Suppression of C3H/HeJ Cell Activation by Lipopolysaccharide Endotoxin

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Earlier studies in our laboratory showed that the lipopolysaccharide (LPS) of Salmonella typhi, which fails to activate B lymphocytes of C3H/HeJ mice, can suppress proliferation and polyclonal antibody synthesis by these cells when they are stimulated by polyclonal activators. In order to determine what stage of the cell cycle was blocked, resting B cells from C3H/HeJ spleens were activated by using different mitogens in the presence of inhibitory concentrations of LPS and analyzed by flow cytometry, using acridine orange to stain DNA and RNA. LPS was found to inhibit the progression of cells into the G1 stage of the cell cycle. Furthermore, 3H]uridine uptake studies showed that RNA synthesis is inhibited during the early phase of activation. These results indicate that inhibition by LPS of the signalling process occurs during a critical period of the cell cycle when the cells become susceptible to the inhibitory effects of LPS. To examine whether LPS acts only on B cells or whether it can suppress other immunocompetent cells from C3H/HeJ mice, studies were carried out on activated thymocytes and macrophages. LPS was found to inhibit thymocyte proliferation stimulated by concanavalin A or the combination of phorbol myristate acetate and ionomycin. Prostaglandin E2 synthesis by macrophages was also blocked by LPS. Thus, LPS is a potent inhibitor of the functioning of the major immunocompetent cells of C3H/HeJ mice.

In previous experiments designed to elucidate the immunoregulatory properties of endotoxin-associated protein (EP) and other polyclonal B-cell activators, we found that when lipopolysaccharide (LPS) was used in conjunction with these cell stimulators, activation of nonresponder C3H/HeJ splenic B lymphocytes was actually inhibited by the LPS (13). No evidence was found that the LPS-stimulated suppressor T cells or macrophages of the C3H/HeJ mouse, which would serve to shut off stimulated B-cell proliferation or polyclonal antibody synthesis by some means. Our working hypothesis, therefore, has been that the observed suppression was the result of a biochemical event(s) in C3H/HeJ B cells that resulted in the interruption of the normal signalling mechanism. The experiments described below are an extension of our original study and serve to emphasize that this suppressive phenomenon blocks progression of most of the EP-activated B cells through the cell cycle by inhibiting the cells from entering into the G1 phase. Furthermore, LPS inhibits other C3H/HeJ cell types, including T cells and macrophages. Consequently, C3H/HeJ cells, which do not respond to wild-type smooth LPS or lipid A, are also susceptible to a process initiated by LPS which turns off activation by other stimulators.

MATERIALS AND METHODS

Animals. C3H/HeJ mice were obtained from the National Cancer Institute (Frederick, Md.). Mice of either sex were used separately at 4 to 6 months of age and maintained on water and Purina Chow ad libitum.

Materials. LPS was prepared by the method by Westphal et al. (18), using as a starting material trichloroacetic acid-extracted endotoxin from Salmonella typhi O:901 (15). Phenol extraction was conducted twice on the aqueous layer containing the LPS followed by ultracentrifugation of the LPS at 100,000 × g for 4 h to remove any contaminating nucleic acid. Amino acid analysis of the purified LPS was performed in the laboratory of Julie Rushbrook of our institution, using an Applied Biosystems 130A amino acid analyzer. The LPS typically contained less than 0.3% peptides. EP was prepared from S. typhi O:901 as described earlier (15). Phorbol myristate acetate (PMA) was obtained from Calbiochem (San Diego, Calif.), acridine orange was obtained from Polyscience Inc., (Warrington, Pa.), and ionomycin was obtained from Sigma Chemical Co. (St. Louis, Mo.). The mouse T-cell recovery kit was obtained from Biotex Laboratories, Inc., (Edmonton, Canada). The prostaglandin E2 (PGE2) enzyme-linked immunosorbent assay (ELISA) kit was obtained from Cayman Chemical (Ann Arbor, Mich.).

