

Endotoxins of Enteric Pathogens Modulate the Functions of Human Neutrophils and Lymphocytes

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The locomotor responses of human peripheral blood neutrophils and lymphocytes were measured by the change from spherical to polarized shapes in the presence of endotoxins (lipopolysaccharide, LPS) of enteric pathogens: *S. dysenteriae* type 1, *V. cholerae* Inaba 569B, *S. typhimurium*, and *K. pneumoniae*. We reported earlier that these endotoxins are chemotactic factors for the neutrophils since they stimulated cell polarization within a few minutes of incubation. Endotoxins had an inhibitory effect upon neutrophil phagocytosis of opsonized yeast and the cells engulfed fewer yeasts. Interestingly, endotoxins increased neutrophil adhesion to clean glass surfaces, but stimulated the cells to exhibit increased random locomotion (chemokinesis) through cellulose nitrate filters and show an enhanced ability to reduce nitroblue tetrazolium (NBT) dye. Unlike neutrophils, lymphocytes direct from blood do not show polarized morphology towards chemotactic factors but the cells acquire locomotor capacity during 24-72 h culture with mitogens such as phytohemagglutinin (PHA), phorbol myristate acetate or concanavalin A. Stimulation of blood lymphocytes with endotoxins did not induce cell polarization in short-term but long-term culture resulted in an increase in the proportion of polarized cells that acquired locomotor morphologies. The majority of these cells were identified as esterase negative B-lymphocytes that migrated through filters. Despite the optimum time of incubation for each of these cell types being different, we found that lymphocytes respond to much lower concentrations of endotoxins than the neutrophils. These findings suggest that endotoxins of enteric pathogens modulate the functions of human blood neutrophils and lymphocytes.

Keywords: Endotoxin, Neutrophil function, Lymphocyte polarization, Lymphocyte phenotype

Introduction

Leukocytes are a heterogeneous group of cells that mediate immune responses. The complex biology of the neutrophils endows them with the ability to sense chemotactic factors, migration, and microbial killing (Sha'afi and Molski, 1988). Different workers have studied leukocyte functions by directly adding the whole bacteria or their antigenic components to the cells. Preincubation of human neutrophils with bacterial endotoxins or LPS produces significant depression of chemiluminescence, oxygen consumption, superoxide production, and bactericidal activity while enhancing nitro blue tetrazolium dye reduction (Proctor, 1979). The whole cells of *V. cholerae* 01, at different cell/bacteria ratios, induced a significant increase in the percentage of polarized neutrophils than induced by other bacteria such as *V. alginolyticus* and *S. typhi* (Valenza *et al.*, 1997). *V. cholerae* can bind to CD4⁺ and CD8⁺ T-cells and B-cells, and the percentage binding of *V. cholerae* to lymphocytes and monocytes was higher than other Gram-negative bacteria such as *Yersinia enterocolitica* and *Salmonella* spp. (Monno *et al.*, 1996). It has been shown that endotoxins bind to neutrophils, both *in vitro* and *in vivo*, resulting an alteration in neutrophil adhesive and locomotor properties (Wilson, 1985). In a recent study, we have shown that endotoxins of enteric pathogens are chemotactic factors for human neutrophils (Islam *et al.*, 2002).

LPS interacts with several cell surface proteins including CD14 when presented as a complex with serum LPS binding protein (LBP). It has been found that L-selectin is a neutrophil surface receptor for LPS and its binding can facilitate production of superoxide anion indicating that L-selectin can mediate both binding and activation of neutrophils (Malhotra *et al.*, 1996). In the presence of serum or LBP, LPS can trigger formation of CD14-CR3 complexes that dissociate as

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neutrophils attach to substrates (Zarewych, 1996). CR3 being an integral membrane protein, usually remains uniformly distributed on unstimulated cells but redistributes to the uropod and retraction fibers during cell locomotion (Francis, 1989). Further study showed that neutrophil adhesion to endothelium, a prerequisite for the process of chemotaxis, involves not only L-selectin but also β -2-integrins including CD11b/CD18 and subsequently cause damage to the endothelium (Finn *et al.*, 1994). LPS presented in complex with either serum proteins or CD14 failed to stimulate the ingestion of IgG coated red cells by neutrophils, however, the number of FcRs and their ability to bind ligand are not affected by treatment with LPS. It has been explained that in the neutrophils, interaction of CD14 with LPS generated intracellular signals that alter the ability of CD11b/CD18 to bind ligand, while the alteration is not sufficient to promote phagocytosis (Detmers, 1994).

