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IN this study levels of prostaglandin E_2 (PGE_2), tumour necrosis factor (TNF) and interleukin-1 (IL-1) alpha in medium from monocyte derived macrophages (MdM) infected with *Chlamydia trachomatis* (L_2/434/Bu or K biovars). TNF and PGE_2 were found in both cases while IL-1 alpha was not detected. Both TNF and PGE_2 levels were higher in the medium of the MdM infected with K biovars. TNF reached maximum levels 24 h post-infection, and then declined, while PGE_2 levels increased continuously during the infection time up to 96 h post-infection. Addition of dexamethasone inhibited production of TNF and PGE_2. Inhibition of PGE_2 production by indomethacin resulted in increased production of TNF, while addition of PGE_2 caused partial inhibition of TNF production from infected MdM.

Key words: *Chlamydia trachomatis*, Monocyte derived macrophages, Prostaglandin E_2, Tumour necrosis factor

TNF and PGE_2 in human monocyte-derived macrophages infected with *Chlamydia trachomatis*

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Introduction

Different biovars of *Chlamydia trachomatis,* an obligate intracellular Gram-negative bacterium, have been associated with clinically distinct infections ranging from hyperendemic trachoma (serovars A, B, and C) to sexually transmitted infections and pneumonia (serovars D to K). Lymphogranuloma venereum is a sexually transmitted disease caused by *C. trachomatis* serovars L1, L2 and L3, which are more invasive than biovars D to K. Lymphogranuloma venereum causes a systemic infection characterized primarily by gross lymphadenopathy, suppurative adenitis, and ulcerative genital tract and rectal diseases.[1]

The mononuclear phagocytes, including both the tissue macrophages and their precursors, the circulating blood monocytes, act as effective microbicidal host defence cells against many pathogenic microorganisms. They have been implicated in regulating the functions of lymphoid and haematopoietic cells, and in most cases, these effects are mediated by soluble factors produced by circulating monocytes and tissue macrophages.[2]

Endotoxin and lipopolysaccharide of the outer membrane of Gram-negative bacteria have been found to potently stimulate human monocytes to produce several substances with important biological activities,[3] including interleukin 1 (IL-1),[4,5] tumour necrosis factor (TNF)[6,7] and prostaglandin E_2 (PGE_2).[8] These factors induce a multitude of biological responses of importance in homeostasis, in host defence mechanisms, and, probably, in the pathogenesis of several diseases.[9–11]

The present study shows that infection of human monocyte derived macrophages (MdM) with L_2 or K biovars induces the production of TNF and PGE_2 but not of IL-1 alpha. The effect of dexamethasone on the production of these factors in MdM has also been examined.

Materials and Methods

Cells: HEp-2 cells, originating from human carcinoma of the larynx (Flow Laboratories, UK, 03-108) were grown in minimal essential medium (MEM) with glutamine and antibiotics (Biological Industries, Beit Haemek, Israel), and 10% foetal calf serum (FCS) (Gibco Laboratories, Grand Island, NY, USA).

Human monocytes were prepared from heparinized blood of normal donors as described previously.[12] Monocytes grown in RPMI-1640 medium supplemented with 10% heat inactivated foetal calf serum, glutamine and antibiotics, incubated at 37°C in an atmosphere of 5% CO_2 for 8–10 days were used as MdM.

Preparation of purified, infectious EB particles: Infectious *C. trachomatis* biovar lymphogranuloma venereum (L2/434/Bu) and serum K elementary body particles were purified from BGM cells as described previously.[12]

Immunoperoxidase assay for titration of C. trachomatis*: C. trachomatis* was titrated on HEp-2 cells as described previously.[12] The final results of titration were expressed as inclusion-forming units per millilitre.

Chlamydial infection in MdM and control cells: MdM (8×10^5 cells/well) and HEp-2 cells (2×10^5 cells/well) were grown in 24-well plates (Nunc, Denmark). Twenty-four h later the cells, treated or not treated with either dexamethasone (10^{-6} M), indomethacin (10^{-6} M) or PGE_2 (10^{-6} M), were infected or not infected with L_2 or K biovars at a multiplicity of infection (M.O.I.) of 1–2. Two h later the unadsorbed *Chlamydiae* were removed, and fresh medium with or without dexamethasone (10^{-6} M), indomethacin (10^{-6} M) or PGE_2 (10^{-6} M), respectively, was added to the wells. At various time intervals (2, 24, 48 and 96 h post-infection) medium from the wells was harvested and centrifuged for 5 min at 20 000 × ***g***. The supernatant was frozen at −70°C until PGE_2, TNF or IL-1 alpha were measured.