Purification of B cells. Mice were sacrificed by cervical dislocation, and single-cell suspensions were prepared from the spleens as previously described (15). The erythrocytes were lysed by Tris-ammonium chloride treatment, and T cells were depleted by treatment with anti-Thy 1.2 monoclonal antibodies (Sigma) and rabbit Low-Tox complement (Cedarlane Laboratory, Hornby, Canada). Dense resting B cells were obtained by Percoll (Pharmacia, Uppsala, Sweden) density gradient separation of the T cell-depleted suspensions. Cells in the band showing a density of 1.076 to 1.08 were selected and washed twice with RPMI 1640 medium supplemented with 5% fetal calf serum (FCS), 100 U of penicillin per ml, and 100 μg of streptomycin per ml. By functional tests for T cells using the concanavalin A (ConA) mitogenicity assay, such B-cell preparations routinely were >98% pure.

Purification of T cells. Single-cell suspensions were pre-
pared from spleens. Erythrocytes were lysed by treatment with Tris-ammonium chloride, and T cells were obtained by passage through Biotex mouse T-cell recovery columns. Cells were washed twice with RPMI 1640 culture medium containing 5% FCS before being cultured in microtiter plates.

**Macrophage stimulation.** Resident peritoneal macrophages were obtained by washing the peritoneal cavity with RPMI 1640 culture medium containing 10% FCS. The peritoneal exudate cells (4 × 10^6/ml) were incubated in 1-ml volumes in 24-well flat-bottom plates for 2 h, washed free of nonadhering cells, and cultured with an appropriate stimulant for 24 h. The supernatants were then recovered, centrifuged to remove any cells, and stored at −20°C until assayed for PGE2.

**DNA and RNA synthesis.** Purified resting B cells were cultured in 96-well U-bottom microtiter plates (Falcon Labs, Lincoln Park, N.J.) at 2.5 × 10^9 cells per well in 0.2 ml of RPMI 1640 culture medium supplemented with 5% FCS (GIBCO, Grand Island, N.Y.). Resting B cells were stimulated with EP or other mitogens in the presence or absence of LPS, which was added at various times to the cultures as indicated for the experiment. The cells were incubated at 37°C in 10% CO2 for 48 h. DNA synthesis was measured by [³H]thymidine incorporation by adding 1 μCi of [³H]thymidine (ICN Biochemicals, Costa Mesa, Calif.) in the last 18 h of incubation. For RNA synthesis, resting B cells were cultured in a volume of 1 ml in tubes at 2.5 × 10^6 cells per ml, and [³H]uridine (ICN Biochemicals) incorporation was measured by adding 1 μCi of [³H]uridine at various times as described for the experiment. The cultures were harvested onto glass fiber filters by using a cell harvester (Brandel, Gaithersburg, Md.), and [³H]thymidine and [³H]uridine incorporation was measured with a Beckman liquid scintillation counter (model 6000 IC).

Thymocytes (2.5 × 10^9 per well) or T cells were cultured in RPMI 1640 culture medium containing 10% FCS, 100 U of penicillin and 100 μg of streptomycin per ml, and 10^-5 M mercaptoethanol with the appropriate stimulant for 72 h. DNA synthesis was measured by [³H]thymidine incorporation as described above. In all experiments variation about the mean of triplicate cultures did not exceed ±10%. Unless otherwise noted, experiments were repeated at least three times.