It has been shown that both spleen and peripheral blood lymphocytes (PBL) of mice respond to endotoxin-stimulation by blastogenesis and antibody production (Grey *et al.*, 1972). In humans, endotoxin weekly stimulates DNA synthesis in lymphoid cells from spleen, lymph node, tonsil, and bone marrow (Ringden, 1976) but is ineffective in stimulating PBL to blast transformation (Greaves *et al.*, 1974; Ringden, 1976). The immunological effects of the cell wall LPS preparations of *V. cholerae* Inaba 569B, Ogawa NIH 41, and NAG 4715 strains obtained by the hot phenol-water procedure have been examined in mice and found that the LPS has potencies as B-cell mitogens, adjuvants, immunosuppressants, polyclonal B-cell activators, and phagocytic stimulants for macrophages (Nakano *et al.*, 1977). The *S. typhimurium* mitogen (not LPS) has been found to be a potent stimulus for murine B-cell proliferation (a process that showed minimal macrophage dependence) but has no significant effect on proliferation of T-cells (Brooks and Vitetta, 1987). However, lymphocyte proliferation to porins isolated from *S. typhimurium*, *S. typhi*, and *E. coli* have been examined by ^3H -thymidine incorporation assay and found that the porins are able to activate T-lymphocytes from mice immunized with *S. typhi* outer-membrane proteins (Gonzalez *et al.*, 1993). However, in a recent study, the growth-induced shape change of lymphocytes have been studied and found that human peripheral blood lymphocytes respond to endotoxins by acquiring locomotor morphologies in culture (Seheli *et al.*, 2002). In the present study, we investigated the effects of endotoxins of enteric pathogens on the functions of human neutrophils and lymphocytes by directly adding these to the cells and compared the doses of the stimuli and the optimal conditions required for each type of cells.

Materials and Methods

Source of endotoxins The endotoxin from *Shigella dysenteriae* type 1 was obtained by the phenol-water extraction procedure (Westphal and Jann, 1965), and those from other enteric pathogens

were procured from Sigma. Stock solutions of endotoxins were prepared in Hanks Balanced Salt Solution containing 10 mM morpholinopropane sulfonic acid (HBSS-MOPS) at 5 mg/ml and stored at -20°C until used. Different concentrations of endotoxins were freshly prepared from the stock prior to addition to the cells.

Isolation of neutrophils and lymphocytes Peripheral blood was drawn from normal, healthy human adults and the cells were purified by the standard procedure of dextran sedimentation followed by centrifugation on Ficoll-Hypaque. The MNCs comprising mostly of lymphocytes were collected from the interface and neutrophils were collected as cell pellet. These cells were washed separately with excess of HBSS-MOPS, twice, each time changing the tubes.

Polarization assays for neutrophils and lymphocytes

Neutrophils in HBSS-MOPS were taken in several tissue culture plastic tubes (Fisher Scientific, Fairlawn, USA) and different concentrations of endotoxins were added to the cells. N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) at 10^{-8} M was used as the standard chemotactic factor for the neutrophils in these experiments. The cells were incubated at 37°C , fixed with 2% glutaraldehyde, washed and then examined under the microscope. The details of the assay and scoring methods were described elsewhere (Shields and Haston, 1985; Islam *et al.*, 1997; Islam *et al.*, 2002). The polarized cells were expressed as the percentage of the total cells examined.

The MNCs consisting >90% lymphocytes (data presented in results) were used in polarization assays without further separation into lymphocytes and monocytes. In the short-term, the cells in HBSS-MOPS were incubated separately with different concentrations of endotoxins. Colchicine at different concentrations was used in these experiments as the positive inducer for polarization. For culture experiments, the medium was supplemented with heat-inactivated (56°C for 30 min) autologous plasma (20%), penicillin (100 IU/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and L-glutamine (2 mM), all procured from Sigma. Different concentrations of endotoxins starting from 10-50 $\mu\text{g}/\text{ml}$ were added to the cells. The effects of endotoxins toward lymphocyte polarization in culture were assessed and compared to those stimulated by the standard mitogen PHA (1 $\mu\text{g}/\text{ml}$, Sigma, St. Louis, USA).

