iNOS. Figure 1a depicts the increase in HO-1, iNOS and COX-2 protein expression when RAW 264.7 macrophages were stimulated with zymosan (0.3 mg ml^{-1}) for different time periods. The time course indicated that HO-1 protein expression was apparent by 6 h and reached a maximum at 18 h, whereas iNOS was induced later and increased with time. In contrast, high levels of COX-2 protein were already observed at 6 h and also increased with time. These changes in protein expression paralleled the production of PGE_2 and nitrite (Figure 1b). Cell activation was also followed by measuring degranulation as β-D-glucuronidase release. As shown in Figure 2, zymosan stimulation of RAW 264.7 macrophages was concentration-dependent in the range tested (0.1 – 0.6 mg ml^{-1}). Maximal levels of PGE_2 and nitrite were reached by stimulation with 0.3 mg ml^{-1} zymosan, although for degranulation the highest effect was already observed at 0.2 mg ml^{-1}.

Effects of eicosanoids

Exogenous PGE_2 did not exert significant effects on HO-1, COX-2 or iNOS expression induced by zymosan (Figure 3 and Table 1). We next determined the influence of exogenous LTs at the time of maximal HO-1 expression and observed that challenging macrophages during zymosan stimulation with either LTB_4 or LTC_4 in the nanomolar range, had

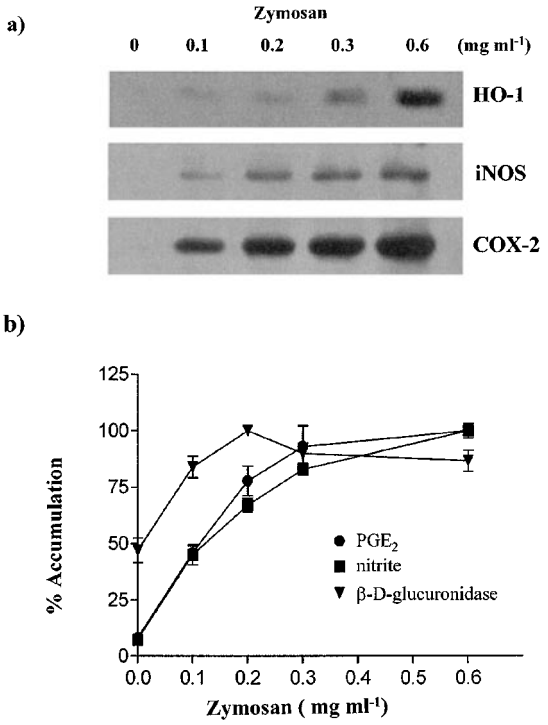

Figure 2 Concentration-dependence of zymosan stimulation. Cells were incubated with zymosan (0.1, 0.2, 0.3 and 0.6 mg ml^{-1}) for 18 h. (a) Effect on HO-1, iNOS and COX-2 protein expression. Results are representative of three experiments. (b) Effect on nitrite, PGE_2 and β-D-glucuronidase accumulation. Percentages were calculated with respect to the maximum level measured (67.7 ± 2.1 ng ml^{-1} PGE_2, 1585.8 ± 20.6 ng ml^{-1} nitrite and 19.9 ± 0.9 × 10^6 F.U. ml^{-1} of β-D-glucuronidase). Data are the mean ± s.e.mean of three experiments.