**Cell cycle analysis by cytofluorometry.** The cell cycle analysis was done by measuring cellular RNA and DNA content, using acridine orange dye, by the method of Darzynkiewicz et al. (2). Acridine orange binding to double-stranded DNA results in green (530-nm) fluorescence, while binding to single-stranded RNA gives red (>600-nm) fluorescence. Purified resting B cells (2.5 × 10^6/ml) from C3H/HeJ mice were cultured in medium as described above with various mitogens and with or without LPS. At specific time intervals between 0 and 66 h, 1 ml of FCS was added to the culture. The cells were then fixed with 6 ml of 70% cold ethanol and kept at 4°C until analyzed. The flow cytometric analysis was done using an argon ion laser (388 nm) for excitation and measuring green and red fluorescence with a Cytofluorograph HIS (Ortho Instruments, Westwood, Mass.) linked to a model 2140 computer (Ortho Instruments). The fluorescence from 10,000 cells was measured. The numbers of cells in various phases of the cell cycle were determined according to the DNA and RNA content of the cells. Replicate determination for 10,000 cells from the same sample were in agreement within 1%. Duplicate cultures deviated less than 5% from the mean.

**Assay for PGE2.** PGE2 in the macrophage culture supernatant was estimated with an ELISA kit (Cayman Chemical). In brief, a 96-well microtiter plate was coated with mouse antibolin antibody. Wells were then washed, and to each well 50 μl of each of the following was added: dilutions of the culture supernatants, acetylcholinesterase-labeled PGE2, and anti-PGE2 antibody. Plates were incubated overnight at room temperature and washed, and then a constant amount of acetylcholine derivative was added and the color was allowed to develop for 3 h. The color change was read at a 420-nm wavelength with a microtiter reader (ARTEK Systems Corp.), and PGE2 was estimated by using a standard curve that was prepared by using known amounts of pure PGE2.

**Cytotoxicity assay.** Cell viability was measured by vital dye staining with eosin Y. C3H/HeJ spleen cells or thymocytes were suspended at 2.5 × 10^6 cells per ml in culture medium. Cells were cultured with or without stimulants at the appropriate concentrations and LPS at 100 μg/ml. After 24 h of culture, 10 replicate cultures per group were pooled and the cells were centrifuged. The cell pellet was resuspended in 1 ml of medium, and 0.1 ml was added to an equal volume of a solution of 0.1% eosin Y in 3% FCS. In this manner, a sufficient number of cells were available to assess viability by direct counting in a hemocytometer chamber.

**RESULTS**

**Suppression of DNA synthesis in activated B lymphocytes.** When resting B cells of C3H/HeJ mice are cultured in the presence of various concentrations of EP a dose response is obtained as shown in Fig. 1. However, when various concentrations of LPS are added at the initiation of the culture in the presence of EP, suppression of DNA synthesis likewise occurs in a dose-dependent manner (Fig. 1). Aside from the fact that C3H/HeJ mice and their cells are highly resistant to the toxic effects of LPS, the concentrations of LPS which suppress DNA synthesis in C3H/HeJ B cells act to stimulate DNA synthesis in LPS responder B cells from C3H/OuJ mice (data not shown). In addition, direct measurements of the viability of C3H/HeJ spleen cells and thymocytes after exposure to a highly suppressive concentration of LPS showed no increase in cell death over the controls. After 24 h of culture, neither the total cells recov-
The stimulation index is the ratio of PMA- or ionomycin-stimulated (B) cells by LPS. B cells (2.5 x 10⁶/0.2 ml) were stimulated with EP (100 ng) or PMA (50 ng) and ionomycin (1 μM) for 48 h. LPS (50 μg) was added at the initiation of culture or 12 h after the stimulation with mitogens. DNA synthesis was measured by [³H]thymidine incorporation. The stimulation index is the ratio of experimental to control counts per minute of [³H]thymidine incorporation.

