

THE ABILITY OF BACTERIAL LIPOPOLYSACCHARIDE TO MODULATE THE INDUCTION OF UNRESPONSIVENESS TO A STATE OF IMMUNITY

CELLULAR PARAMETERS*

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There exists a body of evidence which details that the antibody response to a number of different antigens requires the cooperation between thymus-derived lymphocytes (T cells) and bone marrow-derived lymphocytes (B cells) in a reaction whose net effect is the production of specific antibody by B cells (1). Both of these lymphocyte classes can be made specifically unresponsive to a given antigen (2), although each specific population displays a distinct kinetic pattern in the induction and maintenance of unresponsiveness (3).

Bacterial lipopolysaccharides (LPS)¹ are mitogenic for B lymphocytes (4, 5), a phenomenon which may be related to their ability to circumvent the requirement of T cells for antibody formation to antigens which normally require cellular cooperation. The latter has been demonstrated both in vivo or in vitro using such T cell-dependent antigens as sheep erythrocytes (6-8), haptens (9, 10), and serum proteins (11). LPS can also prevent immunological unresponsiveness. This effect was originally described by Claman (12), who observed that mice which were given LPS after a normally tolerogenic dose of bovine gamma globulin (BGG) did not become tolerant. Golub and Weigle (13) subsequently defined the temporal relationship between the injection of tolerogen and LPS and furthermore demonstrated that the effect of LPS on the induction of tolerance was independent of its activity on the phagocytic index of the reticuloendothelial system.

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¹ *Abbreviations used in this paper:* AHGG, aggregated human gamma globulin; BGG, bovine gamma globulin; BSS, balanced salt solution; DHGG, deaggregated human gamma globulin; HGG, human gamma globulin; LPS, lipopolysaccharides; PFC, plaque-forming cells.

The cellular basis for the interference of the induction of immunological unresponsiveness with LPS remains undefined. To study these parameters, particularly with respect to the effect of LPS on the state of B- and T-cell tolerance, the system of unresponsiveness presently investigated was the state of tolerance to human gamma globulin (HGG) which can be induced in adult mice (14). HGG is an antigen particularly suited for such studies since the cellular events occurring in the induction of the tolerant state have been well characterized (3). The experiments to be reported demonstrate that LPS interferes with the establishment of unresponsiveness by converting the induction of tolerance to a state of immunity. The priming which occurs is restricted to the B-cell population without apparent influence on the induction of specific unresponsiveness in T cells. These findings are interpreted as the ability of LPS to circumvent a required T-cell helper function and to modulate a tolerogenic stimulus into an immunogenic signal.

Materials and Methods

Animals.—A/J and AKR male mice, 5 wk of age, were obtained from the Jackson Laboratory, Bar Harbor, Maine. C3H male mice were obtained from the L. C. Strong Laboratory, Del Mar, Calif. The mice were maintained on Purina chow pellets and chlorinated water acidified to a pH of 3.0 with HCl (15).

Antigen: Tolerogen and Immunogen.—HGG was obtained through the courtesy of the American Red Cross National Fractionation Center. Before use, IgG was purified by DEAE-cellulose chromatography. The fraction was used to prepare either deaggregated HGG (DHGG), referred to as tolerogen, or aggregated HGG (AHGG), referred to as immunogen (16).

Irradiation.—Mice were given 1000 R whole body irradiation in a small animal irradiator (Gamma Cell 40, Atomic Energy of Canada Ltd., Ottawa, Canada). Mice to be used as lethally irradiated recipients in reconstitution experiments were 10–12 wk of age.

Preparation of Cell Suspensions.—Lymphoid tissues were removed from mice killed by exsanguination. Single cell suspensions were made from spleens or thymuses by gently grating these tissues against a stainless steel screen. Bone marrow was obtained from the femurs and tibia by flushing out the marrow with balanced salt solution (BSS) using a syringe fitted with a 25-gauge needle. Bone marrow so obtained was rendered into a single cell suspension by repeated aspiration and extrusion from a syringe. Each of these cell suspensions was filtered through a nylon netting and washed three times in BSS. In the case where cells were used in transfer experiments, the BSS contained 100 μ g streptomycin and 100 U penicillin per ml. Cells appropriately diluted were refiltered through nylon before the transfer.

Hemolytic Plaque Assay.—Antibody-forming cells to HGG were enumerated using a modification (17) of the Jerne plaque assay (18) in which protein antigens are covalently coupled to goat erythrocytes (Colorado Serum Co., Denver, Colo.). Indirect plaque-forming cells were developed using squirrel monkey antimouse γ -globulin used at a concentration previously determined to be optimal in the assay.

Production of Anti- θ Serum.—AKR mice were injected intraperitoneally with a series of seven injections of 20×10^6 C3H thymocytes given over a period of 11 wk. 7 days after the last injection, the animals were bled and individual sera were tested (19) for their anti- θ titer. High titer sera were pooled before use.

