Aspirin inhibits *Chlamydia pneumoniae*-induced NF-κB activation, cyclo-oxygenase-2 expression and prostaglandin E$_2$ synthesis and attenuates chlamydial growth

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Infection with *Chlamydia pneumoniae* has been implicated as a potential risk factor for atherosclerosis. This study was designed to investigate the mechanisms of the anti-chlamydial activity of aspirin. A reporter gene assay for NF-κB activity, immunoblot analysis for cyclo-oxygenase (COX)-2 and radioimmunoassay for prostaglandin E$_2$ (PGE$_2$) were performed. Following infection of HEp-2 cells with *C. pneumoniae*, NF-κB was activated, COX-2 was induced and PGE$_2$ was elevated. Aspirin inhibited NF-κB activation at a concentration of 0·1 mM, partially inhibited COX-2 induction and blocked PGE$_2$ synthesis completely. In addition, high doses of aspirin (1 and 2 mM) inhibited chlamydial growth in HEp-2 cells, decreasing the number and size of inclusion bodies; this effect could be overcome by adding tryptophan to the culture. Indomethacin also blocked the synthesis of PGE$_2$, but had no effect on COX-2 expression or chlamydial growth. These results indicate that aspirin not only has an anti-inflammatory activity through prevention of NF-κB activation but also has anti-chlamydial activity at high doses, possibly through depletion of tryptophan in HEp-2 cells.

INTRODUCTION

Chlamydia pneumoniae, a Gram-negative obligate intracellular bacterium, is known to be a respiratory pathogen (Grayston, 1992; Grayston *et al.*, 1986, 1990). This microorganism can cause chronic infection and has recently been linked to atherosclerotic disease. Since Saikku *et al.* (1988) first reported serological evidence of an association between *C. pneumoniae* and coronary heart disease, a number of serological investigations have shown a positive relationship between *C. pneumoniae* infection and cardiovascular and cerebrovascular diseases (Cook *et al.*, 1998; Miyashita *et al.*,

1998; Wimmer *et al.*, 1996), such as carotid atherosclerosis (Melnick *et al.*, 1993). Furthermore, *C. pneumoniae* has been detected in atherosclerotic but not normal arteries by several techniques (Kuo *et al.*, 1993; Ouchi *et al.*, 1998; Shor *et al.*, 1992; Yamashita *et al.*, 1998) and isolated from coronary (Ramirez, 1996) and carotid (Jackson *et al.*, 1997) atheromas. These findings suggest that chronic *C. pneumoniae* infection might be a trigger and/or enhancer of inflammatory reactions in the vessel wall and thus a causative agent of atherosclerosis.

Several lines of evidence indicate that the dimeric transcription factor NF-κB plays an important role in atherosclerosis (Brand *et al.*, 1996; Collins, 1993; Dichtl *et al.*, 1999; Gawaz *et al.*, 1998; Ross, 1993). NF-κB controls the expression of various genes involved in inflammation and proliferation, such as intercellular adhesion molecule-1 (ICAM-1) (Baeuerle & Henkel, 1994; Dichtl *et al.*, 1999; Poston *et al.*, 1992), vascular cell adhesion molecule-1 (VCAM-1) (Cybulsky & Gimbrone, 1991; Dichtl *et al.*, 1999; Neish *et*

Abbreviations: COX, cyclo-oxygenase; PGE$_2$, prostaglandin E$_2$.

al., 1992), cyclo-oxygenase-2 (COX-2) (Appleby et al., 1994; D'Acquisto et al., 1997; Pistritto et al., 1999) and inducible nitric oxide synthase (iNOS) (Chu et al., 1998; Marks-Konczalik et al., 1998). In its inactive state, the prototypic NF-κB dimer, consisting of subunits p50 and p65 (Rel A), is present in the cytoplasm bound to an inhibitory protein, IκB (Baeuerle & Baltimore, 1988). NF-κB is activated to translocate to the nucleus after degradation of IκB (Traenckner et al., 1994) in response to a number of stimuli including inflammatory cytokines (Baeuerle & Henkel, 1994; Marks-Konczalik et al., 1998), pathogenic micro-organisms (Krull et al., 1999; Sharma et al., 1998; Speir et al., 1998) and bacterial LPS (Kol et al., 1999; Pistritto et al., 1999).