**Effect of LPS on PKC-mediated DNA synthesis.** Recent work in our laboratory has shown that DNA and RNA synthesis stimulated by EP in C3H/HeJ B cells is mediated to a large extent by protein kinase C (PKC) activation (1). Since the synergistic combination of PMA and the Ca²⁺-ionophore ionomycin activates PKC and drives both B and T cells to synthesize DNA (1, 8), we chose to determine whether LPS would affect PKC-mediated DNA synthesis in C3H/HeJ B cells. The results depicted in Fig. 2 show that the suppression of DNA synthesis in B cells stimulated by PMA and ionomycin (Fig. 2B) follows a pattern similar to that seen in EP-activated B cells (Fig. 2A). When LPS is added at the initiation of the cell culture (0 h), about 66% inhibition occurs in PKC-activated cells compared with approximately 70% inhibition in cells stimulated with EP. Substantial inhibition was observed (40 and 55%, respectively) when LPS was added to both types of cultures after 12 h of activation, indicating that some cells in the G1 phase of the cell cycle that were stimulated either by PMA and ionomycin or by EP may still be susceptible to the shutdown and therefore cannot enter the S phase.

**LPS inhibition of B-cell progression through the cell cycle.** In order to determine when in the cell cycle the inhibitory effect of LPS on B cells took place, we used cytofluorometry to assess the relative amounts of RNA and DNA per cell. By this method, we could establish the stage of the cell cycle through which the cells were progressing in the presence of the EP mitogen when they were blocked with LPS that was added at the initiation of the culture. As shown in Fig. 3, resting B cells activated by EP show a steady increase in the percentage of cells in G1 beginning at 12 h; however, for cells exposed to LPS, inhibition of the percentage of cells entering the G1 phase was seen first at 12 h. This inhibition persisted at later time points, and as a consequence, fewer cells initiated DNA synthesis when exposed to LPS, as shown by the differences in the percentages of cells in the S phase (Fig. 3B).

**LPS suppression of RNA synthesis in B cells.** To confirm the inhibitory effect of LPS on the progression of C3H/HeJ B cells through G1, we measured RNA synthesis in EP-activated cells exposed to LPS. As shown in Table 1, when resting B cells were exposed to EP and LPS at the initiation of the microtiter plate culture, [³H]uridine incorporation was significantly reduced within the first 12 h of the culture period, as well as at later times up to 24 h, before most of the cells engage in DNA synthesis.

**LPS suppression of thymocyte activation.** In view of the definite suppressive activity of LPS for C3H/HeJ B cells, the question which naturally followed was whether this inhibi-

![FIG. 2. Inhibition of DNA synthesis in EP-stimulated (A) and PMA- plus ionomycin-stimulated (B) B cells by LPS. B cells (2.5 x 10⁶/0.2 ml) were stimulated with EP (100 ng) or PMA (50 ng) and ionomycin (1 μM) for 48 h. LPS (50 μg) was added at the initiation of culture or 12 h after the stimulation with mitogens. DNA synthesis was measured by [³H]thymidine incorporation. The stimulation index is the ratio of experimental to control counts per minute of [³H]thymidine incorporation.](http://iai.asm.org/)

![FIG. 3. Effect of LPS on progression of EP-stimulated resting B cells through the cell cycle. B cells (2.5 x 10⁶/ml) were stimulated with EP (1 μg/ml) in the presence (■) or absence (□) of LPS (250 μg/ml) at 37°C. Simultaneous quantitation of DNA and RNA in situ was done by staining Triton X-100-permeabilized cells with acridine orange as described in Materials and Methods. Fluorescence of individual cells was measured in a Cytofluorograph IIS, and the percentages of cells in the various phases of the cell cycle were determined based on the RNA and DNA content per cell. (A) Percent cells in G1 phase; (B) percent cells in S phase.](http://iai.asm.org/)
tion was limited to B cells or extended to other cell types of the immune system. When thymocytes were cultured with the T-cell mitogen ConA or with PMA and ionomycin, DNA synthesis was obtained in the usual fashion; however, in the presence of LPS, inhibition occurred (Table 2). Of interest is the fact that with higher levels of activation with either ConA or with PMA and ionomycin, less inhibition was observed with a constant amount of LPS. Similar results were obtained with purified splenic T cells (data not shown).