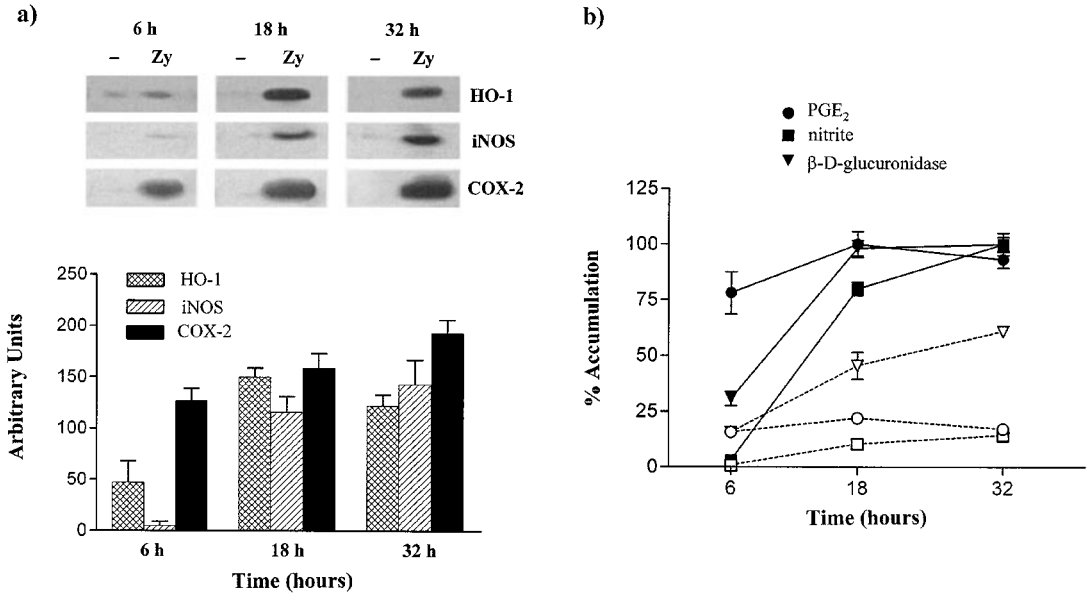

Figure 1 Time-course of zymosan stimulation in RAW 264.7 macrophages. Cells were incubated for 6, 18 and 32 h in the presence of zymosan (Zy, 0.3 mg ml^{-1}). (a) Effect on HO-1, iNOS and COX-2 protein expression. Band intensity is represented as arbitrary units. (b) Effect on nitrite, PGE_2 and β-D-glucuronidase accumulation. Percentages were calculated with respect to the maximum level measured (21.0 ± 1.2 ng ml^{-1} PGE_2 2529.7 ± 75.3 ng ml^{-1} nitrite and 20.5 ± 1.0 × 10^6 F.U. ml^{-1} of β-D-glucuronidase). Open symbols and broken lines represent unstimulated cells. Data are the mean ± s.e.mean of three experiments.

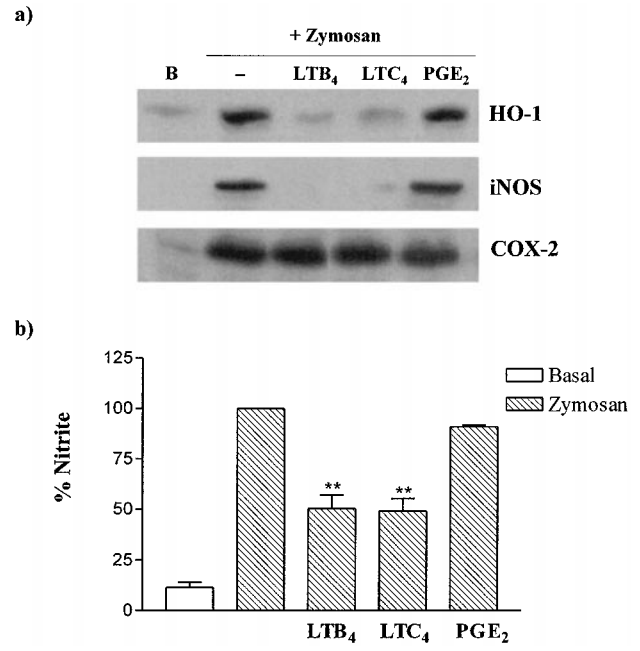

Figure 3 Effect of LTB_4, LTC_4 and PGE_2 on zymosan-induced HO-1, iNOS and COX-2 protein expression (a), and nitrite production (b). (**$P<0.01$). Cells were incubated with LTB_4 (30 nM), LTC_4 (30 nM) or PGE_2 (30 nM) in the presence of zymosan (0.3 mg ml^{-1}) for 18 h. Nitrite production was calculated as percentage with respect to control (zymosan). Data are the mean±s.e.mean of four experiments.

