Opposite Effects of Bacterial Lipopolysaccharide on Fc-Receptor-Mediated Phagocytosis of Two Bone Marrow-Derived Macrophage Cell Lines, BDM-1 and BDM-1W3

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ABSTRACT. We have reported the isolation and characterization of three factor-dependent macrophage cell lines from bone marrow cells of C3H/HeN mice. We have since isolated a subclone, BDM-1W3, from one of these cell lines. We found previously that BDM-1W3 has a different sensitivity to bacterial lipopolysaccharide (LPS) for growth than its parental cell line, BDM-1. In this report, we show that LPS inhibits BDM-1W3 phagocytosis of antibody-coated sheep erythrocytes (Fc-mediated phagocytosis), whereas it enhanced Fc-mediated phagocytosis by BDM-1. It was observed that a loss of Fc-receptor capacity parallels a loss of phagocytic activity in LPS-treated BDM-1W3 cells. LPS stimulated phagocytosis of latex beads by BDM-1 and BDM-1W3, suggesting that Fc-mediated phagocytosis and phagocytosis of latex beads differ in their regulatory mechanisms. When BDM-1 cells were cultured with LPS, they underwent drastic morphological changes, whereas LPS-treated BDM-1W3 cells did not change significantly. Gamma interferon enhanced FC-mediated phagocytosis by BDM-1, while it had no significant effect on that by BDM-1W3. These cell lines should be useful for studying signal transduction mechanisms in LPS-mediated macrophage activation.
MATERIALS AND METHODS

Reagents. Lipopolysaccharide (LPS) from Salmonella typhi was purchased from Difco (Detroit, MI). Mouse recombinant interleukin 3 (IL-3) and IFN-γ were purchased from Genzyme (Boston, MA). Human recombinant macrophage colony-stimulating factor (M-CSF) was provided by Dr. S. Nagata (Osaka Bioscience Institute, Osaka, Japan), and M. Takahashi (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan).

Cells and culture media. L929 and WEHI-3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Flow Laboratories, Inc.) supplemented with 5% fetal calf serum (FCS, Hyclone). Conditioned media from L929 cells (LCM) and WEHI-3 cells (WEHI3CM) were prepared as described (16). The detailed properties of a factor-dependent macrophage cell line, BDM-1, have been reported (17). A subclone, BDM-1W3, was isolated from BDM-1. BDM-1 cells were plated in McCoy's 5A medium (M5A medium) (Flow Laboratories Inc., McLean, VA) supplemented with 20% FCS in the presence of calcium ionophore A23187 (25 ng/ml) (Sigma Chemical Co., St. Louis, MO), 0.6 μg/ml of 12-o-tetradecanoyl-phorbol-13-acetate (TPA) (LC Service Corp., Woburn, MA), and 50% WEHI3CM, and the cells were successively transferred every 5 days. The growth rate of the cells gradually decreased until 3 weeks in culture. After 3 weeks, however, a few cells survived and proliferated with a slow growth rate. In order to improve the growth rate, 10% LCM was added to the culture. As the cells grew out, distinct colonies were marked, picked out, and grown further with LCM in culture. One clone was subsequently cloned by limiting dilution in M5A medium with 20% FCS and 50% WEHI3CM and designated as BDM-1W3. After isolation, BDM-1W3 cells were cultured in M5A medium with 20% FCS and 50% WEHI3CM. BDM-1 cells were cultured in M5A medium with 20% FCS and 50% LCM.

Preparation of macrophages. Mouse peritoneal exudate macrophages (PEM) were obtained from C3H/HeN mice by injection of sterile proteose peptone broth (2.5 ml). Four days later, cells were collected from the peritoneal cavity with 5 ml of M5A medium, washed, suspended, and plated in M5A medium supplemented with 20% FCS and 25% LCM. The cells were cultured for 4 days and then used for the assay of phagocytosis. The cell populations contained more than 95% macrophages as determined by morphology and esterase staining.

Bone marrow cells were prepared from C3H/HeN mice by flushing the femurs with a 26-gauge needle and M5A medium. After washing by centrifugation, the cells were plated in M5A medium with 20% FCS and 25% LCM. After incubation for 9 days, adherent cells (BMM) were collected by trypsin treatment. The cell monolayers contained more than 95% macrophages.