Determination of TNF, PGE_2 and IL-1 alpha: TNF concentrations were determined by ELISA (Biokine TNF Test Kit, T Cell Sciences, Inc., Cambridge, MA, USA). Cell free culture medium from the kinetic studies was subjected to RIA for PGE_2 analysis as described previously.[13] IL-1 alpha concentrations were determined by ELISA (Endogen Inc., Boston, MA, USA).

Results

PGE_2 production from MdM infected with Chlamydiae: MdM untreated or treated with dexamethasone (10^{-6} M) or indomethacin (10^{-6} M), were infected or mock infected with *C. trachomatis* (L_2 or K biovars) at a M.O.I. of 1–2. At various time intervals (2, 24, 48, 96 h post-infection) medium was harvested and PGE_2 levels were determined by RIA in the cell-free medium. Fig. 1 shows that the amount of PGE_2 in the medium from L_2 and K infected MdM increased during the infection time. Infection with the K biovar resulted in higher levels of PGE_2 secretion. Infection of the MdM with a higher M.O.I.[5] of both L_2 or K biovars resulted in an increase of PGE_2 production (data not shown). Daily medium replacement resulted in lower PGE_2 production per 24 h from the infected MdM compared with the infected cells in which no medium was replaced (data not shown). Addition of dexamethasone (10^{-6} M) or indomethacin (10^{-6} M) to the chlamydial infected MdM (K or L_2) resulted in complete inhibition of PGE_2 production. The effect of dexamethasone and indomethacin on PGE_2 production observed in MdM infected with the K biovar was much more pronounced than that observed in MdM infected with the L_2 biovar. This phenomenon was caused by the reduced ability of the L_2 biovar to stimulate PGE_2 production in MdM compared to the K biovar. Fig. 2 shows the effect of dexamethasone and indomethacin on PGE_2

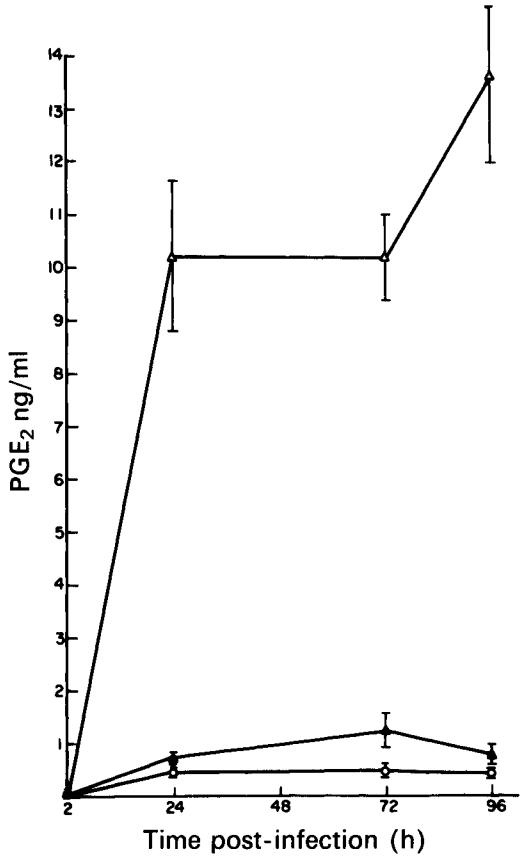

FIG. 1. PGE_2 release from MdM infected with *C. trachomatis* L_2 (343/Bu) or K biovars at a M.O.I. of 1–2. △, PGE_2 release from MdM infected with K biovar; ▲, PGE_2 release from MdM infected with L_2 biovar; ○, mock infected MdM.