Acetyl salicylic acid (aspirin), widely used to treat inflammation, can specifically inhibit the activation of NF-κB through binding to IκB kinase-β (IKKβ), an enzyme that phosphorylates IκB, leading to its degradation (Kopp & Ghosh, 1994; Yin et al., 1998). Recent experiments have shown that aspirin inhibits cytomegalovirus-induced NF-κB activation and prevents replication of the virus (Speir et al., 1998). In addition, aspirin and another non-steroidal anti-inflammatory drug, indomethacin, inhibit COX-2 activity and so block the formation of prostaglandins (Gierse et al., 1999). Recently, it has been demonstrated that C. pneumoniae infection in endothelial cells triggered activation of NF-κB, induced phosphorylation of p42/p44 MAPK and induced the expression of adhesion molecules such as ICAM-1, VCAM-1 and endothelial-leukocyte adhesion molecule-1 (E-Selectin). This was followed by subsequent rolling, adhesion and migration of leukocytes (Krull et al., 1999).

In the present study, we have demonstrated the effect of aspirin on chlamydial growth and activation pathways of NF-κB, COX-2 and prostaglandin E$_2$ (PGE$_2$).

METHODS

Cell culture. HEp-2 (ATCC CCL-23), a human epithelioid larynx carcinoma-derived cell line, was maintained in Iscove's modified Dulbecco's medium (IMDM; Gibco-BRL) supplemented with 10 % heat-inactivated fetal calf serum (FCS; PAA Laboratories), 100 µg ampicillin ml^{-1} and 50 µg gentamicin ml^{-1}. Cells pre-cultured at 37 °C with 5 % CO$_2$ in 75 or 25 ml culture flasks (Sumilon) were allowed to adhere to tissue-culture plates (Sumilon) prior to use.

Chlamydial strain. C. pneumoniae strain J138, isolated in Japan in 1994 (Shirai et al., 2000), was grown in HEp-2 cells and elementary bodies (EBs) were purified by discontinuous centrifugation in urografin (Caldwell et al., 1981). EBs, diluted with a sucrose/phosphate/glutamate buffer supplemented with 10 % FCS, were stored at −80 °C until use. Titration was performed and titres were expressed as inclusion-forming units (i.f.u.) ml^{-1}.

Inoculation of C. pneumoniae. The stock chlamydial suspension was thawed and diluted in IMDM. For the growth assay, HEp-2 cells (5 × 10^4 per well) grown in IMDM in 96-well tissue-culture plates for 24 h received 1·5 × 10^3 i.f.u. per well, resulting in an m.o.i. of 0·03. For other experiments, cells grown in 24-well plates received 2·0 × 10^5 i.f.u. per well, while cells grown in 6-well plates received 8·0 × 10^5 i.f.u. per well, resulting in an m.o.i. of 0·4 in each case. The inoculum was centrifuged at 700 **g** for 1 h at 22 °C followed by incubation at 36 °C for a

further hour. After the extracellular bacteria were removed, infected cells were further incubated in IMDM with or without aspirin (acetyl salicylic acid) or indomethacin (1-[p-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid) (Sigma) at the concentrations indicated. The concentrations of drugs used in this study were similar to those used in a previous analysis (Yin et al., 1998). Non-infected cells were treated similarly with the drugs.

NF-κB reporter gene assay. Plasmid pNFκB-Luc, carrying five NF-κB DNA-binding sites driving the expression of the luciferase gene, and plasmid pCMVβ, carrying the β-galactosidase gene (Siebenlist et al., 1994), were purchased from Stratagene. HEp-2 cells (2 × 10^5) grown in 24-well plates for 24 h were co-transfected with 600 ng of the reporter plasmid pNFκB-Luc and 60 ng of the control plasmid pCMVβ using the Trans IT-LT polyamine transfection reagent (Mirus) (Budker et al., 1996; Gupta et al., 1996) followed by incubation for 20 h. The transfected cells were infected with C. pneumoniae at an m.o.i. of 0·4. At the indicated time-points (0, 8, 24 and 48 h), cells were washed with PBS and lysed in 150 µl lysis buffer (Pica Gene). The cell extracts were assayed for luciferase in a luminometer (Microlumat Lb 96P; Berthold) and for β-galactosidase after heat-inactivation of endogenous β-galactosidase (Shaper et al., 1994). Relative luciferase activity was defined as the mean ± SD (n = 5) of the activity ratio of luciferase/β-gal. TNF-α was purchased from Genzyme.