Assay methods to study neutrophil functions

Neutrophils pretreated with endotoxins were placed on microscope slides and then allowed to phagocytose opsonized baker's yeast (*Saccharomyces cerevisiae*, pretreated with fresh autologous plasma and then washed) at 37°C , as described elsewhere (Islam *et al.*, 1997 and 2002). The cells attached to at least three yeasts were counted as phagocytic cells and the number of yeast bound to one hundred neutrophils were determined by counting 300 randomly selected cells. The details of the assay methods for adhesion and respiratory burst had been described in our previous publications.

Locomotion assays for neutrophils and lymphocytes

Neutrophils in HSA (Sigma) were allowed to migrate through cellulose nitrate filters (pore size: 3 μm , Millipore, Billerica, USA) in response to uniform 1 mg/ml concentration of different

endotoxins. Lymphocytes cultured for 48 h with endotoxins (20 µg/ml) were allowed to migrate through filters (pore size: 8 µm) in response to uniform endotoxins, as present in the culture filtrate together with other medium supplements. At the end of 90 minutes incubation for neutrophils and 4 h for lymphocytes at 37°C, the distance into the filter attained by the leading front of cells was measured.

Esterase staining of MNCs Mononuclear cells were stained for the presence or absence of α -naphthyl acetate esterase (ANAE, according to the procedure of Islam and Wilkinson, 1989; Seheli *et al.*, 2002) in order to identify them on the basis of their phenotype. Monocytes were scored as diffusely stained cells with reddish brown color while T-lymphocytes identified as showing discrete reddish brown spots in the cytoplasm. B-lymphocytes were scored as esterase negative since they could not give reaction with the substrate, α -naphthyl acetate (Sigma), due to lack of the enzyme in the cells.

Lymphocyte separation Lymphocytes were separated into T-enriched and B-enriched populations by passing the MNCs through nylon wool columns (Seheli *et al.*, 2002) and used in culture experiments. These cells were also stained for ANAE.

Statistical analysis Data analyses were carried out using the Statistical Package for Social Sciences (version 10.0 for Windows, SPSS Inc., Chicago, USA). A comparison of the two groups (treated with or without endotoxins) was carried out using *t*-test for paired samples. The differences were considered significant when *p* was ≤ 0.05 .

Results

Neutrophil polarization in endotoxins All the endotoxins of enteric pathogens presented in this study induced dose-dependent polarization in the neutrophils (Fig. 1). Endotoxins as little as 100 ng/ml induced cell polarization, and a substantial proportion of neutrophils, 22-45%, polarized at 100 µg/ml. The maximum proportions of neutrophils were polarized with the endotoxins at a concentration of 1 mg/ml and the polarized cells were 66% with the endotoxin from *K. pneumoniae*, 69% with that from *V. cholerae*, and 71% with that from *S. dysenteriae*. The endotoxin of *S. typhimurium* was less effective in inducing neutrophil polarization and some 50% of the cells became polarized at 2 mg/ml. The time course of polarization showed the optimum time for each of these endotoxins to be 30 min at 37°C, as reported earlier (Islam *et al.*, 2002).

Lymphocyte polarization in short- and long-term assays It was observed that about 15% of the lymphocytes straight from blood suspended in HBSS-MOPS became polarized during a 30 min assay while about 45% of the cells responded to colchicine at 10^{-5} M concentration. There was no short-term effect of endotoxins up to 500 µg/ml on lymphocyte

