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THE EFFECT OF MONOCYTE-DERIVED MACROPHAGES ON THE GROWTH OF *RICKETTSIA CONORII* IN PERMISSIVE CELLS

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Summary. - We examined whether monocyte-derived macrophages (MdM) incubated with rickettsia-infected HEp-2 or BGM cells a) affect *R. conorii* (Boutonneuse fever) growth, and b) secrete TNF and IL-1 alpha. BGM and HEp-2 cells were infected with *R. conorii* at multiplicities of infection (MOI) of 1-0.01. After 2 hr of adsorption, the cells were washed and MdM were added at an effector to target ratio of between 3 and 5. At 2, 24, 48, and 96 hr post-infection (p.i.) cells were scraped off; cell-free medium was collected and TNF and IL-1 levels were determined by ELISA and RIA, respectively. MdM caused a 50-70 % reduction in the yield of *R. conorii* in HEp-2 cells as compared to the control (infected HEp-2 cells incubated without MdM). This reduction was more pronounced at MOI 0.1 and 0.01, than at MOI 1. In contrast, no reduction in the rickettsial yield was observed in the BGM cells incubated with MdM. TNF and IL-1 alpha levels in the cell-free medium from infected HEp-2 cells incubated with MdM were higher (2-5 fold) than those from infected BGM cells incubated with MdM. These data suggest the possibility that, and the mechanisms whereby, MdM may modulate rickettsia replication *in vivo.*

Key words: Spotted fever group rickettsia; macrophages; tumour necrosis factor; interleukin-1

Introduction

Rickettsia conorii (RC) and Israeli Spotted Fever (ISF) belong to the spotted fever group of rickettsiae, and are transmitted to humans by bites from ticks (Walker, 1989). The rickettsiae cause vasculitis by infecting the endothelial cells of small arteries, veins, and capillaries (Yagupsky *et al.,* 1989). Mononuclear phagocytes, including both tissue macrophages and their precursors, circulating blood monocytes, act as effective microbicidal host defense cells against many pathogenic microorganisms. They have been implicated in regulating

the functions of lymphoid and hematopoietic cells, and in most cases, these effects are mediated by soluble factors produced by circulating monocytes and tissue macrophages (Nathan, 1987). 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 acitivities, including interleukin 1 (IL-1), tumour necrosis factor alpha (TNF), and prostaglandin E_2 (PGE_2) (Morrison and Ulevitch, 1978; Mannel, 1986; Kunkel *et al.*, 1986; Auron *et al.*, 1987; Beutler and Cerami, 1988). These factors induce a multitude of biological responses of importance in homeostasis, in host defensive mechanisms, and, probably, in the pathogenesis of several diseases (Wood and Hamerman, 1985; Bernheim, 1986; Nerup *et al.*, 1987; Auron *et al.*, 1987; Beutler and Cerami, 1988).

In the present study, we examined whether effectors such as monocyte-derived macrophages (MdM) can alter the course of RC replication in infected cells and whether rickettsial infected cells can stimulate MdM to secrete TNF and IL-1.

Materials and Methods

Cells. BGM (an African green monkey kidney line) and HEp-2 cells (originating from human carcinoma of the larynx) (both obtained from Flow Lab., England) were grown in RPMI 1640 and in MEM, respectively, with glutamine and antibiotics (Biological Industries, Beit Haemek, Israel), and 10 % foetal calf serum (FCS) (Gibco Laboratories, Grand Island, N.Y.).

Human monocytes were prepared from heparinized blood of normal donors as described previously (Manor and Sarov, 1986). Monocytes grown in antibiotic free RPMI 1640 medium supplemented with 10 % heat inactivated foetal calf serum and 1 % glutamine and incubated at 37 °C in an atmosphere of 5 % CO_2 for 8-10 days were used as monocyte-derived macrophages (MdM).

Rickettsia propagation. Rickettsia propagations were carried out as previously described (Manor and Sarov, 1990).

Plaque assay for Rickettsiae. The plaque assay was performed as previously described (Manor and Sarov, 1990).