Immunoblot analysis of cyclo-oxygenase. HEp-2 cells were grown in 6-well plates, infected with C. pneumoniae and then treated with drugs. At the indicated time-points, cells were washed with cold PBS, harvested and lysed in SDS sample buffer (62·5 mM Tris/HCl, pH 6·8, 2 % SDS, 10 % glycerol, 5 % β-mercaptoethanol, 0·1 % bromophenol blue). Samples were sonicated three times for 5 s each in a cold-water bath with a sonicator (Bioruptor; Cosmo Bio) and heated to 100 °C for 5 min. Samples, containing 20 µg protein, were electrophoresed in 10 % SDS-polyacrylamide gels and separated proteins were transferred on to a nitrocellulose membrane. The membrane was blocked for 3 h with 5 % non-fat dried milk in Tris-buffered saline with 0·1 % Tween 20 at room temperature and incubated overnight at 4 °C with rabbit polyclonal IgG anti-COX-2 or anti-COX-1 antibodies (Santa Cruz Biotechnology). The appropriate secondary horseradish peroxidase-conjugated antibody (Amersham Pharmacia Biotech) was added and complexes were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech).

Determination of PGE$_2$. HEp-2 cells were grown in 24-well plates and infected with C. pneumoniae (2·0 × 10^5 i.f.u. per well). At the indicated time-points, PGE$_2$ levels in culture supernatants were measured using a PGE$_2$ ^{125}I radioimmunoassay (BIOTRAK; Amersham Pharmacia Biotech) as described in the manufacturer's instruction manual.

Determination of NO. NO (nitrite plus nitrate) was determined using an HPLC NO-detector system (ENO-20; Eicom Co.). Aliquots of 10 µl culture supernatant, after centrifugation at 1000 **g** for 1 min, were injected into the HPLC system, consisting of an ion-exchange column to separate nitrite and nitrate and a cadmium-reduction column to reduce nitrate to nitrite. Total nitrite was mixed with Griess reagent and the A$_{540}$ was measured using a flow-through spectrophotometer.

Growth of C. pneumoniae. Growth of C. pneumoniae was determined by counting inclusion bodies. After the infection was established, the inoculum was replaced with IMDM with or without aspirin or indomethacin and incubated further for up to 48 h at 36 °C. The cells were fixed, stained and examined under a fluorescence microscope at a magnification of ×200 for the presence of inclusion bodies. Immunofluorescence staining of C. pneumoniae was performed with the C. pneumoniae-specific mAb RR402 (Washington Research), as described previously (Matsushima et al., 1999). Numbers of i.f.u. were determined

on the basis of the mean number of inclusion bodies per field determined in five fields per well from five samples. Relative chlamydial growth (%) was calculated as 100 × (i.f.u. of treated sample/i.f.u. of untreated control). L-Tryptophan was purchased from Sigma.

Statistical analysis. Statistical comparisons were made using the Mann–Whitney U test for the growth assay and Student's unpaired t-test for the other experiments. Values of $P < 0.05$ were considered significant.

RESULTS

Inhibition of *C. pneumoniae*-induced NF-κB activation by aspirin

NF-κB activation in *C. pneumoniae*-infected HEp-2 cells was measured by NF-κB reporter gene assay (Fig. 1). NF-κB activity (indicated as relative luciferase activity) was increased at 8 and 24 h post-infection and decreased to non-infected levels after 48 h. Conversely, TNF-α treatment of the cells (positive control; maximal activation of NF-κB of the treated cells is usually observed after a few hours) showed maximal activation of NF-κB after 8 h without infection but the activity decreased sharply after 24 h and, to a marginal extent, after 48 h. When aspirin (0.1 or 2.0 mM) was added to infected HEp-2 cells, *C. pneumoniae*-induced NF-κB activation was inhibited to non-infected levels.