**LPS suppression of macrophage activation.** Our previous studies (13, 14) have shown that certain R types of LPS, including *Salmonella minnesota* R595 LPS (5), can stimulate to a limited degree the proliferation of B cells and interleukin-1 production by macrophages of C3H/HeJ mice. In order to determine whether the wild-type smooth LPS could inhibit C3H/HeJ macrophage activation, we employed a sensitive assay for the production of another macrophage product, PGE2. As shown in Fig. 4, R595 LPS activated PGE2 production in C3H/HeJ macrophages in a dose-response fashion, with a maximum stimulation index of about 26 at 50 μg/ml. However, in the presence of a smooth *Salmonella typhi* LPS, PGE2 production stimulated by R595 LPS was strikingly reduced. Therefore, not only are the biochemical events associated with the proliferation of C3H/HeJ B and T cells blocked by smooth LPS but the conversion of arachidonic acid to PGE2 in C3H/HeJ macrophages is also blocked.

**TABLE 1.** Suppression of RNA synthesis by LPS in EP-activated resting B lymphocytes

<table>
<thead>
<tr>
<th>EP (1 μg/ml) LPS (250 μg/ml)</th>
<th>Pulse time of [3H]uridine incorporation (total % cpm ± SEM)</th>
<th>% Inhibitionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>−</td>
<td>8,043 ± 741</td>
</tr>
<tr>
<td>+</td>
<td>0 − 12 h</td>
<td>11,006 ± 92</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>8,637 ± 303</td>
</tr>
<tr>
<td>+</td>
<td>12 − 24 h</td>
<td>17,298 ± 904</td>
</tr>
<tr>
<td>+</td>
<td>12 − 24 h</td>
<td>11,596 ± 302</td>
</tr>
</tbody>
</table>

 a C3H/HeJ resting B cells (2.5 × 10⁶/ml) were cultured in tubes.

**FIG. 4.** Effect of *S. typhi* LPS on production of PGE2 by C3H/HeJ peritoneal macrophages stimulated by *S. minnesota* R595 LPS. Culture supernatants were assayed for PGE2 by ELISA after 24 h of culture in the absence of *S. typhi* LPS (○) in the presence of 50 μg of *S. typhi* LPS (□). The stimulation index represents the ratio of the PGE2 produced by stimulated cells to the PGE2 produced by unstimulated controls.

**DISCUSSION**

In our previous studies dealing with the phenomenon of LPS suppression of C3H/HeJ splenic B lymphocytes activated by protein mitogens, we found that these cells appeared most susceptible to inhibition when they were in an activated state (13). However, the cell cultures consisted of a mixture of B cells, T cells, and macrophages, which suggested the possibility of accessory cells playing a role in the suppression observed. Consequently, T cells were depleted by using anti-Thy 1.2 antibody and complement. Reduction of the inhibitory effect was not observed in T-cell-depleted cultures, and in fact, the level of inhibition was enhanced as much as 30% when R595 LPS was used as a stimulant and smooth LPS from *S. typhi* O-910 was used as an inhibitor (14). Furthermore, triple adsorption with carbonyl iron to remove adherent macrophages from spleen cell suspensions also had no effect on the level of inhibition induced by *S. typhi* LPS (data not shown). Confirmation of these results has been obtained in the experiments reported here by the use of highly enriched resting B cells which were activated by EP and also inhibited by LPS, strongly suggesting that accessory cells are not involved in this inhibitory phenomenon but rather that the smooth LPS is acting directly on B cells. Depletion of these accessory cells did not alter the suppression effects of LPS, and in the experiments reported here, purified resting B cells activated by EP were also inhibited by LPS, thereby eliminating accessory T cells or macrophages as a factor.