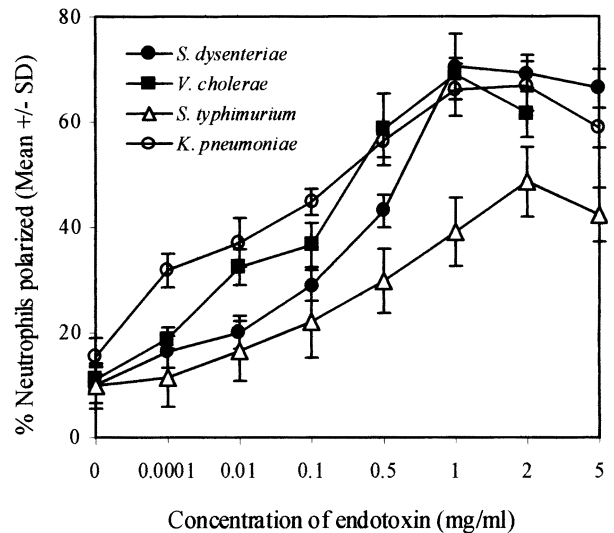


Fig. 1. Dose-response curves for human neutrophil polarization in endotoxins of enteric pathogens. The peak response was observed at 1 mg/ml when 66% of the cells were polarized with the endotoxin of *K. pneumoniae* (○), 69% with *V. cholerae* Inaba 596B (■), and 71% with *S. dysenteriae* type 1 (●). The optimum dose for the endotoxin of *S. typhimurium* was 2 mg/ml (△) when approximately 50% of the cells were polarized. The points on the ordinate represent the proportions of neutrophils polarized in the medium.

polarization (data not shown). In long-term experiments, lymphocytes were cultured in the presence of an activator of growth such as PHA that was used as a positive control. Endotoxins (10-50 µg/ml) were tested for their probable long-term effects on growth-induced shape change of lymphocytes. Lymphocytes did not show any appreciable change in morphology up to 50 µg/ml of endotoxins even after 6 h of incubation. Some cells showed polarized morphology while a number of them had unhealthy features or even died in culture in presence of 50 µg/ml endotoxins during 3-5 d in culture. For this reason, cells were cultured in the presence of 20 µg/ml endotoxins for a period of 72 h and the results are shown in Fig. 2. It was found that all of the endotoxins induced lymphocyte polarization in long-term assays. The morphology of the endotoxin-stimulated polarized lymphocytes was large and typical of locomotor cells with the appearance of constriction rings in the cell body. Lymphocytes assuming polarized morphologies in endotoxin-added cultures were actively changing shapes. Polarized morphology was found to be directly related to acquiring locomotor capacity of the lymphocytes as studied by locomotion assays.

Effects of endotoxins on neutrophil phagocytosis, adhesion, and NBT reduction Neutrophils pretreated with different endotoxins phagocytosing opsonized yeast varied from about 67-90% while the corresponding values for control cells were between 85-95%. It was found that all of the endotoxins significantly suppressed the proportions of the neutrophils

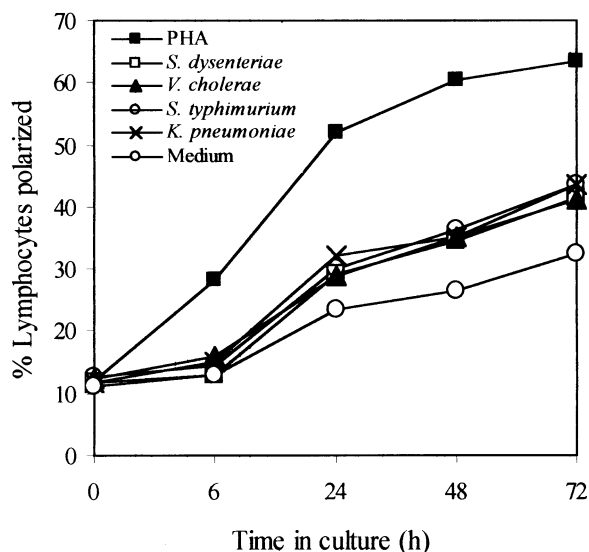


Fig. 2. Effects of endotoxins of *S. dysenteriae* type 1 (□), *V. cholerae* Inaba 569B (▲), *K. pneumoniae* (×), *S. typhimurium* (○), and the mitogen PHA (■) on human lymphocyte polarization in culture. The endotoxins used in culture experiments were at 20 µg/ml and PHA was at 1 µg/ml. The symbol (△) represents the proportions of lymphocytes polarized in the culture medium. Statistical analyses of the data showed that endotoxins significantly induced lymphocyte polarization as the values of *p* were ≤0.001 at 24, 48, and 72 h.

phagocytosing opsonized yeast ($p < 0.05$). Endotoxin-treated neutrophils were also found to bind to significantly fewer yeast particles ($p \leq 0.01$, Table 1). These results showed that endotoxins had an inhibitory effect on the phagocytic functions of the cells. A reason for this was elucidated by the study of cell adhesion since all of the endotoxins enhanced neutrophil adhesion to glass surfaces ($p \leq 0.01$) that might have suppressed their phagocytic function. Interestingly, the NBT dye reduction ability of the cells was significantly enhanced ($p \leq 0.005$) by the bacterial endotoxins (Table 1).

