IMMUNOLOGIC RESPONSIVENESS OF THE C3H/HeJ MOUSE: DIFFERENTIAL ABILITY OF BUTANOL-EXTRACTED LIPOPOLYSACCHARIDE (LPS) TO EVOKE LPS-MEDIATED EFFECTS*

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The lipopolysaccharide (LPS)[1] component of the cell wall of gram-negative bacteria possesses a broad range of biological activities and has been utilized to probe both subcellular and cellular phenomena (1–6). It is known to stimulate cell-specific activity in a diversity of cell types, including platelets (7), polymorphonuclear leukocytes (8), lymphocytes (9), fibroblasts (10), and macrophages (11) among others. Activation of lymphocytes has been studied in a number of systems: LPS has been shown to be a B-lymphocyte mitogen (12), a polyclonal B-lymphocyte activator (13), and an in vivo adjuvant (14) enhancing the antibody response to simultaneously administered antigens. Work by Claman (15) established that administration of LPS after tolerogen interfered with the in vivo induction of tolerance. Golub and Weigle (16) subsequently defined the temporal relationship between the injection of tolerogen and LPS. Moreover, Louis, et al. (17) showed that this regimen modulated the unresponsive state to one of immunity, and that this effect was limited to B cells, whose ensuing secondary response was T-independent in the face of continuing T-cell tolerance. Chiller and Weigle (18) demonstrated that LPS could be used to terminate tolerance to human gamma globulin (HGG) late in tolerance, at a time when T cells were still tolerant but B cells were responsive. Under these circumstances, LPS was believed to be able to bypass the tolerant T cells and activate HGG-specific B cells.

In 1968, Sultzer (19) described a mutant C3H mouse, the C3H/HeJ, which

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[1] *Abbreviations used in this paper:* AHGG, aggregated human gamma globulin; DHGG, deaggregated human gamma globulin; HGG, human gamma globulin; LAP, lipid A-associated protein; LPS, lipopolysaccharide; LPS-B, butanol-extracted lipopolysaccharide; LPS-P, phenol-extracted lipopolysaccharide; PFC, plaque-forming cells; POL, polymerized flagellin; SRBC, sheep red blood cells; TCA, trichloroacetic acid; TNP, 2,4,6-trinitrophenyl.

appeared to be resistant to the effects of LPS. Further work with this strain has shown that it is refractory to virtually all LPS-mediated effects studied, including endotoxic shock (20), pattern of LPS-induced polymorphonuclear leukocyte changes (21), protection from bacterial infection (22), mitogenesis (23), polyclonal B-cell activation (24), inhibition of tolerance induction (25, 26), and in vivo adjuvanticity (26).

Recent work from this laboratory by Skidmore et al. (27) has demonstrated that the ability of C3H/HeJ spleen cells to be stimulated to mitogenesis by LPS is dependent upon the method employed in extraction of LPS. Thus, LPS extracted with the phenol-water technique of McIntire et al. (28) was found to be ineffective, whereas LPS extracted by the butanol-water technique of Morrison and Leive (29) or the trichloroacetic acid (TCA) technique of Boivin et al. (30) proved to be a potent mitogen. Nevertheless, there was a definite quantitative difference between the responses of C3H/HeJ mice and those of LPS-responsive C3H/St mice to butanol-extracted LPS. The biochemical basis of these observations was clarified by the work of Morrison et al. (31) and Sultzer and Goodman (32), who showed that butanol- and TCA-extracted LPS preparations contained a low molecular weight protein which was tightly bound to the lipid-A moiety of LPS, whereas the phenol-extracted preparation lacked this protein. Their observations demonstrated that in the C3H/HeJ mouse, this protein was the seat of the mitogenic activity of butanol and TCA extracts; the LPS from which the protein was separated was rendered nonmitogenic. Therefore, the current studies were undertaken to learn whether or not the potency of butanol-extracted LPS in C3H/HeJ mice extended to several of the other immunological activities which LPS mediates in responder strains.