Rickettsial strains. RC, Casablanca strain, was kindly provided by Dr.C. Wisseman, Microbiology Department, University of Maryland, U.S.A.

Enzyme-linked immunosorbent assay (ELISA) for TNF. TNF concentrations were determined by ELISA (Biokine TNF Test Kit, T Cell Sciences, Inc., Cambridge, MA, U.S.A.).

ELISA for IL-1. IL-1 alpha concentrations were determined by ELISA (Endogen Inc. Boston).

The effects of MdM on RC replication. The replication of RC was examined as follows. HEp-2 cells ($2x10^4$ cells per well) were seeded in 96-well microdilution plates (Nunc). Two days later, the cells were infected at a multiplicity of infection (MOI) of 0.1-1. After 1.5 hr of adsorption at 37 °C, RC-infected cells were washed three times with RPMI 1640 to remove unadsorbed RC. Then, $1x10^5$ MdM [an effector target (ET) ratio 5:1] were added to the RC infected HEp-2 cells. Immediately after RC adsorption and at 24, 48, and 96 hr after infection, triplicate samples were removed from individual wells by scraping and frozen at -70 °C until TNF and IL-1 alpha were measured. Controls included HEp-2 and BGM cells infected with RC and uninfected HEp-2 and BGM cells treated with MdM.

Results and Discussion

The importance of macrophages in the host response to microbial infection has been demonstrated (Skamene and Gross, 1983). In general, two macrophage activities, intrinsic and extrinsic, have been defined. The first is the resistance to organism replication within macrophages, which has been found in many procaryotic and eucaryotic microorganisms (Armstrong and Hart, 1975; Nacy and Meltzer, 1979) and in many viral systems (Morgensen, 1979). Extrinsic activity was first demonstrated in viral systems and was defined as antiviral effects on other virus-infected cells (Morahan *et al.*, 1980; Morse and Morahan 1981; Leibson *et al.*, 1986). Morahan *et al.* (1980) and Morse *et al.* (1981) showed that addition of mouse peritoneal macrophages suppressed the growth of herpes simplex virus in infected cells. Leibson *et al.* (1986) showed a reduction in herpes simplex virus yield from human fibroblasts treated with human mononuclear cells. We have recently shown extrinsic activity of human macrophages on cytomegalovirus replication in human fibroblasts (Manor and Sarov, 1988*a*) and on *C. trachomatis* replication in HEp-2 cells (Manor and Sarov, 198*b*). The present study is the first demonstration of extrinsic activity upon the obligate intracellular parasite rickettsia.

MdM caused reductions in rickettsial yield in HEp-2 cells (Fig. 1), of 30–60 % and 55–80 % when the cells were infected at MOI 1 and MOI 0.1, respectively, but only a slight reduction or none at all in BGM cells. In general, the reduction in the rickettsial yield in HEp-2 cells was more pronounced in the HEp-2 cells infected with RC at MOI 0.1 than at MOI 1.

In the present study, it has been shown that direct contact of MdM with infected cells and factors such as IL-1 and TNF, which have been shown to be produced by macrophages (Mestan *et al.*, 1986) might play a role in the mechanism of MdM extrinsic activity. Both IL-1 and TNF levels were higher (2–5

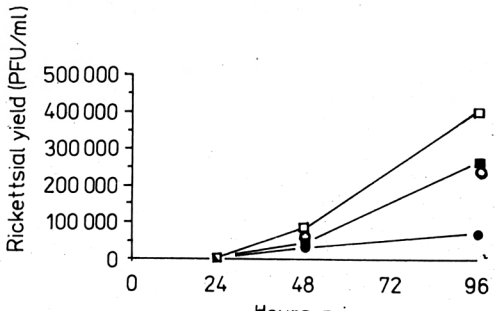

Fig. 1
Effect of MdM on rickettsial yield in HEp-2 or BGM cells infected with RC at a multiplicity of 0.1.MdM were added to HEp-2 or BGM cells at an E:T ratio of 5:1 immediately after rickettsial adsorption Control infected HEp-2 cells without MdM □; Infected HEp-2 cells with MdM ●; Control infected BGM cells without MdM ■; Infected BGM cells with MdM ○.