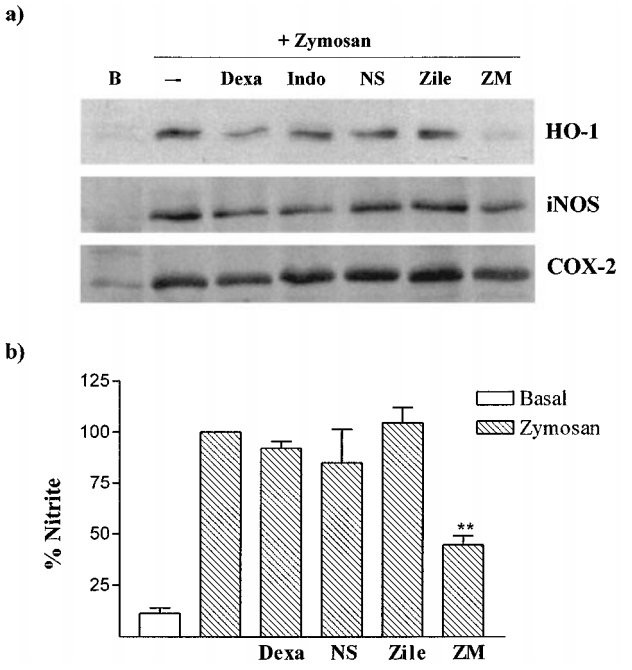

Figure 4 Effect of dexamethasone, indomethacin, NS398, zileuton and ZM 230,487 on zymosan-induced HO-1, iNOS and COX-2 protein expression (a), and nitrite production (b) (**$P<0.01$). Cells were incubated with dexamethasone (Dexa, 1 μM), indomethacin (Indo, 1 μM), NS398 (NS, 10 μM), zileuton (Zile, 10 μM) or ZM 230,487 (ZM, 5 μM) in the presence of zymosan (0.3 mg ml^{-1}) for 18 h. Dexamethasone was added 1 h before zymosan. Nitrite production was calculated as percentage with respect to control (zymosan). Data are the mean±s.e.mean of 3–9 experiments.

Table 1 Effect of LTB_4, LTC_4 and PGE_2 on zymosan-induced HO-1, iNOS and COX-2 protein expression

	HO-1	*iNOS*	*COX-2*
Basal	4.9±1.6	1.8±1.2	6.8±0.2
Zymosan	100	100	100
LTB_4	15.5±4.5**	13.7±9.0**	98.0±12.0
LTC_4	21.5±3.2**	9.0±15.0**	99.3±4.0
PGE_2	85.0±4.6	101.0±12.0	102.0±9.5

Band intensity was calculated as percentage with respect to control (zymosan). Data are the mean±s.e.mean of four experiments. **$P<0.01$.

Table 2 Effect of dexamethasone, indomethacin, NS398, zileuton and ZM 230,487 on zymosan-induced HO-1, iNOS and COX-2 protein expression

	HO-1	*iNOS*	*COX-2*
Basal	4.9±1.6	1.8±1.2	6.8±0.2
Zymosan	100	100	100
Dexamethasone	25.0±5.3**	77.6±7.9*	71.3±11.5*
Indomethacin	71.0±17.1*	84.7±11.2	87.1±16.5
NS398	67.3±15.05*	112.8±9.5	107.0±8.1
Zileuton	119.0±29.0	114.3±8.9	91.5±5.0
ZM 230,487	13.5±8.4**	71.3±15.1*	86.0±9.4

Band intensity was calculated as percentage with respect to control (zymosan). Data are the mean±s.e.mean of 3–9 experiments. *$P<0.05$, **$P<0.01$.

inhibitory effects on HO-1 and iNOS expression and nitrite levels. None of these treatments alone significantly modified the expression of these enzymes or metabolite levels (data not shown).