Materials and Methods

Mice. C3H/St male mice, 6–12 wk of age, were obtained from the L. C. Strong Laboratory, Del Mar, Calif. C3H/HeJ male mice, 6–12 wk of age, were obtained from The Jackson Laboratory, Bar Harbor, Maine. All mice were maintained on Wayne Lab-Blox F6 pellets (Allied Mills, Inc., Chicago, Ill.) and chlorinated water acidified with HCl to a pH of 3.0 (33).

LPS. Escherichia coli K235 was generously supplied by Dr. Arthur Hirata and Mr. Jerry Petruska of Abbott Diagnostics, North Chicago, Ill. LPS was extracted from these bacteria by the butanol-water technique (LPS-B) of Morrison and Leive (29). LPS extracted from *E. coli* K235 by a phenol-water technique (LPS-P) was kindly donated by Abbott Diagnostics, through Dr. Floyd McIntire at the University of Colorado School of Dentistry, Denver, Colo.

Lymphocyte Cultures. The preparation of spleen cell suspensions and the constituents of the serum-containing culture media employed in these studies have been previously described (34). For measurement of polyclonal B-cell activation, lymphocytes were incubated in plastic culture trays (model 3008; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) at a cell density of 1.0×10^7 viable spleen cells/ml in a 1.0-ml volume. Culture trays were incubated at 37°C, in a humidified atmosphere containing a mixture of 5% CO_2 in air. Cultures were fed daily with 20–40 μl of nutritional cocktail (35). The direct plaque forming cell (PFC) response to sheep erythrocytes and to trinitrophenyl (TNP) was assayed using a modification of the hemolytic plaque assay of Jerne and Nordin (36).

HGG. HGG was purified from Cohn Fraction II, as previously described (37), and was obtained through the courtesy of the American Red Cross National Fractionation Center with the partial support of National Institutes of Health grant HE 13881 HEM. The methods for preparation of HGG, aggregated by heating to 63°C for 25 min, or deaggregated by ultracentrifugation at 150,000 *g* for 150 min, have been detailed previously (38). The numbers of indirect PFC to HGG in individual mice were assayed on goat erythrocytes conjugated to HGG with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, as detailed elsewhere (39). Assessment of

indirect PFC was accomplished by use of a rabbit anti-mouse IgG amplifying serum.

Cell Transfer. Groups of primed and unprimed recipient mice were exposed to 1,000 rads of whole body irradiation emitted from a Gamma Cell 40 small animal irradiator (Atomic Energy of Canada, Ltd., Ottawa, Canada). This irradiator utilizes a cesium-137 source, emitting a central dose of 109 R/min. Donor cells were prepared as single cell suspensions from the spleens of 90-day tolerant mice, washed twice in sterile balanced salt solution, and viable cells enumerated by trypan blue dye exclusion. 35×10^6 viable spleen cells were transferred to each recipient.

Results

Polyclonal Activation of C3H/HeJ and C3H/St Spleen Cell Cultures by Phenol- and Butanol-Extracted LPS Preparations. The abilities of a substance to function as a lymphocyte mitogen and as a polyclonal B-cell activator appear to be closely linked for most systems studied (13, 40). For this reason, the capacities of phenol- and butanol-extracted K235 LPS preparations to elicit polyclonal antibody responses were compared in C3H/HeJ and C3H/St mice. 1.0×10^7 viable spleen cells/ml were cultured in a 1.0-ml vol in the presence of incremental concentrations of LPS-P or LPS-B. 48 h later, the direct PFC responses to sheep erythrocytes (SRBC) and to TNP were assessed. The data presented in Figs. 1 and 2 indicate that while phenol-extracted LPS is an effective polyclonal B-cell activator for cultures of C3H/St spleen cells, it is ineffective in the C3H/HeJ mouse over the entire range of concentrations examined. Butanol-extracted LPS, on the other hand, is active in both strains. The polyclonal response increased directly with the concentration of LPS-B (and LPS-P in the C3H/St) for both antigens tested. In all cases, LPS-B was a more potent polyclonal B-cell activator than was LPS-P.

