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Inhibition of Growth of *Chlamydia trachomatis* by Tumor Necrosis Factor Is Accompanied by Increased Prostaglandin Synthesis

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Development of *Chlamydia trachomatis* (L_2/434/Bu) in HEp-2 cells was inhibited by treatment of the cells with recombinant human alpha tumor necrosis factor (TNF). In the infected cultures that were treated with TNF, high concentrations of prostaglandin E_2(PGE_2) were detected, exceeding by far the concentrations found in TNF-treated but uninfected cells or in infected cells that were not treated with TNF. PGE_2 levels increased gradually for 2 days after infection. Raising the tryptophan concentration in the culture medium, which reversed the inhibition of chlamydial replication by TNF, also blocked the increase in PGE_2 formation. However, neutralizing antibodies to beta interferon, which also interfered with the antichlamydial effect of TNF, did not decrease PGE_2 formation. Excessive formation of PGE_2 by cells infected with chlamydiae and treated by TNF might be related to some of the complications associated with chlamydial infection.

Tumor necrosis factor (TNF) contributes in a variety of ways to the defense of the host against infectious agents and to its recovery from injury. Yet, in certain situations, TNF may be extremely harmful (17, 24). There is only fragmentary information on the mechanisms that underly these deleterious effects. Several studies have suggested that prostaglandins, whose synthesis is enhanced by TNF, play a critical role (8, 12). Other studies have shown that vulnerability of the host to the lethal effects of TNF is increased by certain pathogens. In mice bearing tumors (1a) or infected with certain bacteria (9) or with malaria parasites (4) and in mice injected with bacterial endotoxin (19), the lethal dosage of TNF is significantly lower than that in healthy animals. Recently, we showed that growth of *Chlamydia trachomatis*, obligate intracellular bacteria which are involved in the etiology of certain ophthalmic and venereal diseases and can be associated with reactive arthritis (13), induces the formation of TNF in macrophages (15). This cytokine, in turn, was found by us to inhibit the intracellular growth of these parasites by a mechanism apparently involving induced beta interferon (IFN-β) and tryptophan-degrading enzymes (23).

In the present study we provide evidence that, in cells infected with chlamydiae, TNF induces the production of prostaglandin E_2 (PGE_2) much more effectively than in uninfected cells. Such a potentiation effect of a pathogen on the prostaglandin-inducing activity of TNF may contribute to the increased vulnerability of hosts infected with parasites to the deleterious effects of the cytokine.

MATERIALS AND METHODS

***C. trachomatis* growth.** *C. trachomatis* (L_2/434/Bu) elementary bodies were purified as described previously (21). HEp-2 cells, derived from a human carcinoma of the larynx, were obtained from Flow Laboratories, England, and grown in minimal essential medium containing 10% fetal calf serum, 2 mM L-glutamine, and antibiotics (cell culture medium). The medium contained less than 0.1 U of endotoxin per ml, as determined by the *Limulus* amoebocyte lysate assay.

TNF. Human recombinant TNF (6×10^7 U/mg of protein) containing less than 0.125 U of endotoxin per ml (as determined by the *Limulus* amoebocyte lysate assay), produced by Genentech Co., South San Francisco, Calif., was kindly provided by G. Adolf, Boehringer Institute, Vienna, Austria.

Antibodies. The mouse monoclonal antibodies to human IFN-β were obtained from Inter-Yeda Inc., Rehovot, Israel.

Infection of HEp-2 cells. The cells were seeded into 96-well microdilution plates at 3×10^4 cells per well. After 48 h of incubation at 37°C in an atmosphere of 5% CO_2 in air, the cells were infected at a multiplicity of infection of 1 in the presence of infection medium (minimal essential medium containing 2.5% fetal calf serum, 1% glucose, 2 mM L-glutamine, vancomycin [0.1 μg/ml], and gentamicin [16 μg/ml]). Control cultures were mock infected. In some experiments, chlamydiae were heat inactivated for 15 min at 56°C before infection. After 1 h of adsorption at 37°C, the inoculum was removed, and the cells were incubated further in infection medium with or without additives as indicated. Two days after infection, the cells were scraped into the medium. The contents of triplicate wells were pooled and stored at −70°C until they were assayed for chlamydial yield (see below). In parallel wells, the culture medium was harvested, clarified by centrifugation, and frozen until determination of the PGE_2 content.