Induction of COX-2 by *C. pneumoniae* and its inhibition by aspirin

Since NF-κB is one of the controlling factors of COX-2 expression, we then determined COX-2 levels in *C. pneumoniae*-infected HEp-2 cells by immunoblotting (Fig. 2a). Amounts of COX-2 increased from 8 to 48 h after infection in a time-dependent manner compared with the basal level in non-infected cells. On the other hand, levels of the housekeeping COX-1 protein were not affected by chlamydial
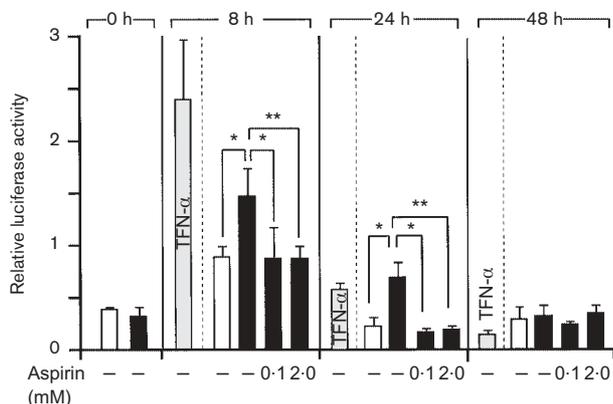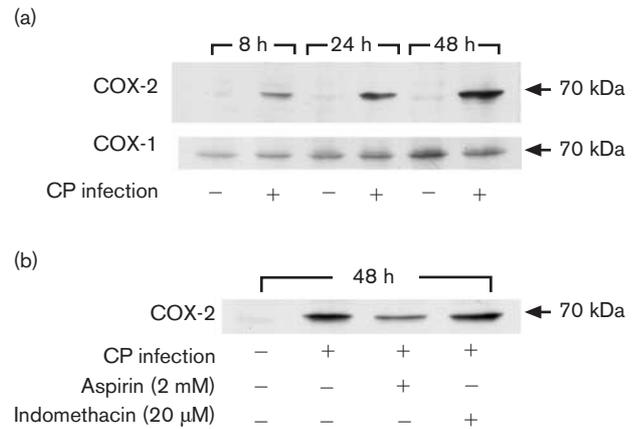
Fig. 2. (a) Immunoblot analysis of cyclo-oxygenases COX-2 and COX-1 in HEp-2 cells with and without *C. pneumoniae* (CP) infection. (b) Effects of aspirin (2 mM) and indomethacin (20 μM) on the induction of COX-2 at 48 h of *C. pneumoniae* infection. Similar results were obtained in several separate experiments.

infection. Addition of aspirin (2 mM) partially decreased *C. pneumoniae*-induced COX-2 expression after 48 h of infection (Fig. 2b), possibly because COX-2 expression is activated by other transcription factors. Indomethacin had no effect on COX-2 expression (Fig. 2b).

PGE$_2$ is produced in response to *C. pneumoniae* infection

At 8–48 h after *C. pneumoniae* infection, large amounts of PGE$_2$ were produced in culture supernatants of the HEp-2 culture, peaking at 24 h (Fig. 3). Aspirin and indomethacin, both non-selective COX inhibitors, completely blocked the *C. pneumoniae*-induced production of PGE$_2$ due to inhibi-
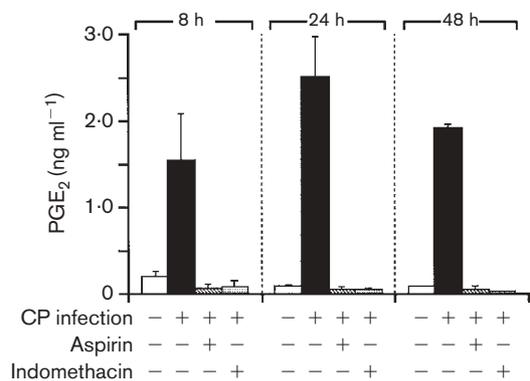
Fig. 1. Effect of aspirin on activation of NF-κB as determined by relative luciferase activity in HEp-2 cells after infection with *C. pneumoniae* (filled bars) or without infection (control culture, open bars). TNF-α (10 ng ml^{-1}) was added as a positive control without infection (shaded bars). Statistical significance of differences: *, $P < 0.05$; **, $P < 0.01$.

Fig. 3. Production of PGE$_2$ in culture supernatants of HEp-2 cells in the absence or presence of aspirin (2 mM) or indomethacin (20 μM). HEp-2 cells were grown in 24-well plates and infected with *C. pneumoniae* (CP). Aspirin or indomethacin was added immediately after infection. Data are shown as means ± SD of two separate experiments performed in triplicate.

tion of COX-2 activity, although indomethacin inhibits the enzyme activity of COX-2 but not its expression, as shown in Fig. 2(b).