Ability of Phenol- and Butanol-Extracted LPS Preparations to Enhance the Response of C3H/HeJ and C3H/St Mice to HGG. LPS, and in particular its lipid-A constituent, have been shown to possess the capacity to enhance the PFC response to protein antigens such as bovine serum albumin (14) or heat-aggregated human gamma globulin (AHGG) (41). Therefore, the capacity of LPS-B to function as an adjuvant for the PFC response to HGG was evaluated in C3H/HeJ and C3H/St mice. Groups of six mice of either strain were immunized with 400 μg of AHGG i.v., followed 3 h later by an i.v. injection of saline or a variable amount of LPS-B. An additional group received antigen followed by 100 μg of LPS-P. The results of this type of experiment are seen in Fig. 3. In the responder strain (C3H/St), the degree of enhancement of the indirect PFC response to HGG was directly related to the concentration of LPS-B injected (the direct PFC response to this antigen is generally of very low magnitude, and reflected no LPS adjuvant effect in any case). In the C3H/HeJ, however, LPS-B failed to augment the response to HGG, regardless of the dose employed. The inability of LPS-P to act as an adjuvant in this strain, as observed by others (25), was confirmed in the present experiments (Fig. 3). In other experiments, when high doses of LPS-B were injected into C3H/HeJ mice after antigen administration, a slight increase in PFC was observed, but was not found to be statistically significant.

Inhibition of Induction of Tolerance to HGG by LPS-B in C3H/HeJ Mice. Previous work from this laboratory by Louis et al. (17) has shown that injection of LPS into mice tolerized 3 h previously with deaggregated human gamma globulin (DHGG) not only aborted the induction of tolerance, but also

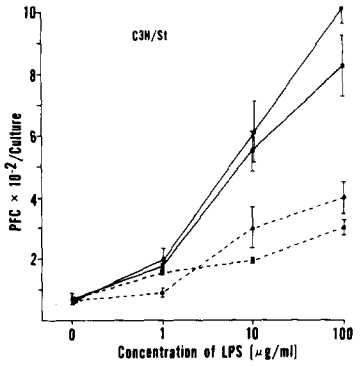

FIG. 1. Polyclonal B-cell activation of C3H/St spleen cell cultures. 1.0×10^7 viable C3H/St spleen cells were cultured in 1 ml of 5% fetal calf serum-containing medium in the presence of incremental concentrations of LPS-B or LPS-P. The direct PFC responses to SRBC and TNP were assayed 48 h later. Results are expressed as the arithmetic mean of triplicate cultures ± the standard error. (■——■), LPS-B anti-SRBC; (●——●), LPS-B anti-TNP; (■- - -■), LPS-P anti-SRBC; (●- - -●), LPS-P anti-TNP.

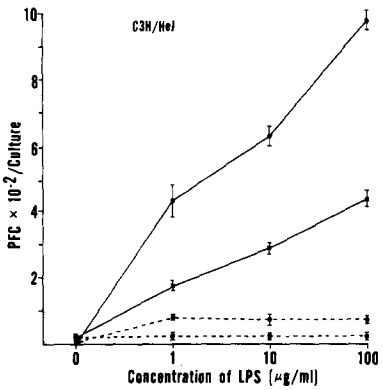

FIG. 2. Polyclonal B-cell activation of C3H/HeJ spleen cell cultures. 1.0×10^7 viable C3H/HeJ spleen cells were cultured in 1 ml of 5% fetal calf serum-containing medium in the presence of incremental concentrations of LPS-B or LPS-P. The direct PFC responses to SRBC and TNP were assayed 48 h later. Results are expressed as the arithmetic mean of triplicate cultures ± the standard error. (■——■), LPS-B anti-SRBC; (●——●), LPS-B anti-TNP; (■- - -■), LPS-P anti-SRBC; (●- - -●), LPS-P anti-TNP.