Anti- θ Treatment of Spleen Cells.—Approximately 10^9 spleen cells were incubated at 37°C

for 30 min in BSS which contained anti- θ serum of a concentration 10 times greater than that effective in killing 95% thymus cells. The spleen cells were then centrifuged and the pellet incubated at 37°C for 45 min with agarose-absorbed (20) guinea pig serum (C) diluted 1:10 with BSS. The cells were then washed twice in BSS before use in adoptive transfer experiments.

Lipopolysaccharide.—LPS from *Escherichia coli* 0111:B4 (lot B35527) was purchased from Difco Laboratories (Detroit, Mich.). Dilutions were made in 0.15 M NaCl.

RESULTS

Effect of LPS on the Induction of Immunological Unresponsiveness to HGG.—A/J mice were injected with a tolerogenic dose of either 2.5 or 1.0 mg DHGG given intraperitoneally (i.p.). 3 h later, the mice were given 50 μ g of LPS intravenously (i.v.), and 30 days later they were challenged i.v. with 400 μ g AHGG. 5 days after the last injection, their response was assayed by enumerating the number of HGG specific plaque-forming cells (PFC) in individual spleens. The following control groups were similarly challenged and assayed. One group of mice received 2.5 mg DHGG i.p. on day 0 followed 3 h later with an injection of 0.2 ml saline. Another group received 1.0 mg DHGG i.p. on day 0 followed 3 h later with an i.v. injection of 0.2 ml saline. A final group received saline on day 0 followed 3 h later with 50 μ g LPS given i.v. The results of this experiment (Fig. 1) reveal that those mice which were injected with

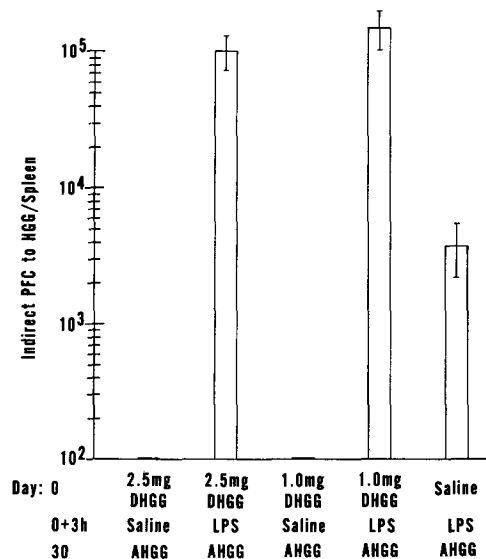


FIG. 1. Secondary response of mice primed with DHGG (tolerogen) and LPS. The abscissa indicates the treatment of various groups of mice in the experiment (six to eight mice per group). The ordinate represents the arithmetic mean of the indirect PFC response to HGG in the spleens of each group of mice. The PFC's were obtained 5 days after challenge with 400 μ g of AHGG. Vertical bars represent the limits of one standard error.

either 2.5 or 1.0 mg DHGG and saline 3 h later were completely tolerant to a subsequent challenge of the immunogenic form of HGG, as revealed by an absence of specific PFC. On the other hand, mice which were treated with tolerogen and then with LPS responded vigorously to a subsequent challenge of AHGG, exhibiting an immune response which was 25 times greater than that obtained in mice treated initially with saline and LPS. These data clearly demonstrate that mice given a tolerogenic form of the antigen and LPS do not become tolerant to that antigen but rather become primed to it.

Effect of LPS on the Induction of Immunological Unresponsiveness As Revealed by a Cell Transfer System.—The following experiments were designed to determine whether the priming effect provided by treatment of mice with tolerogen and with LPS could also be observed in a system where spleen cells from such mice were adoptively transferred to X-irradiated recipients. Groups of donor A/J mice were subjected to one of the following treatments: (a) 0.2 ml saline i.v.; (b) 2.5 mg DHGG i.p.; (c) 1.0 mg DHGG i.p.; (d) 50 μ g of LPS i.v.; (e) 2.5 mg DHGG i.p. and 50 μ g LPS i.v. 3 h later; (f) 1.0 mg DHGG i.p. and 50 μ g LPS i.v. 3 h later. 4 days after these injections, 50×10^6 cells from a pool of spleens obtained from each group of mice were transferred into lethally irradiated syngeneic recipients. All recipients were challenged with 400 μ g AHGG the day of the cell transfer and again 10 days later, and their spleens were assayed individually for PFC to HGG 5 days after the second injection. The results of this experiment (Fig. 2) reveal that recipients of spleen cells obtained from donors injected with 2.5 or 1.0 mg of tolerogen and subsequently with LPS responded vigorously to HGG, at a level which was 4–11 times greater than the response seen in recipients of cells from either saline- or LPS-treated donors. In contrast, recipients of spleen cells obtained from donors injected with either 2.5 or 1.0 mg of tolerogen alone were totally unresponsive to HGG. Therefore, the ability of LPS to modulate the induction of unresponsiveness to a state of immunity can be demonstrated in the neutral environment of an adoptive transfer system.