Effects of inhibitors of eicosanoid synthesis

To determine if products of COX activity could mediate HO-1 induction by zymosan, macrophages were exposed to this stimulus in the presence of the dual COX-1/COX-2 inhibitor indomethacin or the selective COX-2 inhibitor NS398, at concentrations able to abolish PGE_2 production. Both compounds failed to significantly modify HO-1, iNOS or COX-2 protein expression as well as nitrite accumulation (Figure 4 and Table 2). The influence of the 5-LO pathway was assessed by using selective inhibitors with different mechanisms of action, the non-redox agent ZM 230,487 and the iron-ligand inhibitor zileuton. Incubation of RAW 264.7 macrophages with zymosan in the presence of either ZM 230,487 or zileuton, gave divergent results. While HO-1 and iNOS expression was inhibited in cells treated with ZM 230,487, zileuton failed to exert significant modifications. Changes in iNOS were paralleled by changes in nitrite levels. The inhibitory effect of ZM 230,487 was higher on HO-1 expression. In contrast, COX-2 expression was not modified by these compounds. It is known that 5-LO activity is weak in RAW 264.7 macrophages. In our experimental conditions,

LTC_4 levels detected by radioimmunoassay did not change significantly after zymosan stimulation of cells for 1–4 h (data not shown).

In addition, the effect of the glucocorticoid dexamethasone was also studied. This anti-inflammatory compound inhibited HO-1 expression, whereas iNOS or COX-2 were reduced to a lower extent (Figure 4 and Table 2).

Effects of 5-LO inhibitors on NF-κB and AP-1 binding

To establish if the behaviour of 5-LO inhibitors could be related to differences in binding of relevant nuclear factors, such as NF-κB and AP-1, we performed EMSA experiments. As shown in Figure 5, nuclear protein extracts from zymosan-stimulated RAW 264.7 macrophages slowed the migration of an oligonucleotide containing the NF-κB-binding site. This shift was inhibited in cells incubated with either the reference inhibitor MG-132 or ZM 230,438, but not in those treated with zileuton. None of these compounds affected the shift due to AP-1 binding.

Effect of NOS inhibitors

Addition of L-N^G-nitroarginine methyl ester (L-NAME) or the selective iNOS inhibitor, 1400 W significantly reduced HO-1 protein expression and abolished nitrite levels in RAW 264.7 macrophages stimulated with zymosan (Figure 6 and Table 3). In contrast, these agents did not modify COX-2 and L-NAME slightly increased iNOS expression. Other parameters such as degranulation or PGE_2 levels were not affected by NOS inhibitors (data not shown).

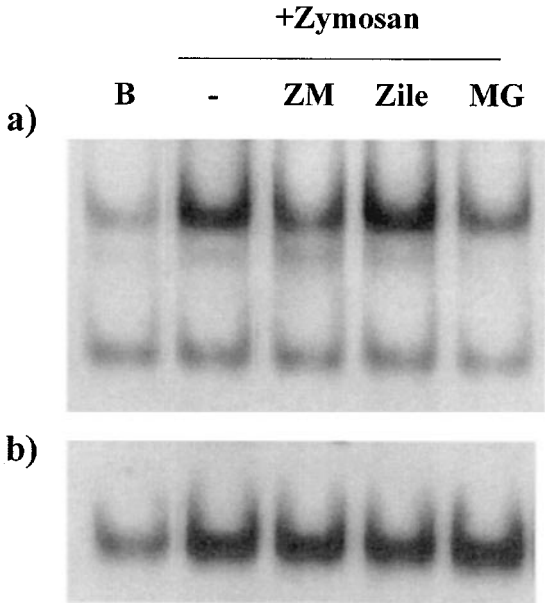

Figure 5 EMSA experiments. (a) NF-κB, (b) AP-1. Cells were preincubated with ZM 230,487 (ZM, 5 μM), zileuton (Zile, 10 μM) or MG-132 (MG, 1 μM) for 15 min before zymosan. (Zy, 0.3 mg ml^{-1}) addition and then incubation proceeded for 2 h. B = unstimulated cells. Results are representative of three independent experiments.

