In this study levels of prostaglandin E₂ (PGE₂), tumour necrosis factor (TNF) and interleukin-1 (IL-1) alpha in medium from monocyte derived macrophages (MdM) infected with *Chlamydia trachomatis* (L₂/434/Bu or K biovars). TNF and PGE₂ were found in both cases while IL-1 alpha was not detected. Both TNF and PGE₂ 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 PGE₂ levels increased continuously during the infection time up to 96 h post-infection. Addition of dexamethasone inhibited production of TNF and PGE₂. Inhibition of PGE₂ production by indomethacin resulted in increased production of TNF, while addition of PGE caused partial inhibition of TNF production from infected MdM.

**Key words:** *Chlamydia trachomatis*, Monocyte derived macrophages, Prostaglandin E₂, Tumour necrosis factor

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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 tumour necrosis factor (TNF)⁵,⁶ and prostaglandin E₂ (PGE₂).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

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**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% CO₂ 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 x 10^5 cells/well) and HEp-2 cells (2 x 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\textsubscript{2} (10^{-6} M), were infected or not infected with L\textsubscript{2} or K biovars at a multiplicity of infection (M.O.I.) of 1–2. Two h later the unadsorbed *Chlamydia* were removed, and fresh medium with or without dexamethasone (10^{-6} M), indomethacin (10^{-6} M) or PGE\textsubscript{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 x g. The supernatant was frozen at -70°C until PGE\textsubscript{2}, TNF or IL-1 alpha were measured.

Determination of TNF, PGE\textsubscript{2} and IL-1 alpha: TNF concentrations were determined by ELISA (Bio-kine TNF Test Kit, T Cell Sciences, Inc., Cambridge, MA, USA). Cell free culture medium from the kinetic studies was subjected to RIA for PGE\textsubscript{2} analysis as described previously. IL-1 alpha concentrations were determined by ELISA (Endogene Inc., Boston, MA, USA).

**Results**

PGE\textsubscript{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\textsubscript{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\textsubscript{2} levels were determined by RIA in the cell-free medium. Fig. 1 shows that the amount of PGE\textsubscript{2} in the medium from L\textsubscript{2} and K infected MdM increased during the infection time. Infection with the K biovar resulted in higher levels of PGE\textsubscript{2} secretion. Infection of the MdM with a higher M.O.I.\textsuperscript{5} of both L\textsubscript{2} or K biovars resulted in an increase of PGE\textsubscript{2} production (data not shown). Daily medium replacement resulted in lower PGE\textsubscript{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\textsubscript{2}) resulted in complete inhibition of PGE\textsubscript{2} production. The effect of dexamethasone and indomethacin on PGE\textsubscript{2} production observed in MdM infected with the K biovar was much more pronounced than that observed in MdM infected with the L\textsubscript{2} biovar. This phenomenon was caused by the reduced ability of the L\textsubscript{2} biovar to stimulate PGE\textsubscript{2} production in MdM compared to the K biovar. Fig. 2 shows the effect of dexamethasone and indomethacin on PGE\textsubscript{2}.
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$^3$ IFU/ml while only 20–50 IFU/ml were detected in the MdM infected with the K biovar. Treatment with indomethacin

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**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. Infection of macrophages
by intracellular parasites might modulate produc-
tion 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. showed that spleen cells from *C. trachomatis*,
pneumonitis agent infected nu/+ and nu/nu mice
produced TNF-$\alpha$. They suggested that TNF-$\alpha$
might play a role in host defence in the murine
model. It has been shown that TNF-$\alpha$ inhibits
chlamydial growth in HEp-2 cells. 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 *Chlamydia* 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 induc-
tion of TNF-$\alpha$ 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 *Chlamydia* 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. $^{31}$ 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
e enhance replication of viruses $^{32}$ and certain
intracellular parasites. $^{33-35}$

In contrast to PGE$_2$ and TNF-$\alpha$, 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 explana-
tion is supported by Kunkel et al.$^{8}$ who showed that
PGE$_2$ regulates macrophage derived TNF gene
expression. These observations support the sug-
gestion that TNF and PGE$_2$ may affect each other's
production. $^{9,38}$ TNF produced by activated macro-
phages 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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