modulated the level of the response to one which is characteristic of antigen-primed animals. Therefore, experiments were undertaken to determine whether or not the responsiveness of C3H/HeJ mice to LPS-B would extend to this parameter also. Groups of six C3H/HeJ mice were injected i.p. with either 2.5 mg of DHGG or saline. 3 h after the tolerizing injection, mice received an i.v. injection of saline, or a variable amount of LPS-B. 30 days later, all mice were challenged with an immunogenic dose of aggregated HGG, and the indirect PFC response to HGG was assayed 5 days after challenge. The results of this type of experiment are illustrated in Fig. 4. The dose-responsive nature of this phenomenon is illustrated for C3H/St mice in Fig. 4A. The data presented in Fig. 4B demonstrate that C3H/HeJ mice receiving tolerogen and no LPS were indeed tolerant, as were those receiving tolerogen followed by LPS-P. Although

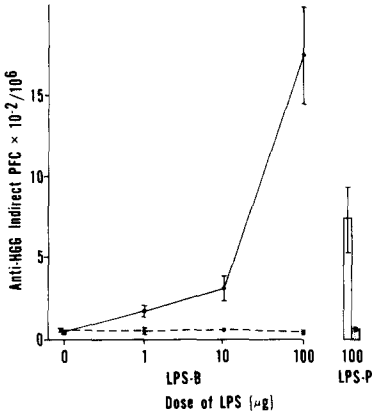

FIG. 3. Adjuvanticity of LPS-B and LPS-P for the in vivo anti-HGG PFC response. Groups of six C3H/St and C3H/HeJ mice were injected with 400 μg AHGG i.v., followed 3 h later by injection of LPS-B, LPS-P, or an equal volume of saline, i.v. The indirect PFC response to HGG was assessed 7 days later. Results are expressed as the arithmetic mean of each group ± the standard error. □, C3H/St; ▨, C3H/HeJ.

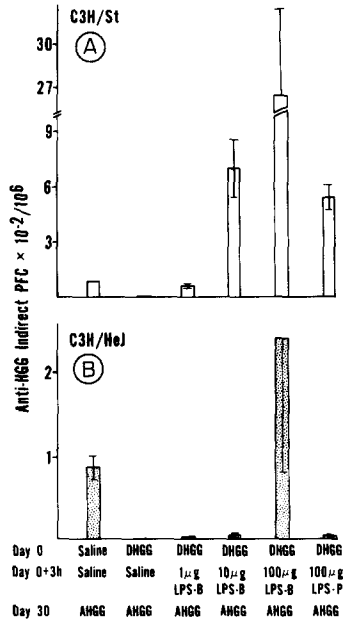

FIG. 4. Ability of LPS-B and LPS-P to inhibit the induction of tolerance to HGG in C3H/St and C3H/HeJ mice. Groups of six C3H/St and C3H/HeJ mice were injected with 2.5 mg DHGG or an equal volume of saline i.p., followed 3 h later by injection of LPS-B, LPS-P, or an equal volume of saline, i.v. All mice were challenged with an injection of 400 μg AHGG i.v. after 30 days, and 5 days later the indirect PFC response to HGG was assessed. Results are expressed as the arithmetic mean of each group ± the standard error. The responses to LPS-P were evaluated in a separate experiment and normalized for control values.

the groups treated with 1 and 10 μg of LPS-B remained unresponsive to HGG challenge, tolerance induction was aborted in all mice receiving 100 μg of LPS-B. Other experiments showed that some, but not all, animals become responsive

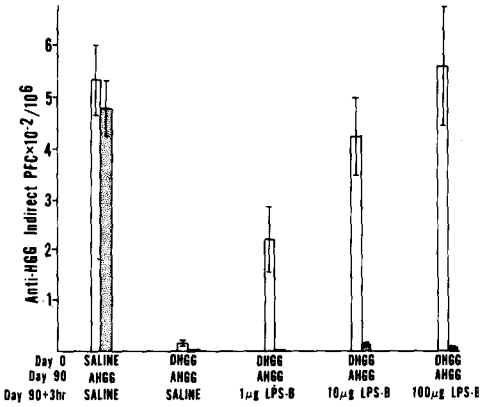

FIG. 5. Ability of LPS-B to bypass tolerant T cells late in HGG tolerance, in C3H/St and C3H/HeJ mice. Groups of six C3H/St and C3H/HeJ mice were injected with 2.5 mg DHGG or an equal volume of saline, i.p. All mice were challenged with an injection of 400 μg AHGG i.v. 90 days later, followed 3 h later by injection of LPS-B or an equal volume of saline, i.v. The indirect PFC response to HGG was assessed 6 days later. Results are expressed as the arithmetic mean of each group ± the standard error. □, C3H/St; ▨, C3H/HeJ.