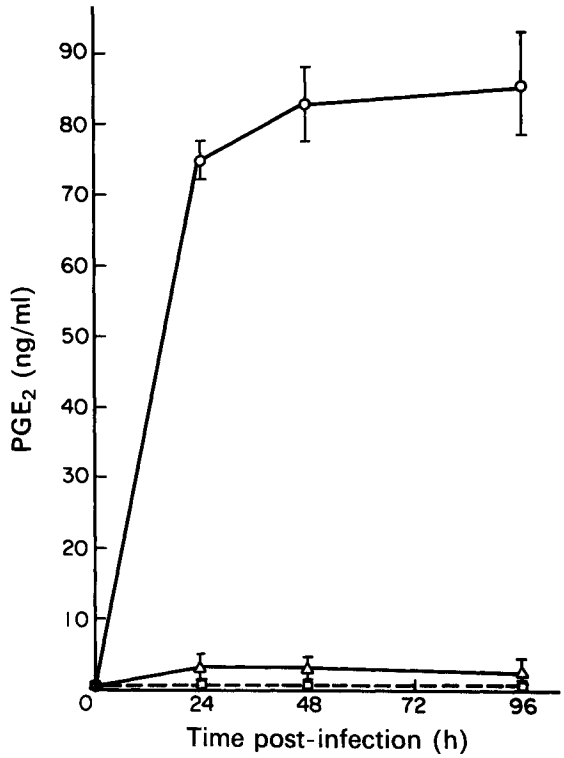

FIG. 2. PGE_2 release from MdM infected with *C. trachomatis* K biovar: the effect of dexamethasone or indomethacin treatment. ○, PGE_2 release from infected MdM without treatment; △, PGE_2 release from infected MdM treated with dexamethasone; □, PGE_2 release from infected MdM treated with indomethacin; - - -, mock infected MdM.

levels in cell free medium from MdM infected with the K biovar. Medium from control mock infected MdM with or without dexamethasone or indomethacin had low levels of PGE_2 (0–0.6 ng/ml).

TNF production by MdM infected with Chlamydiae: TNF was measured by ELISA in parallel with PGE_2 determination. Both L_2 and K biovars induced production of TNF by MdM. TNF production was higher in the MdM infected with the K strain. TNF reached maximum levels 24 h post-infection (Fig. 3) and then declined. Daily replacement of the medium from the infected cells with fresh medium resulted in higher levels of TNF (data not shown). Infection at a higher M.O.I.[5] of both L_2 and K strains resulted in higher TNF production (data not shown).

Addition of dexamethasone (10^{-6} M) to the *Chlamydia* infected MdM resulted in inhibition of TNF production, while addition of indomethacin (10^{-6} M) resulted in an increase of TNF production. Addition of PGE_2 (10^{-6} M) to the *Chlamydia* infected MdM resulted in partial inhibition of TNF production. The effect of dexamethasone and indomethacin on TNF production observed in MdM infected with the K biovar was much more pronounced than that observed in MdM infected with the L_2 biovar. Fig. 4 shows the effect of dexamethasone, indomethacin and PGE_2 on the TNF level in the medium from MdM infected with biovar K. In medium from mock infected MdM treated or not treated with dexamethasone, indomethacin or PGE_2, no TNF was detected.

Determination of IL-1 alpha in the cell free medium of chlamydia *infected or mock infected MdM:* The levels of IL-1 alpha in the medium of K or L_2 infected or mock infected cells was detected by ELISA in parallel with TNF and PGE_2 determination. No difference was found between the IL-1 alpha levels in the mock infected compared to the *Chlamydia* infected MdM medium.

Chlamydial yield in MdM with or without dexamethasone, indomethacin or PGE_2 *treatment:* MdM were treated or not treated with dexamethasone (10^{-6} M), indomethacin (10^{-6} M) or PGE_2 and infected with *C. trachomatis* K and L_2 biovars at a M.O.I. of 1–2. Ninety-six h later triplicates of each treatment were harvested and the chlamydial yield was determined. L_2 reached a yield of 1–2 × 10^5 IFU/ml while only 20–50 IFU/ml were detected in the MdM infected with the K biovar. Treatment with indomethacin