Fc rosettes and Fc-mediated phagocytosis. The method used to opsonize sheep red blood cells (E [IgG]) was described before (16). For attachment of E [IgG], cells (5 x 10⁴ cells) were covered with a suspension of E [IgG] (3 x 10⁷/35-mm
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dish) and incubated for 60 min at 37°C. The dishes were washed in Dulbecco's phosphate-buffered saline (PBS) (Ca²⁺, Mg²⁺ free) for removal of free E[lgG]. The cells which attached more than four E[lgG] were counted as Fc-receptor positive cells. The phagocytosis of E[lgG] (Fc-mediated phagocytosis) was measured as described before (16). The cells (5 x 10⁴ cells/35-mm dish) were plated in MSA medium supplemented with 20% FCS and M-CSF in the presence or absence of LPS. After incubation, a suspension of E[lgG] was added to the cells and incubated for 60 min at 37°C. The extracellular erythrocytes were lysed by the addition of hypotonic DMEM (1:5, v/v in H₂O). After 20 sec, isotonicity was restored by the addition of DMEM, and the cells were fixed immediately in 2.5% glutaraldehyde in PBS. The number of erythrocytes ingested by a cell was counted in random fields; usually 200 cells were examined in each dish.

**Latex particle internalization.** The cells (5 x 10⁴) were plated onto 35-mm dishes in MSA medium with 20% FCS and M-CSF in the presence or absence of LPS, and were cultured for 3 days. After incubation, latex particles (0.8 µm) (Sigma Chemical Co.) were added to cell monolayers at 3 x 10⁶/dish, and the cells were incubated for 1 h at 37°C. The cells were washed 4 times with DMEM and fixed in 2.5% glutaraldehyde. Phagocytosis was quantified by counting intracellular particles in phase-contrast preparations of fixed macrophages. About 100 cells were examined in each dish, and the results were expressed in terms of the number of particles ingested per cell.

**Surface marker analysis.** Cells (1 x 10⁶ cells) in PBS containing 5% heat inactivated FCS and 0.1% NaN₃ were incubated with the monoclonal antibodies for 30 min on ice. They were washed 3 times with chilled PBS containing FCS and NaN₃, then were incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-rat IgG-F(ab⁰)₂ (Zymed Laboratories Inc., San Francisco, CA) for 30 min on ice. After washing, stained cells were analysed in a cell sorter (FACScan). Dead cells were excluded from analysis using 2 µg/ml of propidium iodine. Purified monoclonal antibody (14.8) specific for B cell lineage antigen B220 and a monoclonal antibody (N1/70.15.11.5) specific for macrophage antigen Mac-1 were obtained from Drs T. Takemori and T. Taniyama (National Institute of Health, Tokyo, Japan), respectively. The hybridoma supernatant containing monoclonal antibody against F4/80 antigen was purchased from Serotec (Oxford, U.K.).

![Graph](image_url)

**Fig. 2.** Effect of LPS on Fc-mediated phagocytosis by PEM, BMM, BDM-1, and BDM-1W3. PEM (A), BMM (B), BDM-1 (C), and BDM-1W3 cells (D) were plated in MSA medium supplemented with 20% FCS and human recombinant M-CSF (95 units/ml) at 5 x 10⁶ cells per 35-mm dish. Cells were incubated with (solid bars) or without LPS (10 µg/ml) (open bars) for 3 days; then phagocytic activities were measured. The number of erythrocytes ingested per cell was counted in random fields and 600 cells were examined.

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RESULTS

Characterization of cell surface markers. BDM-1 cells almost exclusively respond to M-CSF (17), while BDM-1W3 cells were dependent for growth on IL-3, GM-CSF, and M-CSF (K. Ohki, O. Kohashi, and A. Nagayama; manuscript in preparation). The two cell lines produce α-naphthyl butyrate esterase and do not produce myeloperoxidase (data not shown). To ensure that the two cell lines are of cells within the mononuclear phagocyte lineage, the cells were examined for surface markers characteristic of myeloid cells or macrophages. Mac-1 antigen is expressed on myeloid cells (20) and Ly-1 B cells (9). F4/80 antigen is a marker for the more mature stages of macrophage development (2, 10). As shown in Figure 1, the two cell lines expressed Mac-1 and F4/80 antigens. These results indicate that the BDM-1 and BDM-1W3 cell lines are from the mononuclear phagocyte lineage. Unexpectedly, the two cell lines expressed B220 antigen, which is restricted to B lineage cells (6). However, it should be noticed that the BDM-1W3 cells expressed more B220 antigen than the BDM-1 cells.