NO production in HEp-2 cells after *C. pneumoniae* infection

Intriguingly, amounts of NO produced by *C. pneumoniae*-infected HEp-2 cells were essentially the same as those produced by non-infected cells, although HEp-2 cells that received a cytokine mixture containing TNF-α, IL-1β and IFN-γ showed increased NO (Table 1). TNF-α alone had no effect (data not shown). A larger inoculum of *C. pneumoniae* was toxic to host cells and was not tested for NO production.

Effects of aspirin on chlamydial growth

When HEp-2 cells were incubated for 48 h in the presence of aspirin, relative chlamydial growth, determined by the number of inclusion bodies, was $71 \cdot 2 \pm 4 \cdot 8$ % (mean $\pm$ SD) with $1 \cdot 0$ mM aspirin and $59 \cdot 7 \pm 5 \cdot 1$ % with $2 \cdot 0$ mM aspirin (Fig. 4a). In contrast, indomethacin did not affect chlamydial growth. A slight increase in chlamydial growth was observed at a low dose ($0 \cdot 1$ mM) of aspirin. A high dose ($2 \cdot 0$ mM) of aspirin not only decreased the numbers of inclusion bodies but also inhibited their enlargement (Fig. 4b, c). Thus, the mean diameter of 30 *C. pneumoniae* inclusion bodies chosen at random was $3 \cdot 2 \pm 0 \cdot 4$ μm in the presence of $2 \cdot 0$ mM aspirin and $5 \cdot 4 \pm 0 \cdot 6$ μm in its absence (significant difference, $P < 0 \cdot 01$). Interestingly, the number of inclusion bodies in aspirin-treated cultures recovered to non-treated levels after incubation for 72 h, but the size of inclusion bodies remained the same (data not shown). The concentrations of aspirin used in these experiments were not toxic to the host cells under the conditions used; cell morphology remained normal and more than 95 % of the cells were viable after the aspirin treatment. However, $5 \cdot 0$ mM aspirin was cytotoxic to HEp-2 cells.

EBs and HEp-2 cells were treated separately with 2 mM aspirin for 1 or 2 h, respectively, prior to infection. This treatment did not affect the subsequent growth of *C. pneumoniae* in HEp-2 cells in the presence or absence of 2 mM aspirin (data not shown), suggesting that aspirin inhibits proliferation of *C. pneumoniae* in host cells following infection.

Effect of tryptophan on aspirin-inhibited chlamydial growth

We found that the inhibition of chlamydial growth by high doses of aspirin was reversed by adding tryptophan to the culture medium in a dose-dependent manner; relative chlamydial growth, inhibited by 2 mM aspirin ($59 \cdot 7 \pm 5 \cdot 1$ %), was increased to $75 \cdot 3 \pm 7 \cdot 4$ % with 10 μg tryptophan ml^{-1} and to almost 100 % with 100 μg tryptophan ml^{-1} (Fig. 5).

DISCUSSION

C. pneumoniae infection activates several signal transduction pathways in the host cell that trigger and/or enhance inflammatory reactions, and may act as a causative agent of atherosclerosis. Several studies, including this one, have shown that *C. pneumoniae* infection increases host-cell NF-κB activity (Krull *et al.*, 1999; Matsushima *et al.*, 1999; Dechend *et al.*, 1999; Molestina *et al.*, 2000). In this study, NF-κB activation was greater at 8 h after infection than at any other time, suggesting that some unknown mechanism(s) might inhibit NF-κB activation during the late phase of chlamydial growth or that *C. pneumoniae* growing in inclusion bodies does not affect the activation of NF-κB, although the infection might stimulate the activation system. We also demonstrated that aspirin at concentrations as low as $0 \cdot 1$ mM inhibited *C. pneumoniae*-induced NF-κB activation in HEp-2 cells. Since aspirin, a common anti-inflammatory agent, inhibits IKK-β activity (Yin *et al.*, 1998), we suggest that the activation of NF-κB by *C. pneumoniae* infection depends mainly on the activation of IKK-β in HEp-2 cells. Another important finding of this study is that *C. pneumoniae* infection did not induce production of NO in HEp-2 cells, despite NF-κB activation. We reported previously that lymphotoxin inhibits *C. pneumoniae* growth in HEp-2 cells,

Table 1. NO production by *C. pneumoniae*-infected and non-infected HEp-2 cells

Results are NO (nitrite+nitrate) (μM) in culture supernatants, determined by HPLC. Data are presented as means $\pm$ SD of three separate experiments. For the *C. pneumoniae*-infected group, HEp-2 cells grown in 24-well plates were infected with *C. pneumoniae* at an m.o.i. of $0 \cdot 4$. For the 'Cytokine mixture added' group, non-infected HEp-2 cells were treated with a cytokine mixture containing 10 ng TNF-α, $1 \cdot 0$ ng IL-1β and 100 U IFN-γ ml^{-1}.