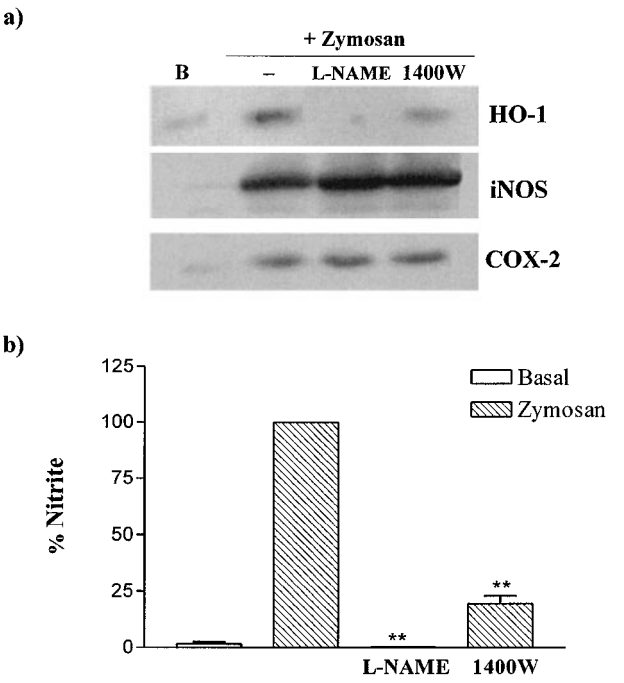

Figure 6 Effect of NOS inhibitors on zymosan-induced HO-1, iNOS and COX-2 protein expression (a), and nitrite production (b) (**$P<0.01$). Cells were incubated with L-NAME (1 mM) or 1400 W (10 μM) in the presence of zymosan (0.3 mg ml^{-1}) for 18 h. Nitrite production was calculated as percentage with respect to control (zymosan). Data are the mean ± s.e.mean of four experiments.

Table 3 Effect of L-NAME and 1400 W on zymosan-induced HO-1, iNOS and COX-2 protein expression

	HO-1	*iNOS*	*COX-2*
Basal	4.9 ± 1.6	1.8 ± 1.2	6.8 ± 0.2
Zymosan	100	100	100
L-NAME	23.3 ± 12.5**	137.9 ± 4.9*	92.0 ± 8.0
1400 W	26.9 ± 10.1**	113.8 ± 12.2	94.8 ± 11.7

Band intensity was calculated as percentage with respect to control (zymosan). Data are the mean ± s.e.mean of three experiments. *$P<0.05$, **$P<0.01$.

Discussion

Results in this study show for the first time that activation of RAW 264.7 cells by unopsonized zymosan upregulates HO-1 protein expression, suggesting that HO-1 induction represents a regulatory mechanism to limit the cytotoxic effects of this inflammatory response. In fact, a protective role of HO-1 has been reported *in vitro* for a variety of stress inducers such as haemoglobin, hypoxia, glutathione depletion, cytokines... (Terry *et al*., 1998; Dennery *et al*., 1997; Shibahara *et al*., 1987). *In vivo*, exogenous administration of HO-1 has been useful in hyperoxia-induced lung injury in rats (Otterbein *et al*., 1999), whereas expression of HO-1 induced by different agents protects renal cells from ischaemia reperfusion injury (Shimizu *et al*., 2000) and has been associated to inhibitory effects in different inflammatory states (Willis *et al*., 1996; Laniado-Schwartzman *et al*., 1997; Mosley *et al*., 1998).

Recently, Otterbein *et al*. (2000) have described the participation of CO generated by HO-1 activity in the anti-inflammatory effects exhibited by this enzyme (Otterbein *et al*., 2000).