Determination of chlamydial yield. HEp-2 cells were seeded into 96-well microdilution plates and infected on the following day with serial dilutions of the samples in infection medium containing 10% fetal calf serum and cycloheximide (1 μg/ml). Fourty-eight hours after infection, the cells were fixed with ethanol, and chlamydial inclusions were stained by an indirect immunoperoxidase method (21). Values are expressed as inclusion-forming units per milliliter of sample.

Determination of PGE_2. The media from control and infected cultures were radioimmunoassayed for PGE_2 as previously described (10). Samples were incubated for 4 h at 4°C with a specific rabbit antiserum against PGE_2, normal

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rabbit serum, a goat antiserum against rabbit immunoglobulins (Calbiochem, Frankfurt, Federal Republic of Germany), and [^{3}H]PGE$_2$ (Amersham, Braunschweig, Federal Republic of Germany). The immune complexes were separated from the unbound [^{3}H]PGE$_2$ by centrifugation, and the radioactivity of the pellet was measured by liquid scintillation spectrometry. PGE$_2$ levels were calculated by comparison with calibrated standards run in parallel.

Analysis of prostaglandins by high-pressure liquid chromatography. Cells (1.5×10^5 in 1 ml of cell culture medium) were seeded into each well of plates with 18-mm wells. After 8 h, [^{3}H]arachidonic acid (New England Nuclear, Dreieich, Federal Republic of Germany) (10 μCi per well) was added to the cultures and allowed to incorporate for 20 h. The cells were then rinsed three times with phosphate-buffered saline and infected or mock infected as described above. After 1 h the infection medium was removed, and the cells were rinsed three times with phosphate-buffered saline and incubated further with infection medium devoid of phenol red and with or without TNF (100 ng/ml). After 48 h, the medium was harvested from the cultures and subjected to solid-phase extraction. Samples were acidified to pH 3.5 with acetic acid and applied to 1-ml C_{18} columns (Bond Elute; Analytichem, Harbor City, Calif.) that had been prewashed with 1 ml of ethanol and equilibrated with 1 ml of 0.1% EDTA. After columns were washed with 1 ml of water, they were eluted with 2 ml of methanol; the eluate containing the eicosanoids was concentrated under a stream of nitrogen. The samples were then analyzed by isocratic reversed-phase high-pressure liquid chromatography at a flow rate of 0.2 ml/min. The mobile phase consisted of acetonitrile (31.9%, vol/vol), acetic acid (0.1%, vol/vol), and H_2O (68%, vol/vol) adjusted to pH 4.5 with sodium acetate; a C_{18} narrow-bore column (1.6 by 250 mm, MZ Analysentechnik, Mainz, Federal Republic of Germany) constituted the stationary phase. Radioactivity of the effluent was monitored with a radioactive flow monitor (Raytest, Straubenhard, Federal Republic of Germany).

Each of the experiments presented in this paper was performed at least three times with essentially identical results. Data for the chlamydial yield and PGE$_2$ determinations are presented as the means ± standard deviations of triplicate determinations. Where the bars for the standard deviation are not drawn they fall inside the symbols. Statistical analysis of data was performed with the Student *t* test.

RESULTS

Growing HEp-2 cells produced spontaneously only low amounts of PGE$_2$ as determined by radioimmunoassay; after incubation for 48 h, the PGE$_2$ concentration in the culture medium was usually less than 0.5 ng/ml. Treatment of the cells with TNF increased the formation of PGE$_2$, although to a lesser extent than that observed in some other cells (5). Infection with chlamydiae also resulted in some enhancement of the synthesis of PGE$_2$ (Fig. 1 and 2).