Group	Time post-infection		
	8 h	**24 h**	**48 h**
Non-infected	$9 \cdot 42 \pm 0 \cdot 08$	$10 \cdot 58 \pm 0 \cdot 02$	$10 \cdot 40 \pm 0 \cdot 59$
C. pneumoniae-infected	$10 \cdot 16 \pm 0 \cdot 56$	$10 \cdot 91 \pm 1 \cdot 18$	$10 \cdot 03 \pm 0 \cdot 58$
Cytokine mixture added	$12 \cdot 62 \pm 0 \cdot 31$*	$13 \cdot 64 \pm 0 \cdot 24$*	$15 \cdot 26 \pm 0 \cdot 23$*

*Statistically significantly different versus non-infected controls; $P < 0 \cdot 05$.
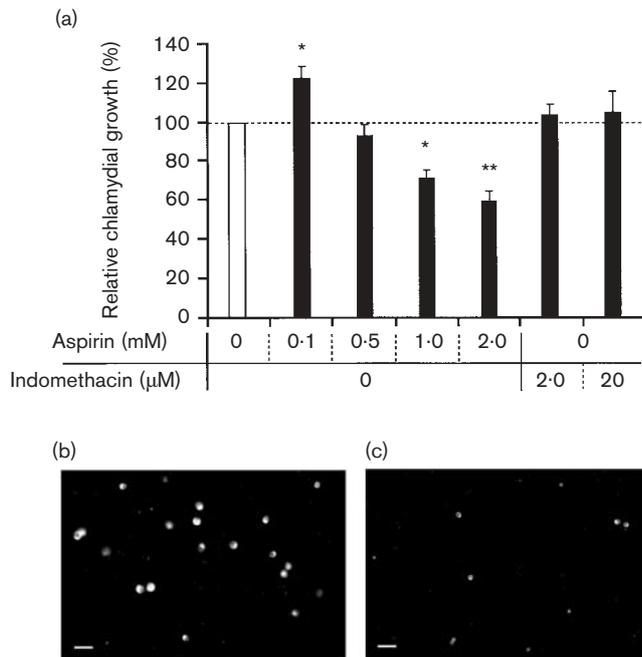
Fig. 4. Effects of aspirin and indomethacin on chlamydial growth in HEp-2 cells. (a) Cells grown in 96-well plates were infected with *C. pneumoniae* and incubated with (filled bars) or without (open bar) aspirin or indomethacin. Staining was performed with mAb RR402. Staining with RR402 was comparable to that with genus-*Chlamydia*-specific mAb CF2. Relative chlamydial growth (%) of infected cells from drug-treated samples (filled bars) is expressed as means ± SD relative to the untreated control (open bar). Statistical significance: *, $P < 0.05$; **, $P < 0.01$ (versus non-treated control). (b)−(c) Immuno-fluorescence staining of *C. pneumoniae* J138 inclusion bodies in the absence (b) or presence (c) of 2 mM aspirin. HEp-2 cells were infected and incubated for 48 h. Bars, 10 μm.
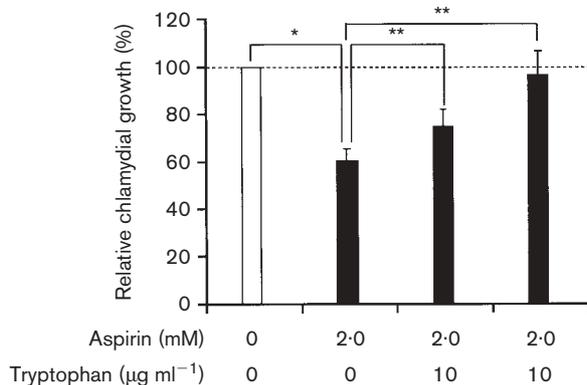