Effects of endotoxins on locomotion of neutrophils and lymphocytes The endotoxins at uniform 1 mg/ml concentration stimulated the neutrophils chemokinetically to

Table 2. Effects of endotoxins on locomotion of neutrophils and lymphocytes

| Cell pretreatment | Neutrophil locomotion | Lymphocyte locomotion |
|------------------------------|-----------------------|-----------------------|
| | Leading front (µm) | Leading front (µm) |
| Control | 59 ± 6 | 39 ± 5 |
| LPS of <i>S. dysenteriae</i> | 75 ± 9 ^a | 55 ± 4 |
| LPS of <i>V. cholerae</i> | 108 ± 9 ^a | 51 ± 12 ^c |
| LPS of <i>K. pneumoniae</i> | 77 ± 14 ^b | 46 ± 11 ^c |

Results are mean ± S.D. of 10 experiments for neutrophils and 7 experiments for lymphocytes. P values are control vs LPS.

^a $p \leq 0.005$; ^b $p < 0.01$; ^c $p \leq 0.05$.

migrate into filters during a 90 min assay (Table 2). Since lymphocytes did not respond to endotoxins in short-term polarization assays, the cells were cultured separately with the endotoxins for 48 h while a proportion of them acquired locomotor morphology; these cells were used in locomotion assays in the presence of uniform 20 µg/ml of the respective endotoxin. It was found that all the endotoxins stimulated the lymphocytes to exhibit increased random locomotion (chemokinesis) through filters (Table 2).

Phenotype of polarized lymphocytes Using ANAE staining for cell phenotype study, the results of 7 experiments showed that the MNCs straight from blood consisted of 67.2% (S.D. = 3.0) T-cells, 22.5% (S.D. = 2.2) B cells, and 10.1% (S.D. = 2.1) monocytes. These cells were used in long-term assays and the cultured cells were stained for ANAE. It was found that a substantial proportion of the nonadherent lymphocytes that came off the culture plates through washing had acquired locomotor morphologies during long-term culture with endotoxins, and these cells consisted of both T- and B-lymphocytes (Table 3). Further, lymphocytes were separated into T-enriched and B-enriched populations that consisted of 80.2% (S.D. = 3.9) T-cells and 41.8% (S.D. = 6.3) B-cells, respectively, in different experiments. Culture of these cells separately with endotoxins showed that compared to the proportions of polarized cells in the T-enriched

Table 1. Effects of endotoxins on neutrophil functions

| Neutrophil pretreatment | Phagocytosis | | Adhesion | Respiratory burst |
|------------------------------|--------------------------|---------------------------------------|---------------------|-------------------------------|
| | % Phagocytic neutrophils | No. of yeast bound to 100 neutrophils | % Adherent cells | NBT reduction, O.D. at 520 nm |
| Control | 90 ± 3 | 587 ± 58 | 59 ± 4 | 0.07 ± 0.02 |
| LPS of <i>S. dysenteriae</i> | 80 ± 8 ^c | 489 ± 115 ^b | 75 ± 9 ^b | 0.15 ± 0.02 ^a |
| LPS of <i>V. cholerae</i> | 77 ± 6 ^a | 464 ± 28 ^a | 72 ± 2 ^b | 0.16 ± 0.03 ^a |
| LPS of <i>K. pneumoniae</i> | 84 ± 4 ^b | 499 ± 21 ^a | 78 ± 7 ^a | 0.17 ± 0.04 ^a |
| LPS of <i>S. typhimurium</i> | 78 ± 9 ^a | 413 ± 47 ^a | 73 ± 6 ^a | not done |

Results are mean ± S.D. of 10 experiments. P values are control vs LPS.

^a $p \leq 0.005$; ^b $p \leq 0.01$; ^c $p < 0.05$.

Table 3. Phenotype of polarized mononuclear cells cultured with endotoxins

| Mononuclear cells cultured for 48 h | Percentage of polarized cells | Percentage of polarized cells identified by ANAE staining | | |
|-------------------------------------|-------------------------------|---|---------|-----------|
| | | T-cells | B-cells | Monocytes |
| Control | 27 ± 6 | 70 ± 2 | 24 ± 3 | 6 ± 1 |
| LPS of <i>S. dysenteriae</i> | 36 ± 5 | 65 ± 5 | 30 ± 4 | 5 ± 2 |
| LPS of <i>V. cholerae</i> | 33 ± 7 | 66 ± 3 | 28 ± 2 | 7 ± 1 |
| LPS of <i>K. pneumoniae</i> | 33 ± 6 | 65 ± 1 | 29 ± 2 | 7 ± 1 |
| LPS of <i>S. typhimurium</i> | 37 ± 8 | 64 ± 1 | 31 ± 5 | 6 ± 2 |

Results are mean ± S.D. of 8 experiments.

population, there were more polarized cells in the B-enriched population.