when treated with 25 μg of LPS-B, and that 50 μg was effective in all cases. Thus, LPS-B is able to activate C3H/HeJ B cells to mitogenesis, to polyclonal antibody production, and to a state of immunity under otherwise tolerogenic conditions, but it is unable to enhance the PFC response to protein antigens.

Inability of LPS-B Preparations to Bypass Tolerant T Cells Late in Tolerance to HGG. The kinetics of the maintenance of tolerance to HGG have been studied in depth (42) and it has been shown that in A/J mice, tolerance persists for more than 4 mo at the level of the intact animal and of the thymus cell, whereas bone marrow-cell tolerance largely abates spontaneously about 50 days after tolerization. Moreover, if a responder strain animal is challenged with antigen plus LPS at a time when its B cells are responsive and its T cells tolerant, the overall tolerance of the animal is terminated, presumably by bypassing tolerant T cells using LPS as an alternate second signal (18).

The ability of LPS-B to function in this manner was investigated in C3H/HeJ and C3H/St mice. Mice were injected i.p. either with saline or a tolerizing dose (2.5 mg) of DHGG. After 90 days, all animals were challenged with 400 μg of the immunogen AHGG i.v., followed 3 h later by the i.v. injection of incremental concentrations of LPS-B. The indirect PFC response of animals so treated was assayed 6 days later. The data presented in Fig. 5 illustrate that while LPS-B is able to activate HGG-responsive B cells in the C3H/St strain in a dose-dependent fashion, it is totally ineffective in the C3H/HeJ strain. Other experiments demonstrated that LPS-P is equally inactive in this strain in this situation. Thus, there appears to be a clear distinction between the ability of LPS-B to inhibit tolerance induction and its inability to bypass tolerant T cells and so terminate tolerance in the C3H/HeJ mouse.

To verify that 90 day-tolerant C3H/HeJ B cells are in fact responsive to HGG, cell transfer experiments were undertaken. Groups of 6 normal C3H/HeJ mice were injected with either 400 μg of AHGG to raise a population of HGG-specific

TABLE I
*Assessment of Tolerance of C3H/HeJ B Cells by Cell Transfer**

Recipient		Donor cells	iPFC/10^6
Priming	Irradiation		
−	+	35×10^6 90 Day Tol	1 ± 0
+	+	35×10^6 90 Day Tol	96 ± 7

* Groups of 6 C3H/HeJ mice were injected with either 400 μg AHGG or saline i.v. 7 days later, all mice were subjected to 1,000 rads of whole body irradiation, and injected with 35×10^6 or no spleen cells from C3H/HeJ mice made tolerant 90 days previously with an i.p. injection of 2.5 mg of DHGG. All mice were boosted with AHGG at cell transfer and again 10 days later. Indirect PFC (iPFC) to HGG were determined 5 days later. All the mice in an additional group, which was primed and irradiated but which received no donor cells, died of radiation injury before plaquing.

T helper cells, or with saline, i.v. 7 days later, all mice were subjected to 1,000 rads of whole body irradiation, and injected with 35×10^6 spleen cells from C3H/HeJ mice made tolerant 90 days previously. All mice were boosted with 400 μg AHGG at the time of cell transfer and again 10 days later. Indirect PFC to HGG were determined 5 days later. The data presented in Table I show that although the tolerance of the transferred cells remains intact, these previously tolerized cells do indeed respond to HGG challenge in the presence of radioresistant HGG-T helper cells. Thus, they must contain an HGG-responsive B-cell population.