Effect of LPS on Fc-mediated phagocytosis. First we examined the effect of LPS on phagocytosis by peritoneal macrophages (PEM) and bone marrow-derived macrophages (BMM). As shown in Figure 2, LPS exerted an inhibitory effect on the phagocytosis by PEM, while a slight stimulation was observed with BMM. We then examined the effect of LPS on phagocytosis by BDM-1 and BDM-1W3. LPS-treated BDM-1 cells engulfed E [IgG] at a higher rate (22 particles/cell) than the untreated cells (9 particles/cell) (Fig. 2C). In contrast to BDM-1, LPS inhibited phagocytosis by BDM-1W3 (Fig. 2D). Phagocytosis by untreated cells (9 particles/cell) decreased after LPS-treatment of BDM-1W3 cells (3 particles/cell). These results indicate that LPS has opposite effects on phagocytosis by these two macrophage cell lines derived from bone marrow cells. To confirm this result, we further examined the effects of LPS on phagocytosis by these cell lines under various experimental conditions. LPS enhanced phagocytosis by BDM-1 dose dependently (Fig. 3A). The stimulation was significant at LPS concentrations as low as 10 ng/ml, and the extent of stimulation was not changed up to the concentration of 1 µg/ml. In contrast to BDM-1, phagocytosis by BDM-1W3 was not inhibited...
at 10 ng/ml; thus higher concentrations of LPS are required to inhibit phagocytosis in BDM-1W3 than to stimulate phagocytosis in BDM-1. A 15% reduction was evident at 100 ng/ml, and the inhibitory effect progressively increased in the presence of increasing concentrations of LPS. When cells were treated with a higher concentration of LPS (10 μg/ml), the stimulatory and inhibitory effects of LPS on phagocytosis increased. Therefore, LPS was routinely used at the concentration of 10 μg/ml.

We examined whether the amount of E [IgG] affected the LPS-induced changes in phagocytosis. The stimulation or inhibition of phagocytosis was observed at various concentrations of E [IgG] in BDM-1 and BDM-1W3 cells (Fig. 3B). Cells were treated with LPS, and their phagocytic activities were tested at different times. After a 24 h treatment, a slight increase in phagocytosis by BDM-1 cells was observed. Phagocytosis extensively increased during 24 to 48 h and then remained at the maximal level for the following 24 h (Fig. 3C). Since LPS-induced stimulation requires time for expression, it was assumed that protein synthesis is necessary for the changes induced by LPS. In fact, cycloheximide, a protein synthesis inhibitor, prevented the increase in phagocytosis by LPS-treated BDM-1 (data not shown). Phagocytosis by LPS-treated BDM-1W3 decreased to about half the initial level during the first 3 h and almost maximal inhibition was attained between 6 and 12 h (Fig. 3C). The phagocytic activity remained at the diminished level until 72 h of incubation. The ability of LPS to inhibit phagocytosis of BDM-1W3 was not simply due to cytotoxicity because LPS did not inhibit the proliferation of BDM-1W3 (K. Ohki, O. Kohashi and A. Nagayama; manuscript in preparation).

Effect of LPS on Fc-receptor capacity in BDM-1W3. The percentage of Fc-rosette-forming cells was decreased after LPS-treatment (Fig. 4): to approximately 65% during the first 12 h and then to only 37% at 72 h of incubation. The decline of cells expressing Fc-receptors paralleled the time course of LPS-induced inhibition of phagocytosis.

Effect of LPS on latex ingestion. Although BDM-1
cells engulfed latex beads at a high rate (18 beads/cell), LPS-treated cells engulfed them at an even higher rate (74 beads/cell) (Fig. 5). LPS slightly stimulated phagocytosis by BDM-1W3. These results indicate that Fc-mediated phagocytosis and phagocytosis of latex beads differ in their regulatory mechanisms.