Growth of *C. trachomatis* in HEp-2 cells was greatly reduced by treatment with TNF (22) (see Fig. 4B). Yet, in spite of the inhibitory effect of the cytokine on chlamydial growth, treatment with TNF markedly enhanced PGE$_2$ formation in the infected cells (Fig. 1), yielding concentrations exceeding by far those found in cultures of noninfected cells after cytokine treatment or in cultures of cells that were infected without being treated with the cytokine. The kinetics showed that the production of PGE$_2$ was induced rather slowly, primarily during the second day of infection and cytokine treatment.

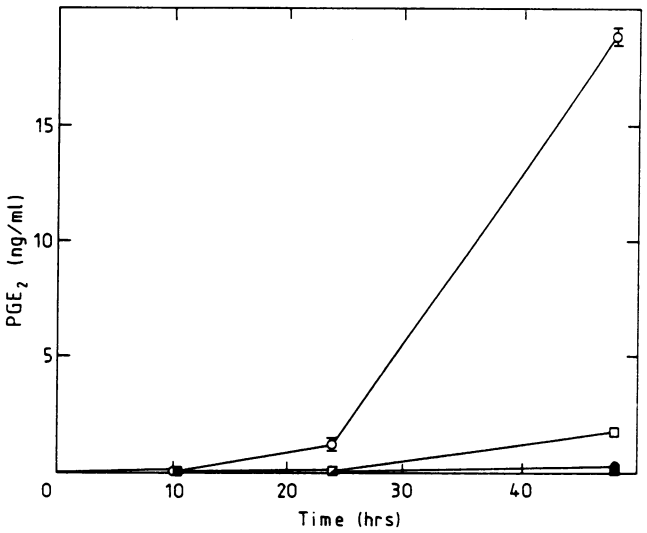

FIG. 1. Effect of TNF and chlamydial infection on PGE$_2$ formation. Chlamydia-infected cells (multiplicity of infection of 1) (○, □) or uninfected cells (●, ■) were incubated with TNF (500 ng/ml) (○, ●) or without TNF (□, ■). After the indicated periods of incubation, PGE$_2$ concentrations in the culture media were determined. Shown are the means ± standard deviations of triplicates from a representative experiment.

The extent of PGE$_2$ formation was proportional to the concentration of live chlamydiae (Fig. 2). Heat-inactivated chlamydiae did not induce PGE$_2$ formation either in the presence or in the absence of TNF (Fig. 2), nor was PGE$_2$ formation induced by bacterial lipopolysaccharide (100 μg/

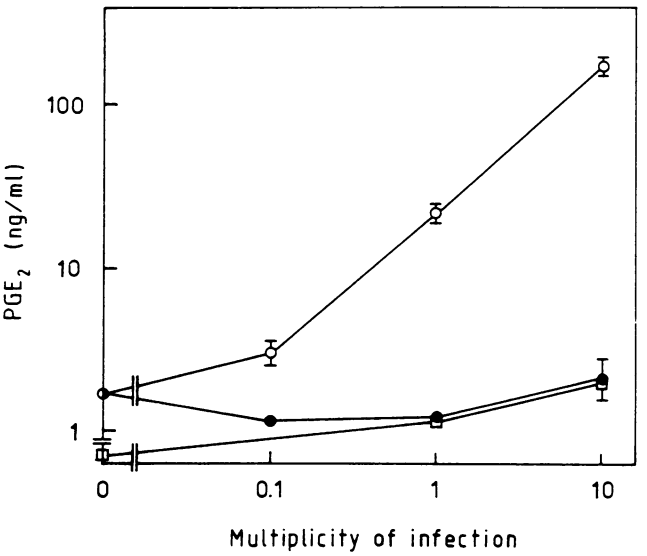

FIG. 2. Effects of different doses of active and heat-inactivated chlamydiae on the induction of PGE$_2$ formation by TNF. HEp-2 cells were inoculated with active chlamydiae (○) or heat-inactivated chlamydiae (●) at the indicated multiplicities of infection and incubated with TNF for 48 h. The concentration of PGE$_2$ in the culture medium was determined thereafter. The PGE$_2$ formation in cultures infected with active chlamydiae without TNF treatment is also shown (□). Symbols represent the means ± standard deviations of triplicates from a representative experiment. Heat-inactivated chlamydiae alone did not induce PGE$_2$ formation.