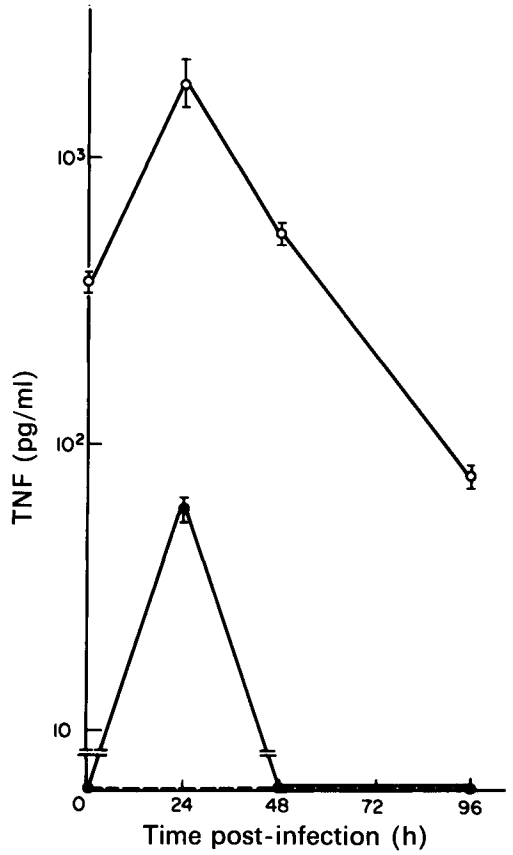

FIG. 3. TNF release from MdM infected with *C. trachomatis* L_2 (343/Bu) or K biovars. ○, TNF release from MdM infected with K biovar; ●, TNF release from MdM infected with L_2 biovar; ---, TNF release from mock infected MdM.

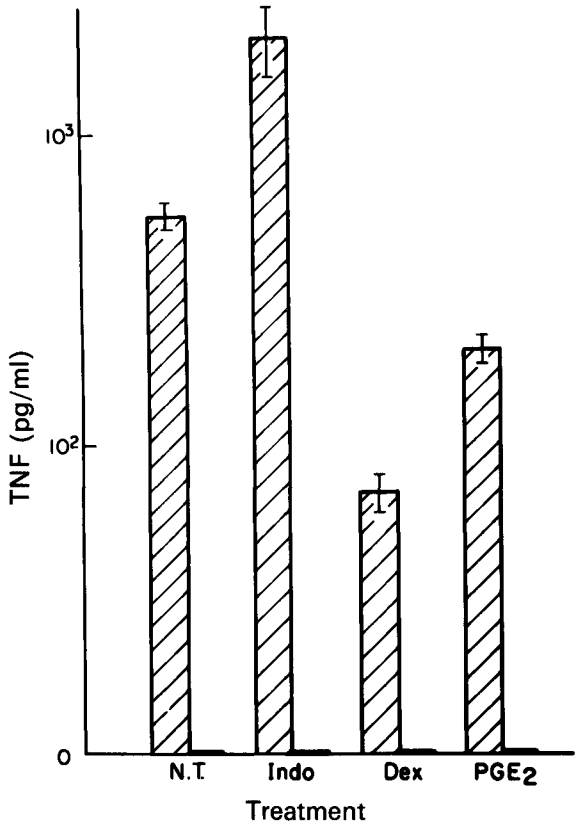

FIG. 4. TNF release from MdM infected with *C. trachomatis* K biovar: the effect of dexamethasone (Dex), indomethacin (Indo) or PGE_2, 24 h post-infection. Control without treatment (NT). ▨, Infected cells; ■, control cells.

and PGE_2 did not affect chlamydial yield, while treatment with dexamethasone resulted in a 2- to 3-fold increase in chlamydial yield of both K and L_2 biovars.

Discussion

Various biovars of *C. trachomatis* differ in their pathogenicity, but the mechanism is still obscure. One possible explanation might be the ability of the pathogen to turn on host defence mechanisms. The mechanisms of the host defence against diseases caused by chlamydial species are not clearly understood, but both humoral and cell mediated immunity are involved.[14] Infection of macrophages by intracellular parasites might modulate production of TNF, PGE_2 and IL-1 alpha, which, in turn, might have a profound effect on the outcome of the infection *in vivo*.