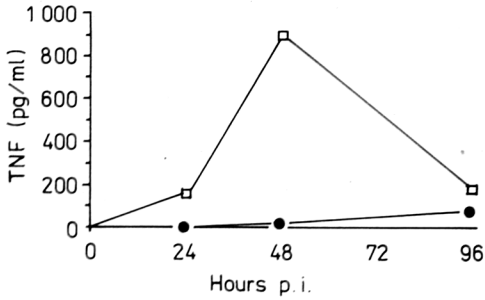

Fig. 2
TNF levels in the supernatant from BGM or HEp-2 cells infected with RC at MOI 1 and incubated with MdM at an E:T ratio of 5:1
TNF levels in supernatant medium from infected HEp-2 cells incubated with MdM □; TNF levels in supernatant from infected BGM incubated with MdM ●.

fold) in the supernatant from HEp-2 cells infected with RC and incubated with MdM than those from the supernatant of rickettsial infected BGM cells incubated with MdM (Figs. 2 and 3). The levels of TNF and IL-1 were higher when the cells (BGM and HEp-2 cells) were infected with RC at MOI 1 than at MOI 0.1 TNF levels reached the maximum level 48 h.p.i. (HEp-2 cells) and then declined while IL-1 levels increased gradually up to 96 h.p.i. An inhibitory activity of TNF has recently been shown against RC (Manor and Sarov, 1990), vesicular stomatitis virus, herpes simplex virus, encephalomyocarditis virus (Mestan *et al.*, 1986), *Trypanosoma cruzi* (De Titto *et al.*, 1986), and *C. trachomatis* in permissive cells (Shemer-Avni *et al.*, 1983). IL-1 like TNF can exert an antiviral effect (Billian, 1987), as well as antichlamydial effect (Shemer-Avni *et al.*, 1990).

In our system, it has been shown that in the HEp-2 cells, infected with rickettsia at MOI 1 and incubated with MdM, TNF levels reach maximum at 48 h.p.i. and then decline. Although some of the rickettsia particles are released at 48 hr h.p.i., most of the rickettsia are still in the cells, as observed by Gimenez stain (data not shown). Furthermore the TNF level at 24 h.p.i. was higher in the supernatant of cells inoculated with a higher MOI. These results might suggesst that there is a signal from the infected cells such as antigen presentation, which induces secretion of TNF from MdM at variable levels according to the intensity of the antigen presentation, which might differ from

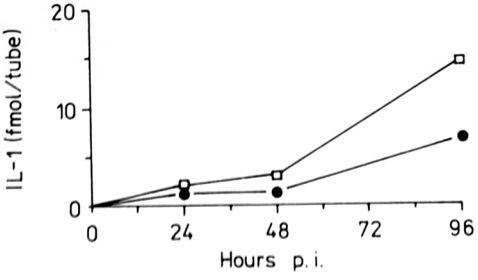

Fig. 3
IL-1 levels in supernatant from BGM or HEp-2 cells infected with RC at MOI 1 and incubated with MdM at an E:T ratio of 5:1
IL-1 levels in supernatant from infected HEp-2 cells incubated with MdM □. IL-1 levels in supernatant from infected BGM incubated with MdM ●.

one cell to another. The fact that TNF increase gradually in HEp-2 cells infected with rickettsia at low MOI supports this suggestion. Clearly further studies are required to explore this possibility.

The reduction in the rickettsial yield in HEp-2 cells but not in BGM cells incubated with MdM might be due to the higher level of TNF and IL-1 secreted by MdM when they were incubated with rickettsia infected HEp-2 cells compared to BGM. The fact that infected cells can stimulate MdM to secrete TNF or IL-1 suggests that *in vivo* not only the pathogen but the infected cells themselves might stimulate TNF and and IL-1 secretion which in turn might inhibit rickettsial growth and dissemination.

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