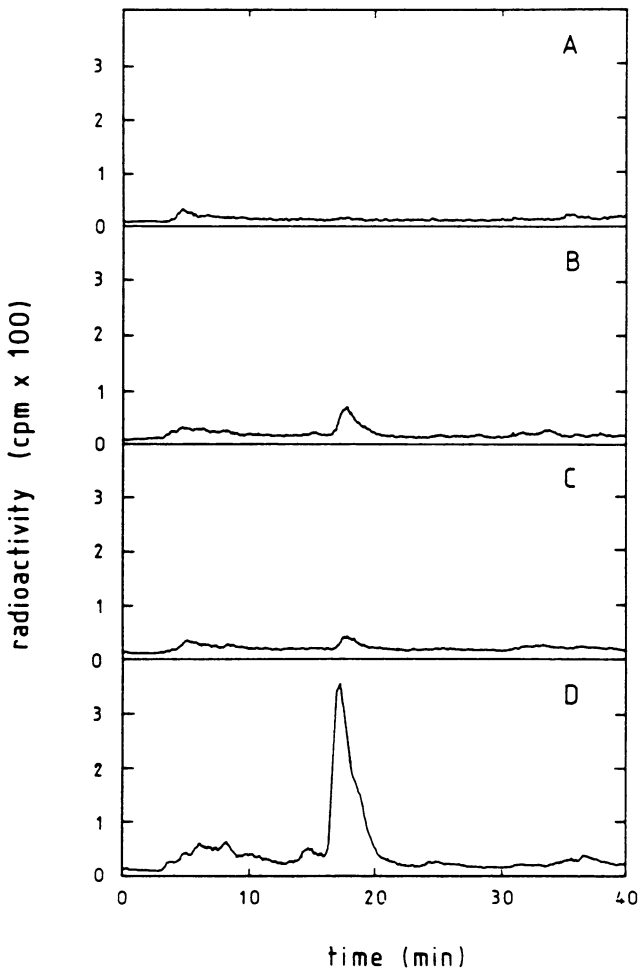

FIG. 3. Analysis of prostaglandins formed by HEp-2 cells on infection and/or TNF treatment by reversed-phase high-pressure liquid chromatography. Cells labeled with [^{3}H]arachidonic acid were cultured for 2 days, and the supernatant was removed and analyzed as described in Materials and Methods. Supernatant was taken from (A) untreated cells, (B) cells treated with TNF (500 ng/ml), (C) cells infected with chlamydiae, and (D) cells infected with chlamydiae and treated with TNF.

ml, from E. coli; Difco Laboratories, Detroit, Mich.) (data not shown).

To gain a more comprehensive view of the arachidonic acid derivatives that are produced by infected cells, HEp-2 cells were labeled with [^{3}H]arachidonic acid before infection and cytokine treatment, and the labeled compounds released by the cells within 2 days of infection were analyzed by reversed-phase high-pressure liquid chromatography (Fig. 3). The major peak of radioactive materials released by the infected cells had a retention time corresponding to that of a PGE_2 standard preparation (17 min). Consistent with the results obtained in the antibody-based assay, PGE_2 formation in TNF-treated, chlamydia-infected cells exceeded by far its formation in control cells, noninfected TNF-treated cells, and infected cells that were not treated with TNF.

The antichlamydial effect of TNF can be suppressed in two ways: by increasing the concentration of tryptophan or by adding neutralizing antibodies against IFN-β (23), suggesting the involvement of tryptophan degradation and of an autocrine function of IFN-β in the antichlamydial effect of TNF.

Increasing the tryptophan concentration to 10-fold that of the normal concentration in the culture medium (10 μg/ml), which strongly suppressed the inhibitory effect of TNF on chlamydial development (Fig. 4B), also resulted in a marked suppression of PGE_2 formation in cells infected with chlamydiae and treated with TNF (Fig. 4A). Prostaglandin formation in noninfected cells was not inhibited.

On the other hand, antibodies to IFN-β with a neutralizing capacity of 500 U/ml, even though they markedly decreased the antichlamydial effect of TNF ($P < 0.001$), had no significant effect ($P > 0.05$) on the high level of PGE_2 formation in the infected, TNF-treated cells (Table 1).