Fig. 5. Effect of tryptophan on inhibition of chlamydial growth by aspirin. HEp-2 cells infected with *C. pneumoniae* were incubated with 2 mM aspirin in the absence or presence of 10 or 100 μg tryptophan ml^{-1} in the culture medium. Data are presented as means ± SD of five separate experiments. Statistical significance of differences: *, $P < 0.01$; **, $P < 0.05$.

possibly by NO production (Matsushima *et al.*, 1999). NO has antimicrobial activity and transcription of the iNOS gene is regulated by NF-κB and AP-1 (Chu *et al.*, 1998; Marks-Konczalik *et al.*, 1998). It is plausible that *C. pneumoniae* may have a system to inhibit NO production by host cells so as to aid survival within infected cells.

Expression of COX-2 is induced by LPS (D'Acquisto *et al.*, 1997), growth factors, cytokines and extracellular stimuli under the control of several transcriptional factors such as AP-2, SP-1 and NF-κB (Appleby *et al.*, 1994; Pistritto *et al.*, 1999). In this study, we found that *C. pneumoniae* infection induced COX-2 synthesis in HEp-2 cells, possibly through NF-κB activation, and this expression was partially inhibited by aspirin but not by indomethacin. *C. pneumoniae* infection enhances PGE$_2$ production, which is completely blocked by aspirin and indomethacin by direct inhibition of COX-2 activity (Gierse *et al.*, 1999). Prostaglandins of the E series, for example PGE$_2$, are produced by the action of COX-2 on arachidonic acid liberated from membrane phospholipids. It has been demonstrated that PGE$_2$ inhibits human T-cell proliferation (Goodwin *et al.*, 1977) and also induces angiogenesis (He & Stuart, 1999). It has also been reported that COX-2 expression is increased in atherosclerotic plaques, predominantly in surrounding areas, in response to *C. pneumoniae* (Song *et al.*, 2000). Our findings suggest that *C. pneumoniae* infection is involved in the progression of inflammatory responses through activation of NF-κB, which induces COX-2 and PGE$_2$.

We also found that aspirin at high doses (1 and 2 mM) inhibited the formation of chlamydial inclusion bodies and proliferation of the bacterium within inclusion bodies in HEp-2 cells. These results suggest that the anti-chlamydial activity of aspirin might be associated with the inhibition of host-cell metabolism, including depletion of essential factors during chlamydial growth. Support for this assumption comes from the finding that the inhibition of chlamydial growth by aspirin was overcome when tryptophan was added to the culture medium. There was no significant effect of tryptophan addition alone on chlamydial growth (i.f.u.) in our culture system in the absence of aspirin (not shown). Tryptophan is an essential amino acid for chlamydiae and reduction of its availability might disturb various metabolic processes, depressing intracellular growth of the pathogen (Pantoja *et al.*, 2000). It has been demonstrated recently that IFN-γ restricts the growth of *C. pneumoniae* in HEp-2 cells through induction of the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase, and the number of typical inclusion bodies decreased with an increase in smaller, less-dense, atypical inclusion bodies containing large, reticulate-like aberrant bodies (Pantoja *et al.*, 2001). Further studies, including ultrastructural morphology of inclusion bodies and indoleamine 2,3-dioxygenase activity in aspirin-treated cells, should be carried out to determine whether the aspirin- and IFN-γ-induced inhibition of *C. pneumoniae* growth is mediated through a common mechanism. It also remains to be clarified whether the inhibition of NF-κB pathways in the presence of aspirin is related to tryptophan catabolism and

whether other mechanisms of tryptophan depletion, independent of NF-κB pathways, are responsible for the attenuation of chlamydial growth.

Very similar results describing the effect of aspirin on chlamydial growth and NF-κB activation by *C. pneumoniae* were published after our initial submission to the journal (Tiran *et al.*, 2002). However, they did not demonstrate rescue from growth inhibition by tryptophan or investigate activation of COX-1 and -2 and PGE$_2$ in response to infection by *C. pneumoniae*, all of which were demonstrated in this study. Our results suggest that *C. pneumoniae*, a possible pathogen involved in atherosclerosis, might trigger and/or enhance inflammatory reactions in atherosclerosis through NF-κB activation and they raise the possibility that aspirin therapy might be useful for *C. pneumoniae* infection in atherosclerosis, in addition to chemotherapy.

ACKNOWLEDGEMENTS

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