In the response to zymosan, iNOS and COX-2 were also induced, leading to significant increases in NO and PGE_2 production. However, according to its role as a stress-inducible protein, HO-1 expression during zymosan stimulation was more rapid in reaching maximal levels than iNOS and COX-2. Our experimental evidence suggests a relationship between HO-1 and endogenous NO which may play a positive modulatory role in HO-1 expression induced by zymosan, likely as a protective mechanism in the macrophage against the biological consequences of high levels of NO generated during phagocytosis. It has been reported previously using stimuli other than zymosan or NO donors, that NO induces the expression of HO-1 in different cellular systems such as vascular smooth muscle (Durante *et al*., 1997), endothelial cells (Foresti *et al*., 1997), HeLa (Bouton & Demple, 2000) and fibroblasts (Alcaraz *et al*., 2000). We have demonstrated in the present work that inhibition of NO synthesis during prolonged stimulation with zymosan (18 h) decreases HO-1 protein expression. Nevertheless other mediators may play a role in HO-1 induction at early times, when there is no significant NO production. To this respect, it is known that reactive oxygen species are quickly generated during zymosan phagocytosis by mouse peritoneal macrophages or RAW 264.7 cells (Herencia *et al*., 2001).

We have also found an interesting negative modulation of HO-1 and iNOS expression by exogenous LTs. The ability of these eicosanoids to modify HO-1 expression may depend on the reduction of iNOS and consequently of NO, observed in our experiments performed in the presence of zymosan for 18 h. On the other hand, our data do not support a role for endogenous 5-LO or COX metabolites in the response to zymosan in RAW 264.7 macrophages. It is interesting to note that inhibition of 5-LO by ZM 230,487 resulted in strong HO-1 reduction, whereas zileuton had no effect. This difference in behaviour is likely due to inhibition of NF-κB binding and independent of 5-LO inhibition. To this respect, Bowie & O'Neill (2000) have recently speculated that ZM 230,487 may have other targets distinct from 5-LO. It is known that upregulation of iNOS and HO-1 gene expression can depend on activation of NF-κB and AP-1 (Alam & Den, 1992; Nunokawa *et al*., 1996; Oshiro *et al*., 1999; Lee *et al*., 2000). Thus, inhibition of NF-κB binding by ZM 230,487 could explain the decrease in HO-1 and iNOS protein expression produced by this drug. This last effect would lead to reduced NO production that may also contribute to the strong inhibitory effect of ZM 230,487 on HO-1 expression.

In this study, we have shown that dexamethasone inhibits HO-1 expression induced by zymosan, which is in line with reports indicating that this glucocorticoid blocks the induction of HO-1 mRNA in response to interleukin-6 in endothelial cells, as part of its anti-inflammatory mechanisms (Deramaudt *et al*., 1999). Long-term expression of HO-1 can result in deleterious effects on cells since it can decrease the availability of haem, with negative effects on haem proteins (Albakri & Stuehr, 1996; Deramaudt *et al*., 1999). Thus, inhibition of HO-1 may be beneficial for the control of chronic inflammation.

During inflammatory responses macrophages are exposed to high concentrations of eicosanoids derived from the LO or COX pathways. We have shown that LTB_4 and LTC_4 potently decreased the expression of HO-1 and iNOS induced by zymosan and these results are consistent with LT products of inflammatory cells having paracrine effects to control macrophage activation after a prolonged exposure to this phagocytic stimulus. Although the role of LTs in modulating innate host defense mechanisms related to NO is poorly understood, it is known that LTB_4 and cysteinyl-LTs contribute to antimicrobial defence and quickly activate NO release and secretory events in human neutrophils (Larfars *et al*., 1999) and mediate the phagocytic response after stimulation of rat alveolar macrophages by *K. pneumoniae* (Mancuso *et al*., 1998). Evidence has also been provided that inhibition of 5-LO during cell activation decreases NO production and cytolytic capacity in thyoglycollate-elicited macrophages (Hubbard & Erickson, 1995), whereas other authors have reported a lack of interaction between LT and NO pathways in RAW 264.7 macrophages stimulated with lipopolysaccharide + interferon-γ (Hulkower *et al*., 1996).

Further studies would be necessary to elucidate the mechanism of action of LTs in this setting although previous work has suggested an anti-inflammatory effect of LTB_4 mediated by peroxisome proliferator-activated receptor-α activation (Devchand *et al*., 1996). We have provided *in vitro* evidence for a regulatory role of LTs working to limit the inflammatory response in RAW 264.7 macrophages.

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