This inhibition of B cells was clearly dose dependent, as depicted in Fig. 1. Furthermore, the inhibition appeared not to be a function of whether the cells were cultured at a level of 2.5 × 10⁶ cells per well or at 10-fold-higher levels in tubes. However, fewer B cells required less LPS for inhibition, and

**TABLE 2.** Suppression of DNA synthesis by LPS in activated thymocytes

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>LPS (μg)</th>
<th>Mean net [3H]thymidine incorporation (cpm) (SI)a</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A (250 ng)</td>
<td>0</td>
<td>11,837 (25.8)</td>
<td>71.0</td>
</tr>
<tr>
<td>Con A (500 ng)</td>
<td>50</td>
<td>3,436 (8.1)</td>
<td>44.0</td>
</tr>
<tr>
<td>PMA (0.1 ng) + ionomycin (0.5 μM)</td>
<td>50</td>
<td>16,007 (34.5)</td>
<td>50.0</td>
</tr>
<tr>
<td>PMA (0.5 ng) + ionomycin (0.5 μM)</td>
<td>0</td>
<td>7,968 (17.7)</td>
<td>33.1</td>
</tr>
</tbody>
</table>

 a Numbers in parentheses indicate the stimulation index (SI), or the ratio of experimental to control counts per minute of [3H]thymidine incorporation.

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if the activation was enhanced with higher concentrations of stimulant, less inhibition was observed with a constant amount of LPS, as shown by the data for activated thymocytes in Table 2. Nevertheless, the amount of LPS used to suppress C3H/HeJ cells would activate normal LPS responder C3H/OuJ cells. Furthermore, the viability experiment with thymocytes and spleen cells showed no significant differences between cells exposed to a high concentration of LPS and those not exposed during a 24-h period prior to the time when activated cells would initiate DNA synthesis. Therefore, we conclude that the inhibition we have observed is probably not due to nonspecific cell death or disruption of cell membranes caused by LPS. In addition, this inhibition phenomenon is not restricted to S. typhi LPS since we have also observed similar levels of inhibition of splenic B-cell DNA synthesis with LPS from Escherichia coli O127:B8 and Salmonella typhimurium (data not shown).

In contrast to our work, Flebbe et al. (4) reported that LPS from E. coli O111:B4 did not inhibit [3H]thymidine uptake in C3H/HeJ spleen cells stimulated by an Ra-LPS and Rd-LPS from S. minnesota. Their experiment, conducted in microtiter cell cultures, however, was limited to a single low dose of E. coli LPS with double the number of cells, compared with our culture system reported here, suggesting that less than optimal conditions were operative in their experiment.

In other instances an LPS precursor has been shown to inhibit the activation of proliferation of C3H/HeJ spleen cells. Tomai et al. (16) reported that lipid X and its monooaclyl glucosamine phospholipid derivative inhibited the proliferation of C3H/HeJ cells stimulated by monophosphoryl lipid A or an Re glycolipid of E. coli. However, lipid A-associated protein activation of these cells was not inhibited, and optimal inhibition was observed when the inhibitors were added prior to the mitogens, suggesting competitive inhibition of a putative lipid A receptor (16). More recently, Erwin et al. (3) reported that a nonmitogenic deacetylated Neisseria meningitidis LPS blocked the mitogenic activity of Salmonella LPS in normal responder C3H/HeN spleen cells; however, this inhibition was also LPS specific and not observed with ConA-stimulated spleen cells. In our hands, the most efficient inhibitor has been the complete wild-type smooth LPS. Our preparations have been processed to remove nucleic acid and protein contaminants, neither of which are known as inhibitors in large or trace amounts. Indeed the protein isolated from smooth LPS of various species is well documented to be a potent polyclonal activator of B cells, surpassing LPS in some instances (12). Therefore, we consider the LPS, and not trace contaminants, to be the active inhibitor. As to what may be responsible for this suppression, we have conducted numerous experiments, the results of which will be published later, that provide evidence that the major components of LPS can block C3H/HeJ splenic B-cell activation. Acid hydrolysis of LPS to produce free lipid A and polysaccharide revealed that both components can suppress the activation of C3H/HeJ cells but on a weight basis to a lesser extent than complete LPS. Even more intriguing has been the finding that mild alkaline hydrolysis of R595 LPS produces a material that can still activate LPS responder C3H/OuJ cells, but can act as a biochemical event affecting C3H/HeJ cell activation for DNA synthesis. It would appear then that lipid A molecules, which are microheterogeneous in structure (6), may have differing activities depending on the susceptibility of the cell or cell type used. In this regard, Kapp et al. (7) reported that R-form LPS and lipid A were more active in inducing human granulocyte oxygen radical production than smooth LPS and speculated that the presence of O polysaccharide in S-form LPS may have exerted an inhibitory effect on the granulocyte or the R-form LPS was more active because of its lipophilic character.