Discussion

The endotoxins of four enteric pathogens have been studied by their ability to induce morphological polarization in neutrophils and lymphocytes from healthy adults. Neutrophils showed polarized morphology towards these endotoxins in a dose- and time-dependent fashion that would indicate an early event followed by the subsequent chemotactic response of the cells, as reported earlier (Islam *et al.*, 2002). We found that except the inhibitory effect of the endotoxins on neutrophil phagocytosis of baker's yeast, other functions, for example, NBT dye reduction as measure of oxidative metabolism, including adhesion and locomotion of the cells are being enhanced (positively modulated) by endotoxins. We suggest that since endotoxins stimulate neutrophil adhesion to glass surfaces making firm adhesion points on the cell membrane with the substratum, this may have a negative effect on the phagocytic function by interfering with free movement of the cell membrane to bind yeast particles. Previous work suggest that the first step in migration is mediated by selectins on the surface of neutrophils, platelets and endothelial cells while subsequent firm adhesion requires the collaboration of neutrophil integrins and membrane expressed cellular adhesion molecule (Stickle, 1996). It has been found that endotoxins can directly affect neutrophils by increasing their expression of adhesion receptors and priming them for an enhanced oxidative burst after stimulation (Forehand *et al.*, 1989; Zimmerli *et al.*, 1990). Further, endotoxins may be suppressing phagocytosis (negative modulation) via their effects on the cytoskeleton network of the cells (Howard *et al.*, 1990). Our findings indicate that endotoxin-stimulated neutrophils appear to have an enhanced ability to sense and respond to changes in the extracellular environment and may therefore represent an important avenue in augmenting host defence.

Preadherence of neutrophils on fibronectin or the peptide arginine-glycine-aspartate-serine, but not laminin, has been found to prevent the LPS-induced reduction in oxidative receptor expression. Cross-linkage of intracellular Fcγ

receptors with monoclonal antibodies prior to exposure to LPS also prevented the LPS-induced oxidative reduction in Fc receptor expression, demonstrating that an important pathophysiologic property of LPS is to induce an intracellular oxidative-derived reduction in Fcγ receptor expression (Simms and D'Amico, 1994). In contrast to neutrophils, pre-exposing macrophages to Fc fragments, however, failed to reduce binding of *S. typhimurium*, thus eliminating a role of Fc receptor in this process. But macrophages pretreated with neutrophil elastase exhibited a diminished ability to bind *S. typhimurium*, suggesting involvement of complement receptor CR1 (Al-Bahry and Pistole, 1997).

Unlike neutrophils that started responding to microgram concentrations of endotoxins within a few minutes of incubation while the optimal dose being in the range of milligram, lymphocytes did not respond to endotoxins in the short-term. Also the optimal dose was as little as 20 µg/ml and the cells needed to culture for longer periods in order to respond to endotoxins. It has been known that activators of growth such as PHA or anti-CD3 monoclonal antibodies enhance the locomotor capacity of lymphocytes in culture (Wilkinson, 1986). We found that the endotoxins of the enteric pathogens exhibit similar effects since the proportions of lymphocytes acquiring locomotor morphologies increased in culture with endotoxins. It has been reported that activators that drive resting lymphocytes into the G₁ phase of growth also activate the locomotor capacity of the cells in G₁, possibly by stimulating expression of new genes and synthesis of new proteins necessary for locomotion (Wilkinson, 1986; Wilkinson and Higgins, 1987). Thus the long-term effects of endotoxins on lymphocytes may likewise be an effect of gene expression (Wilkinson *et al.*, 1988).