Discussion

The data presented herein establish the dichotomy in the ability of the C3H/HeJ mouse to respond to LPS-B. LPS-B is able to stimulate C3H/HeJ B cells to mitogenesis and to polyclonal B-cell activation, but is unable to function as an adjuvant; it is able to inhibit tolerance induction, but cannot terminate the established tolerant state in this strain. The importance of these observations lies in their demonstration that these capacities are dissociable, very likely both genetically and in terms of the cell types required.

Ever since Sultzer's original description (19) of the C3H/HeJ mouse as an endotoxin-resistant strain, much study has focused upon this strain. However, exceptions to its LPS-refractory state were noted, and in 1975, Skidmore et al. (27) observed that the C3H/HeJ could indeed respond mitogenically to LPS if it were extracted by the butanol-water technique of Morrison and Leive (29), or by the TCA technique of Boivin (30). Skidmore further demonstrated that LPS-B lost its stimulatory potential upon re-extraction with phenol. Recently, Morrison, et al. (31) and Sultzer and Goodman (32) observed that re-extraction of TCA- or butanol-extracted LPS in phenol cleaved a low molecular weight protein from the LPS molecule, and that this protein acted as a mitogen and a polyclonal B-cell activator in the C3H/HeJ mouse. The residual protein-free LPS preparation was shown to be devoid of any such activity.

The ability of LPS-B and the inability of LPS-P to activate C3H/HeJ spleen cells to polyclonal antibody production correlates with their respective mito-

genic capacities. In the responder strain (C3H/St), both LPS preparations are effective polyclonal activators. This is precisely the pattern of mitogenic reactivity demonstrated by Skidmore et al. (27) in these two strains, once again confirming the close biological linkage between mitogenicity and polyclonal B-cell activation. In marked contrast to the capacities of LPS-B to function as a mitogen and polyclonal B-cell activator in the C3H/HeJ, is its inability to function as an in vivo adjuvant of comparable potency to that demonstrated in C3H/St mice. This is true over the entire range of concentrations examined, in contradistinction to the marked effect produced in the C3H/St. Thus it appears that in the C3H/HeJ, LPS-B is able to fulfill some but not all of the biological roles that either LPS-B or LPS-P is able to assume in the C3H/St.

A functional dichotomy is also observed when the effects of LPS-B on induction and termination of tolerance to HGG are studied in vivo in C3H/HeJ mice. In accord with the model established in A/J mice by Louis et al., LPS-B, administered to C3H/HeJ and C3H/St mice after an otherwise tolerogenic dose of DHGG, is able to inhibit the induction of tolerance. Upon challenge, these animals respond as if primed rather than tolerized. The observed difference in the dose-response profiles for inhibition of tolerance induction for the two strains would be predicted on the basis of the ability of C3H/St mice and inability of C3H/HeJ mice to respond to the LPS moiety of LPS-B. Indeed, LPS-P was observed to inhibit tolerance induction in C3H/St but not in C3H/HeJ mice. It should be noted that the inability of LPS-B to act as a classical adjuvant, coupled with its ability to inhibit tolerance induction do not agree with Dresser's concept (43) that the latter phenomenon comprises a stringent definition of adjuvanticity. The data presented here would suggest that these two situations do not always parallel one another, and that inhibition of tolerance induction should not be equated with adjuvanticity. The ability of LPS-B to abort induction of tolerance in C3H/HeJ mice, coupled with the ineffectiveness of LPS-P, follows the same pattern observed for mitogenicity and polyclonal B-cell activation.

In contrast to their effects on tolerance induction, neither LPS-B nor LPS-P is able to bypass tolerant T cells late in tolerance to HGG in order to activate HGG-responsive C3H/HeJ B cells to antibody production. That the kinetic basis for this phenomenon as delineated in A/J mice (42) is also valid for C3H mice is supported by the ability of LPS-B to bypass tolerant T cells in late HGG-tolerant C3H/St mice. The lack of effectiveness of LPS-B in C3H/HeJ mice in this situation correlates with the inability of LPS-B to function as an adjuvant. C3H/HeJ B cells are fully responsive to HGG 90 days after tolerance induction. This was demonstrated by the ability of 90-day tolerant C3H/HeJ spleen cells to respond to HGG when transferred to lethally irradiated recipients which had previously been primed with HGG to generate a population of T helper cells. When T help was omitted, the recipients remained tolerant, underscoring the inability of LPS-B to transmit a T-cell-like signal to responsive C3H/HeJ B cells in the face of continued T-cell tolerance.