DISCUSSION

TNF-induced resistance to viral, procaryotic, or eucaryotic parasites involves, in part, induction of a state of increased antiparasitic activity in cells of the immune system, such as mononuclear and polymorphonuclear phagocytes (6, 7). But TNF also increases the resistance to parasites through a direct effect on the infected cell, as in the case of viral infection (16, 27).

In this way TNF also affects the growth of *C. trachomatis* in HEp-2 cells (22). Simultaneously with a decreased yield of chlamydiae in cells treated with TNF, these cells also exhibited a marked potentiation of the production of PGE_2 which was dependent on both their TNF treatment and infection by the parasite (Fig. 1). High-pressure liquid chromatography revealed that, indeed, the major metabolite of arachidonic acid in these cells is PGE_2. It remains to be seen whether in other pathogen-infected cells the formation of different prostanoids in response to TNF is enhanced as well.

The antiviral activity of TNF has been shown to be partly due to endogenous formation of IFN-β (16). Also, the antichlamydial effect of TNF is decreased by antibodies against IFN-β (23). However, PGE_2 formation was not affected by those antibodies (Table 1), indicating that it is independent of IFN-β.

The mechanism underlying the antichlamydial activity induced by TNF appears to involve an increase in the degradation of tryptophan (23). A similar mechanism has been suggested for the antichlamydial effects of IFN-γ (3, 20), and the enhanced expression of the tryptophan-degrading enzyme indoleamine-2,3-dioxygenase has been ascribed to this mechanism. In support of this notion, the addition of tryptophan reverses the antichlamydial effect of IFN-γ (3, 20) and of TNF (23). A tryptophan supply might be essential for the development of *C. trachomatis*; its depletion from the growth medium inhibits the parasitic cycle. In addition, tryptophan can function as a radical scavenger (28), possibly preventing some radical-dependent defense mechanism from operating against the chlamydiae. The finding that tryptophan also inhibits TNF-induced PGE_2 synthesis in chlamydia-infected cells (Fig. 4A) raises a third possibility. PGE_2 has been shown to have antichlamydial activity, possibly mediated by increased cyclic AMP levels in the infected cells (25). It therefore seems possible that the induction of PGE_2 by TNF plays a role in the antichlamydial effect of the cytokine. On the other hand, the finding that antibodies against IFN-β decreased the antichlamydial effect of TNF without affecting PGE_2 formation indicates that high PGE_2 levels alone are not sufficient to explain the inhibition of chlamydial growth by TNF.

Whether endogenous PGE_2 formation indeed contributes to the suppression of ongoing chlamydial infection in cells