While the conventional view holds that LPS is ignored by T-cells, it has been found that administration of LPS to mice not only activates all B-cells, but also engages most CD4⁺ and CD8⁺ T-cells, as measured by the expression of the activation markers CD69 and CD25 and by size increase, followed by massive T-cell apoptosis in the days following LPS exposure (Castro *et al.*, 1998). Lymphocytes also play a pivotal role in endotoxin-induced neutrophil mediated effects as has been found that there is a necessary requirement of both LPS and T-cells or their products (other than IFN-γ) for enhanced PMN-mediated endothelial cytotoxicity (Tennenberg, 1997). Similar

to this observation, we found that while the nonseparated MNCs were cultured with endotoxins the polarized cells consisted of both T- and B-cells although there were more B-cells than T-cells. However, using B-enriched populations of lymphocytes comprising of about 42% B-cells in culture experiments, there were consistently more B-cells in the polarized population although the increase was not significant (results not shown), suggesting the requirement of T-cells or their products for the effects of endotoxins on B-cells. In conclusion, findings of this study suggest that endotoxins preferentially stimulate the B-lymphocytes, and thus modulate the functions of human neutrophils and lymphocytes.

References

- Al-Bahry, S. N. and Pistole, T. G. (1997) Adherence of *Salmonella typhimurium* to murine peritoneal macrophages is mediated by lipopolysaccharide and complement receptors. *Zentralbl. Bakteriol.* **286**, 83-92.
- Brooks, K. H. and Vitetta, E. S. (1987) Activation of murine B cells with *Salmonella typhimurium* mitogen (STM), lipopolysaccharide (LPS), and dextran sulfate (DxS). I. Cell cycle analysis and induction of cytoplasmic immunoglobulin. *J. Mol. Cell Immunol.* **3**, 215-219.
- Castro, A., Bemer, V., Nobrega, A., Coutinho, A. and Truffa-Bachi, P. (1998) Administration to mouse of endotoxin from gram-negative bacteria leads to activation and apoptosis of T lymphocytes. *Eur. J. Immunol.* **28**, 488-495.
- Detmers, P. A., Zhou, D. and Powell, D. E. (1994). Different signaling pathways for CD18-mediated adhesion and Fc-mediated phagocytosis. Response of neutrophils to LPS. *J. Immunol.* **153**, 2137-2145.
- Finn, A., Strobel, S., Levin, M. and Klein, N. (1994) Endotoxin-induced neutrophil adherence to endothelium: relationship to CD11b/CD18 and L-selectin expression and matrix disruption. *Ann. N. Y. Acad. Sci.* **725**, 173-182.
- Forehand, J. R., Pabst, M. J., Phillips, W. A. and Johnston Jr., R. B. (1989) Lipopolysaccharide priming of human neutrophils for an enhanced respiratory burst. Role of intracellular free calcium. *J. Clin. Invest.* **83**, 74-83.
- Francis, J. W., Todd, R. F. 3rd, Boxer, L. A. and Petty, H. R. (1989) Sequential expression of surface C3b receptors during neutrophil locomotion. *J. Cell Physiol.* **140**, 519-523.
- Gonzalez, C. R., Isibasi, A., Ortiz-Navarrete, V., Paniagua, J., Garcia, J. A., Blanco, F. and Kumate, J. (1993) Lymphocytic proliferative response to outer-membrane proteins isolated from *Salmonella*. *Microbiol. Immunol.* **37**, 793-799.
- Greaves, M., Jannosy, G. and Doenhoff, M. (1974) Selective triggering of human T- and B-lymphocytes *in vitro* by polyclonal mitogens. *J. Exp. Med.* **140**, 1-18.
- Grey, I., Krueger, K. and Spiesel, S. Z. (1972) Stimulation of B-lymphocytes by endotoxin. *J. Immunol.* **108**, 1088-1091.
- Howard, T. H., Wang, D. and Berkow, R. L. (1990) Lipopolysaccharide modulates chemotactic peptide induced actin polymerization in neutrophils. *J. Leukoc. Biol.* **47**, 13-24.
- Islam, L. N., Ahmed, K. M. and Shamy, S. (1997) Modulation of human neutrophil function by *Vibrio cholerae* lipopolysaccharide. *Bangladesh J. Biochem.* **3**, 75-86.