Elucidation of the explanation for the observed pattern of positive and negative LPS responses may be approached from two different directions: cellular and molecular. One might first consider the cellular targets and cellular requirements for these immunological phenomena. C3H/HeJ B cells

have been shown to be unresponsive to the traditional form of LPS (LPS-P) in all biological situations examined. A comparison of the protocols employed reveals that both of the situations in which LPS-B was ineffective involved injection of antigen 3 h before administration of LPS-B. Mitogenesis, polyclonal activation, and inhibition of tolerance induction, on the other hand, employed spleen cell populations which were predominantly resting. It is conceivable, therefore, that once the initial steps of antigen-induced cellular activation have been traversed, C3H/HeJ B cells are not susceptible to the type of positive signal delivered by LPS-B. This may represent a second defect in C3H/HeJ B cells. Alternately, adjuvanticity and termination of tolerance may require collaboration of HGG-specific B cells with a discrete subpopulation(s) of B cells which is either missing or unresponsive in the C3H/HeJ.

A defect in the cellular response to LPS may not necessarily manifest itself at the B-cell level; the unresponsive state may represent a break in cellular communication involving T cells, macrophages, or both. The presence of T cells does not appear to be mandatory for the mitogenic response to LPS (12, 44). Although there is some evidence to the contrary in the rabbit (45), T cells do not appear to be important in polyclonal activation (41, 46, 47). However, the PFC response to HGG is T-dependent, and there is some evidence to indicate that augmentation of the response to T-dependent antigens by LPS may involve T cells (46, 48). Ness et al. (49) have shown that the adjuvant effect of LPS for synthetic polypeptide antigens has both T-independent and T-dependent facets. A C3H/HeJ T-cell defect, manifested expressly in the T-dependent aspects of LPS-induced adjuvanticity, might explain the absence of an adjuvant effect seen with LPS-B in this strain.

The role of T cells in inhibition of tolerance induction mediated by nonspecific B-cell activators appears to be minimal. This is demonstrated by the work of Schrader (50), who has shown that polymerized flagellin (POL) can inhibit tolerance induction in nu/nu mice. In addition, recent evidence from this laboratory by Parks et al. (41), indicates that tolerance induction in C57BL/6J nu/nu mice can be inhibited by LPS.

The role of T cells in LPS-mediated termination of tolerance is not clear. In this situation it is not likely that specific T help is the target cell for the action of LPS, since kinetic studies have shown that the specific T cells are still tolerant. Therefore, any T cell-LPS interaction would have to involve another group of T cells nonspecifically. If T cells constitute an essential component of the events involved in termination of tolerance, then either a defect in T-cell responsiveness to LPS or in B-cell responsiveness to the T cells involved could explain the unresponsiveness observed.

Other evidence has pointed to a defect in the responsiveness of C3H/HeJ macrophages to LPS (22, 51). Several investigators have shown that the presence of macrophages is either irrelevant or suppressive to mitogenic responses (52, 53), and is not required for polyclonal B-cell activation (45). Macrophages constitute an integral part of the response to T-dependent antigens (54), and there is evidence to suggest a contributory role for macrophages in LPS-mediated adjuvanticity (55). However, macrophage dependency of the inhibitory effects of LPS on induction and maintenance of in vivo tolerance to heterologous serum proteins is unknown. Diener et al. (56) have shown that in

vitro tolerance to the T-independent antigen, POL, can be terminated by normal macrophages. Therefore, if macrophages were found to constitute a critical component for in vivo adjuvanticity and termination of tolerance, but not for inhibition of tolerance induction, a defect in macrophage responsiveness to LPS or in B-cell responsiveness to the involved macrophages could explain the observed pattern of C3H/HeJ responses to LPS-B.