The present study shows clearly that L_2 and K biovars induced production of TNF from human MdM (Figs 1 and 3). This production increased when a higher multiplicity of infection of *Chlamydia* was used. Higher levels were observed in the case of K strain infected MdM. This might explain the inability of K strain to grow in MdM, as described by us (Schmitz, Manor, Sarov [Abstract, Germany], and Yong) as well as the difference in the severity of the outcome of the infection *in vivo* between the L_2 and K biovars—L_2 causing a more generalized infection than K. Recently, Williams *et al.*[15] showed that spleen cells from *C. trachomatis*, pneumonitis agent infected nu/+ and nu/nu mice produced TNF-α. They suggested that TNF-α might play a role in host defence in the murine model. It has been shown that TNF-α inhibits chlamydial growth in HEp-2 cells.[16] This suggests that *in vivo*, early in the chlamydial infection, TNF may play a protective role. However, it is also likely that TNF might cause some of the pathological effects seen in the course of chlamydial infection. The capability of *Chlamydiae* to induce TNF production from macrophages is not unique to chlamydiae, but has been demonstrated recently in a wide range of intracellular pathogens such as viruses,[17] bacteria,[18–20] eukaryotic parasites,[21] and fungi.[22] Various molecules have also been found to be able to induce TNF in macrophages, such as bacterial lipopolysaccharides and endotoxin.[23] Further studies are required to characterize the chlamydial component responsible for the induction of TNF-α in human macrophages.

This study shows that L_2 and K also induced human MdM to produce PGE_2 (Fig. 1). The level of PGE_2 increased when a higher multiplicity of infection of *Chlamydiae* was used. The PGE_2 level was higher when MdM were infected with the K strain. PGE_2 has been shown to be produced by macrophages infected with viruses,[24,25] bacteria,[26] and intracellular parasites,[27,28] and may cause profound metabolic and functional changes in these cells.[29]

Dexamethasone inhibited TNF and PGE_2 production (Figs 2 and 4). These results are in agreement with those described by Beutler *et al.*[30] and by Danon *et al.*,[31] respectively who have shown that dexamethasone inhibits TNF and PGE_2 production at the transcriptional level.

Only L_2 was found to replicate in MdM.[12] Treatment of the cells with dexamethasone enhanced the yield of infectious chlamydial particles in these cells. These results cannot be simply explained by the dexamethasone inhibition of TNF production from chlamydial infected MdM. This conclusion is based on the findings that addition of an excess of PGE_2, which inhibits TNF production, or indomethacin, which enhances TNF production, did not affect the chlamydial yield in MdM. The mechanism by which dexamethasone enhances chlamydial yield in MdM needs further investigation. Corticosteroids have been found to enhance replication of viruses,[32] and certain intracellular parasites.[33–35]

In contrast to PGE_2 and TNF-α, no IL-1 was detected in the media from MdM infected with either K or L_2 biovars. These results differ from those reported by Rothermel *et al.*,[36] who showed that *C. trachomatis* induced production of IL-1 by human monocytes. This difference might be due to the difference in the M.O.I. used by Rothermel as compared to the M.O.I. in our system (1–2) or to the differentiation state of the cells used by Rothermel (monocytes) compared to ours (MdM). Roux Lombard *et al.*[37] showed that blood monocytes cultured for several weeks, produced much less IL-1 than freshly isolated monocytes. Furthermore, they showed that monocytes cultured for a few weeks produced a specific IL-1 inhibitor.

The level of TNF detected in the medium of the L_2 or K strains infected MdM reached a maximum at 24 h post-infection and then declined (Fig. 2). When the infected MdM were washed daily, the TNF level remained high throughout the entire experimental period (data not shown). A possible explanation is that the high level of PGE_2 depressed TNF production, and that washing the cells eliminated the interference of PGE_2. This explanation is supported by Kunkel *et al.*[8] who showed that PGE_2 regulates macrophage derived TNF gene expression. These observations support the suggestion that TNF and PGE_2 may affect each other's production.[9,38] TNF produced by activated macrophages may be responsible for increased synthesis of PGE_2, which, in turn, limits macrophage activation in an autoregulatory manner.[24,25] A delicate balance between TNF and PGE_2 produced by macrophages might play a major role in the

outcome and severity of chlamydial infection *in vivo*. Animal model studies are required to examine the possible therapeutic effect of prostaglandin inhibitors and antibodies to TNF on the outcome of chlamydial infections.

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