- Islam, L. N., Nabi, A. H. M. N., Ahmed, K. M. and Sultana, N. (2002) Endotoxins of enteric pathogens are chemotactic factors for human neutrophils. *J. Biochem. Mol. Biol.* **30**, 482-487.
- Islam, L. N. and Wilkinson, P. C. (1989) Evaluation of methods for isolating human peripheral blood monocytes. Studies on chemotactic locomotion and other functional characteristics. *J. Immunol. Meth.* **121**, 75-84.
- Malhotra, R., Priest, R. and Bird, M. I. (1996) Role for L-selectin in lipopolysaccharide-induced activation of neutrophils. *J. Biochem.* **320**, 589-593.
- Monno, R., Valenza, M. A., Panaro, M. A., Lisi, S., Marcuccio, L., De Vito, D. and Mitolo, V. (1996) Spontaneous binding of *Vibrio cholerae* to human leucocytes. *Microbios.* **88**, 169-176.
- Nakano, M., Tanabe, M. J., Hori, H., Kondo, S. and Hisatsune, K. (1977) Immunological properties of *Vibrio cholerae* lipopolysaccharides. *Microbiol. Immunol.* **21**, 611-619.
- Proctor, R. A. (1979) Endotoxin *in vitro* interactions with human neutrophils: depression of chemiluminescence, oxygen consumption, superoxide production, and killing. *Infect. Immun.* **25**, 912-921.
- Ringden, O. (1976) Activation of human lymphocyte subpopulations by rabbit anti human β_2 -microglobulin and by lipopolysaccharide. *Scand. J. Immunol.* **5**, 891-900.
- Seheli, K., Kanta, H. Y. and Islam, L. N. (2002) Activation by mitogens and endotoxins of human peripheral blood lymphocytes: morphological polarization, locomotion and cell type study. *Dhaka Univ. J. Biol. Sci.* **11**, 49-58.
- Sha'afi, R. I. and Molski, T. F. (1988) Activation of the neutrophil. *Prog. Allergy* **42**, 1-64.
- Shields, J. M. and Haston, W. S. (1985) Behaviour of neutrophil leukocytes in uniform concentrations of chemotactic factors: contraction waves, cell polarity and persistence. *J. Cell Sci.* **74**, 75-93.
- Simms, H. H. and D'Amico, R. (1994) Regulation of intracellular polymorphonuclear leukocyte Fc receptors by lipopolysaccharide. *Cell. Immunol.* **157**, 525-541.
- Stickle, J. E. (1996) The neutrophil. Function, disorders and testing. *Vet. Clin. North. Am. Small Anim. Pract.* **26**, 1013-1021.
- Tennenberg, S. D. and Weller, J. J. (1997) Endotoxin-induced neutrophil-mediated endothelial cytotoxicity is enhanced by T-lymphocytes. *J. Surg. Res.* **69**, 11-13.
- Valenza, M. A., Monno, R., De Vito, D., Mitolo, V., Lisi, S., Marcuccio, C., Marcuccio, L. and Panaro, M. A. (1997) Polarizing response of human polymorphonuclear cells to *Vibrio cholerae*. *Cytobios.* **90**, 193-201.
- Westphal, O. and Jann, K. (1965) Bacterial lipopolysaccharides: extraction with phenol water and further application of the procedure; in *Methods in Carbohydrate Chemistry*, Whister, R. L. and Wolfrom, M. L. (eds.), Vol. **5**, p. 88, Academic Press Inc., New York, USA.
- Wilkinson, P. C. (1986) The locomotor capacity of human lymphocytes and its enhancement by cell growth. *Immunol.* **57**, 281-289.
- Wilkinson, P. C. and Higgins, A. (1987) OKT3-activated locomotion of human blood lymphocytes: a phenomenon requiring contact of T cells with Fc receptor-bearing cells. *Immunol.* **60**, 445-451.
- Wilkinson, P. C., Lackie, J. M., Haston, W. S. and Islam, L. N. (1988) Effects of phorbol esters on shape and locomotion of

- human blood lymphocytes. *J. Cell Sci.* **90**, 645-655.
- Wilson, M. E. (1985) Effects of bacterial endotoxins on neutrophil function. *Rev. Infect. Dis.* **7**, 404-418.
- Zarewych, D. M., Kindzelskii, A. L., Todd, R. F. 3rd and Petty, H. R. (1996) LPS induces CD14 association with complement receptor type 3, which is reversed by neutrophil adhesion. *J. Immunol.* **156**, 430-433.
- Zimmerli, W., Reber, A. M. and Dahinden, C. A. (1990) The role of formylpeptide receptors, C5a receptors, and cytosolic-free calcium in neutrophil priming. *J. Infect. Dis.* **161**, 242-249.