The second direction from which the differential pattern of responsiveness may be approached involves the molecular constitution of the probe. Three possible molecular models could explain the activity of the LPS-B preparation in C3H/HeJ mice. First, all activity could reside in the lipid-A-associated protein (LAP) portion of the complex; the LPS component functioning simply as an inert carrier. Second, binding of LAP could alter the configuration of the LPS molecule, exposing a site or sites that would be capable of activating the C3H/HeJ B cell. Finally, some of the complex's effects could result from the action of LAP, whereas others would be attributable to LPS activated by LAP.

If one were to postulate that the immunological effects of LPS-P are attributable to several distinct sites of action, one site would presumably be responsible for mitogenesis and polyclonal B-cell activation; this site might or might not include that involved in inhibition of tolerance induction. There is, in fact, some evidence that more than one site of action is involved in the biological activities of LPS: Jacobs and Morrison (57) have shown evidence for the dissociation of mitogenesis and T-independent immunogenicity, Poe and Michael (58) have produced evidence for the functional dissociation of LPS-induced mitogenesis from polyclonal activation, and Parks et al. (41) have found a pattern of dissociation of the properties of LPS in the C57BL/6J nude mouse which parallels that described here. This first site, responsible for mitogenesis and polyclonal activation, would be inhibitable by complexing LPS with polymyxin B (59). A second site, then, would be the hypothetical seat of LPS adjuvanticity and its ability to bypass tolerant T cells. If the LAP moiety of LPS-B functions as an independent mitogen bound to an inert carrier, then LAP and possibly certain other B-cell mitogens would only be able to reproduce the immunological effects due to the first site. This would produce the pattern of responses observed. Alternately, LAP may activate the first site on the LPS molecule relative to the C3H/HeJ receptor but not the second. Finally, if there are three active sites on LPS, LAP may be responsible for mitogenesis and polyclonal activation in the C3H/HeJ, it may activate the site responsible for inhibition of tolerance induction, but be unable to activate the site involved in adjuvanticity and termination of tolerance.

Summary

The lipopolysaccharide (LPS)-protein complex extracted from the cell wall of *Escherichia coli* K235 by the butanol-water technique has been shown to evoke a mitogenic response in bone marrow-derived (B) lymphocytes from the C3H/HeJ mouse strain. These mice are resistant to the effects of LPS extracted with phenol. Therefore, the ability of butanol-extracted LPS to modulate a spectrum of C3H/HeJ B-cell functions was investigated. Both butanol-extracted (LPS-B) and phenol-extracted (LPS-P) LPS preparations activated responder C3H/St spleen cell cultures to polyclonal antibody production, while only LPS-B

activated C3H/HeJ spleen cells. Both LPS-P and LPS-B acted as adjuvants when injected after aggregated human gamma globulin (HGG) in C3H/St mice, but neither preparation was effective as an adjuvant in C3H/HeJ mice. LPS-P injected with deaggregated HGG (tolerogen) into LPS-sensitive mice has been shown previously to inhibit the induction of tolerance to HGG. In the present studies, it was shown that LPS-B, but not LPS-P, was able to inhibit tolerance induction to HGG in the C3H/HeJ, whereas both preparations were effective in the C3H/St. LPS has also been shown to bypass tolerant T cells in LPS-sensitive mice late in tolerance to HGG at a time when B cells are responsive. However, in the C3H/HeJ, neither LPS-B nor LPS-P was capable of this function. The responsiveness of these B cells to HGG was demonstrated in transfer experiments. Thus, in the C3H/HeJ, LPS-B stimulates mitogenesis, polyclonal B-cell activation, and inhibition of tolerance induction, but cannot act as an effective adjuvant or as a bypass mechanism to activate B cells in the presence of tolerant T cells. The explanation for this pattern of responses may be attributable to yet another cellular defect in the C3H/HeJ mouse.

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