In our initial attempts to analyze the underlying features of this inhibitory effect, we examined the progression of stimulated B cells through the cell cycle by the use of cytofluorometry in the presence or absence of inhibiting concentrations of LPS. From the results obtained, it appears that many of the B cells were blocked from entry into G1 by LPS, whereas increasing numbers of EP-stimulated cells accumulated in G1 in the absence of LPS. As a consequence fewer EP-stimulated cells were found in S phase upon exposure to LPS, as was expected. Apparently signal events turned on relatively early in the cell cycle are affected by LPS. In addition, when B cells were stimulated by EP or by PMA and ionomycin for 12 h and then LPS was added, significant inhibition of DNA synthesis occurred as well (Fig. 2). This suggests that after the initial signals have occurred, other biochemical events can be suppressed, rather than nonspecific effects such as the complexing of LPS with EP or PMA and ionomycin, which would prevent access of the stimulant to the cell surface. Candidates for such later events could be (i) RNA synthesis, which is compromised during the first 24 h of cell culture (Table 2), or (ii) PKC activation, since specific PKC inhibitors can block PKC activation and subsequent DNA synthesis both early and 12 h after cells have been stimulated by EP or by PMA and ionomycin (1).

The suppression phenomenon we have described is not meant to explain the lack of a mitotic response of C3H/HeJ B cells or macrophage activation. Rather C3H/HeJ cells still can be considered unreactive to LPS as a consequence of a genetic deficiency (11, 17) which most likely is due to lack of a signal receptor or the presence of a receptor which is incompetent in transducing the necessary signal. However, when the C3H/HeJ B cell receives a positive signal from an unrelated mitogen such as EP or PMA and ionomycin, exposure to LPS at relatively high concentrations turns on a negative signal(s) which blocks the cell from continuing through the cell cycle to proliferation.

Although by their genetic nature, C3H/HeJ cells provide a system to study how LPS can suppress activated cells, there have been reports of LPS directly inhibiting other cells. At concentrations of LPS which are mitogenic for normal B cells, the continual proliferation of certain B-lymphoma cell lines can be blocked (9, 10). In these studies the LPS was not cytotoxic, and growth resumed after LPS was removed; however, the stage of differentiation of the cell lines affected their susceptibility to the LPS effect. Thus, Ralph et al. (10) showed that R8 and RAW 112-2 are stem cell lines that were resistant to LPS, whereas RAW 8 and R7 pre-B cells were susceptible. Whether the signal pathway(s) affected by LPS in the B-lymphoma cells is similar to that presumed to occur in activated C3H/HeJ cells remains to be determined. Nevertheless, the comparison is intriguing and worthy of study.

Of additional interest is the fact that this suppression is not limited to B lymphocytes. T cells, thymocytes, and macrophages activated by various means can be inhibited by LPS. Since peritoneal macrophages are not known to proliferate, the characteristics of LPS effects on these cells may differ from those which lead to B-cell or T-cell division. While this suggests that multiple signal pathways may be altered by LPS in C3H/HeJ cells it is also possible that a signal pathway common to both processes may be involved, such as PKC activation. Clearly, further studies are needed to delineate the mechanism of this suppression phenomenon.
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