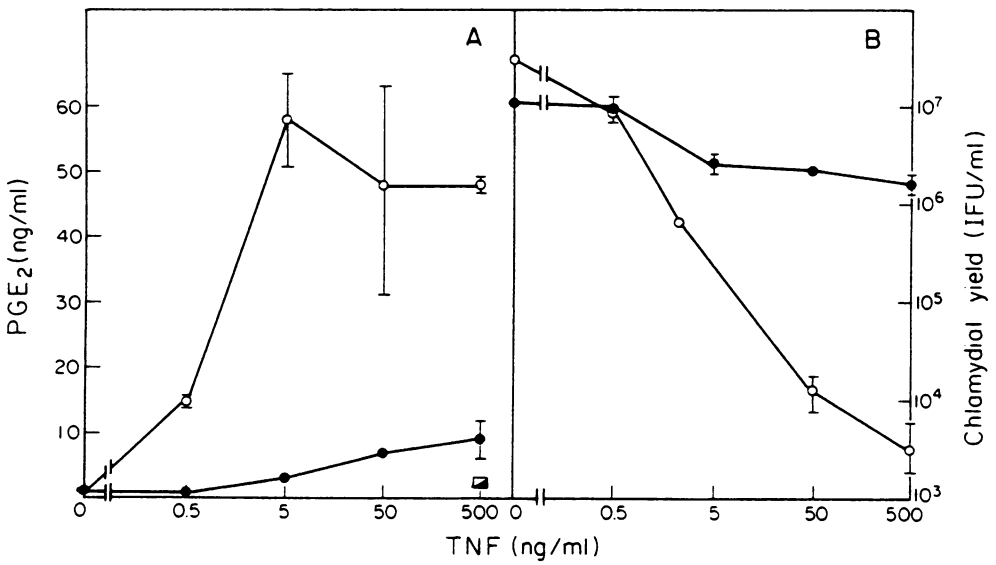

FIG. 4. Effect of TNF and tryptophan on (A) PGE$_2$ formation and (B) chlamydial yield. HEp-2 cells were infected with chlamydiae (○, ●) for 1 h, and then the cells were treated with TNF in the presence of either 10 μg (○) or 100 μg (●) of tryptophan per ml. Data for uninfected cells treated with TNF in the presence of either 10 or 100 μg of tryptophan per ml are also shown (◪). The data show the means ± standard deviations of triplicates from a representative experiment.

exposed to TNF has to be clarified by further experiments. However, since PGE$_2$ has been shown to inhibit chlamydial growth (25), it is conceivable that this PGE$_2$ can contribute to the defense against chlamydiae, at least by enhancing the resistance to the pathogen in neighboring, as yet uninfected, cells.

It is typical of many elements in an inflammatory reaction that their effects on the host can be of an ambivalent nature: on the one hand they may participate in the defense of the host against invading pathogens, but on the other hand they may have adverse effects. Such an ambivalent role is known for TNF as well as PGE$_2$, and part of the in vivo toxicity of TNF has been linked to prostaglandin formation (8, 12). Since chlamydiae can induce the formation of TNF in experimental situations (15, 26), one may speculate that also during natural infection TNF is formed, which in turn induces high levels of PGE$_2$ in the infected cells, leading to some of the complications of chlamydial infection.

A manifestation of the consequences of such events might be the high incidence of premature labor and abortions in chlamydia-infected humans (18) and ruminants (1), which possibly reflects the labor-inducing activity of prostaglandins. Indeed, in some cases of abortion and premature labor, increased levels of PGE$_2$ have been observed in the amniotic and allantoic fluids (14, 18).

Chlamydial infection has been found to be associated with sexually aquired reactive inflammatory arthritis (11). It is possible that in that form of rheumatoid arthritis, chlamydial infection leads to the formation of TNF and consequently PGE$_2$, mediators which have been found in the synovial fluids of patients with rheumatoid arthritis (2) and which are thought to contribute to some of the symptoms of the disease, such as pain and bone resorption.

In summary, the present study demonstrates that, in cell culture, infection by *C. trachomatis* in the presence of TNF can result in marked production of PGE$_2$ in cells that under normal conditions are only poor producers of prostaglandins. Further investigation is necessary for evaluating the relevance of this in vitro model to the in vivo situation.

TABLE 1. Influence of antibodies against IFN-β on the antichlamydial effect of TNF and on PGE$_2$ formation

Treatment	Chlamydial yield (IFU/ml)[a]	PGE$_2$ (ng/ml)[a]	
		With chlamydiae	Without chlamydiae
Untreated cells	$2.8 \times 10^7 \pm 3 \times 10^5$	4.3 ± 0.2	0.5 ± 0.1
TNF (500 ng/ml)	$1.2 \times 10^4 \pm 1 \times 10^2$[b]	226 ± 75[b]	2.6 ± 0.6[b]
TNF (500 ng/ml) + Abs[c] to IFN-β	$1.7 \times 10^6 \pm 2 \times 10^5$[b]	320 ± 83[b]	1.2 ± 0.7
Abs to IFN-β	$3.5 \times 10^7 \pm 4 \times 10^6$	11 ± 2[d]	0.4 ± 0.1

[a] Values represent the means ± standard deviations of triplicate determinations. Data were analyzed by the Student *t* test. IFU, Inclusion-forming units.
[b] $P < 0.01$ in comparison with untreated cells.
[c] Abs, Antibodies.
[d] $P < 0.05$ in comparison with untreated cells.

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