**Effect of LPS on the morphology of BDM-1 and BDM-1W3 cells.** When BDM-1 cells were treated with LPS (10 µg/ml) for 6 days, drastic morphologic changes were observed (Figs. 6A, 6B). The LPS-treated cells were larger than the untreated cells, and some cells had long, thin pseudopods. The BDM-1W3 cells were rounded and adhered to dishes less tightly than did BDM-1 cells. LPS-treatment did not induce any significant morphological changes in BDM-1W3 cells (Figs. 6C, 6D).

![Phase-contrast microscopy of BDM-1 and BDM-1W3 cells.](image)

**Fig. 6.** Phase-contrast microscopy of BDM-1 and BDM-1W3 cells. Cells were cultured in M5A medium with 20% FCS and M-CSF (95 units/ml) for 6 days with or without LPS (10 µg/ml). A, BDM-1; B, LPS-treated BDM-1; C, BDM-1W3; D, LPS-treated BDM-1W3. Magnification: ×23.

![Effect of IFN-γ on phagocytosis by BDM-1 and BDM-1W3 cells.](image)

**Fig. 7.** Effect of IFN-γ on phagocytosis by BDM-1 and BDM-1W3 cells. Cells were plated in M5A medium in the presence of 20% FCS and M-CSF (380 units/ml) and treated with LPS (5 µg/ml) or IFN-γ (10 units/ml). After incubation for 48 h, phagocytic activities were measured. The data shown are the means of triplicate determinations (±S.D.).
Effect of IFN-γ on phagocytosis. We examined whether IFN-γ could modulate the phagocytic activities of BDM-1 and BDM-1W3. IFN-γ as well as LPS clearly stimulated Fc-mediated phagocytosis in BDM-1 cells (Fig. 7). When BDM-1W3 cells were treated with IFN-γ, no significant changes in phagocytic activity were observed. Furthermore, IFN-γ did not prevent the inhibition of phagocytosis by LPS-treated BDM-1W3 cells. These results indicate that the inhibition of phagocytosis induced by LPS in BDM-1W3 cells was not mimicked by IFN-γ.

DISCUSSION

We isolated and briefly characterized three factor-dependent macrophage cell lines (17). We have isolated a subclone, BDM-1W3, from one of these cell lines. The parent and subclone cells responded differently to CSFs with regard to growth. The parent, BDM-1, cells responded to M-CSF, but did not respond to IL-3 (17), whereas the BDM-1W3 cells responded to IL-3, GM-CSF, and M-CSF (K. Ohki, O. Kohashi and A. Nagayama; manuscript in preparation). Since M-CSF (CSF-1) is the only mononuclear phagocyte lineagespecific growth factor (21, 23), these cell lines belong to the mononuclear phagocyte lineage. To examine whether BDM-1 and BDM-1W3 cells are differently arrested within the lineage, we compared the surface markers on these cell lines. The two cell lines expressed Mac-1 and F4/80 antigens, and there was no significant difference in the expression of these antigens between them (Fig. 1). However, more B220 antigen was expressed on BDM-1W3 cells than on BDM-1 cells.

In this report, we have shown that LPS differently affected Fc-mediated phagocytosis by BDM-1 and BDM-1W3 cells. Different effects of LPS have been reported previously on phagocytosis by thioglycolate-elicted peritoneal macrophages and on bone marrow-derived macrophages (7, 8, 24). The phagocytosis of E[IgG] by mononuclear phagocytes was enhanced during the course of cell maturation (15). Recently it has been reported that LPS may induce responses by interacting with LPS binding protein in serum that then interacts with receptors. Alternatively, BDM-1W3 is immature compared with BDM-1. This interpretation is consistent with that obtained with the differential LPS sensitivities for BDM-1 and BDM-1W3.

Although the mechanisms by which LPS activates cellular responses have not been elucidated, LPS may induce different cellular changes in mature and immature mononuclear phagocytes. Thus, the macrophage cell lines in this report should be useful for studying the mechanisms of signal transduction induced by LPS.

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REFERENCES