Effect of LPS on the Induction of Immunological Unresponsiveness. Importance of the Interval Between Injection of Tolerogen and Injection of LPS.—In order to ascertain the time interval during which LPS is effective in the conversion from a state of unresponsiveness to a state of immunity, the following experiment was performed. Groups of A/J mice injected with 1.0 mg DHGG i.p. were given 50 μ g LPS i.v. either 3, 24, or 96 h thereafter. Mice injected with saline and 50 μ g LPS served as a control group. 7 days after the injection of DHGG or saline, spleens from each group were prepared as a single cell suspension and 50×10^6 cells were injected into lethally irradiated syngeneic mice. These recipients were injected with 400 μ g AHGG on the day of the transfer and again 10 days later. 5 days after the second injection, their spleens were assayed for PFC specific to HGG. The results of this experiment (Fig. 3) show that the ability of LPS to interfere with the induction of unresponsiveness, of

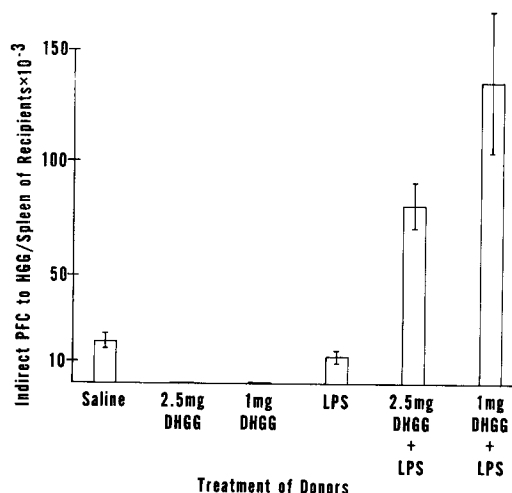


FIG. 2. Effect of LPS on the induction of unresponsiveness as revealed by a cell transfer system. The abscissa indicates the treatment of each group of donors of spleen cells. 4 days after these treatments, 50×10^6 cells from a pool of spleens obtained from each group of mice were transferred into lethally irradiated syngeneic recipients (six to eight mice per group). All recipients were challenged with $400 \mu\text{g}$ AHGG on the day of the cell transfer and again 10 days later. The ordinate represents the arithmetic mean of the indirect PFC response to HGG in the spleens of each group of recipients 5 days after the last injection of AHGG. Vertical bars represent the limits of one standard error.

the times tested, is optimal when it is given 3 h after the injection of tolerogen, is still observed when the two injections are separated by a 24 h interval, and nonexistent when LPS is given 96 h after the tolerogen.

Primary Immune Response of Mice Injected with Tolerogen and with LPS.—The previous data have shown that the interference of tolerance induction with LPS results in a primed immune state demonstrable by a subsequent challenge with AHGG. The following experiment reveals that the modulating effect of LPS is one which will in fact lead to a primary antibody response to HGG. The following protocol was used to reveal this point. Mice were injected with 1.0 mg DHGG i.p. and with $50 \mu\text{g}$ LPS given i.v. 3 h later. Groups of mice were sacrificed at various times after these injections and their spleens assayed individually for PFC specific to HGG. Mice injected with $400 \mu\text{g}$ AHGG or 1.0 mg DHGG served as controls. The kinetics of the primary response observed in these three experimental situations are shown in Fig. 4. Mice injected with tolerogen and LPS began to show splenic PFC on day 8, maximally between days 10 and 14, after which there occurred a gradual decrease in the number of specific antibody-forming cells. Mice injected with the immunogen AHGG developed splenic PFC first on day 4 and responded maximally on day 6, after which the response decreased rapidly. The injection of DHGG alone did not

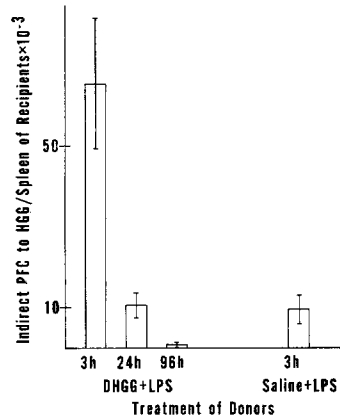


FIG. 3. Effect of LPS on the induction of immunological unresponsiveness; importance of the time interval between the injection of tolerogen and LPS. Treatment of donors (abscissa) comprised of injecting groups of mice with 1.0 mg DHGG i.p. and 50 μ g LPS i.v. either 3, 24, or 96 h later. A control group received saline and 50 μ g LPS 3 h later. 7 days after this treatment, 50×10^6 spleen cells from each group of mice were transferred into lethally irradiated syngeneic recipients (five to eight mice per group). The recipients were injected with 400 μ g AHGG on the day of the transfer and again 10 days later. The ordinate indicates the arithmetic mean of the indirect PFC response to HGG in the spleens of each group of recipients 5 days after the last injection of AHGG. Vertical bars represent the limits of one standard error.

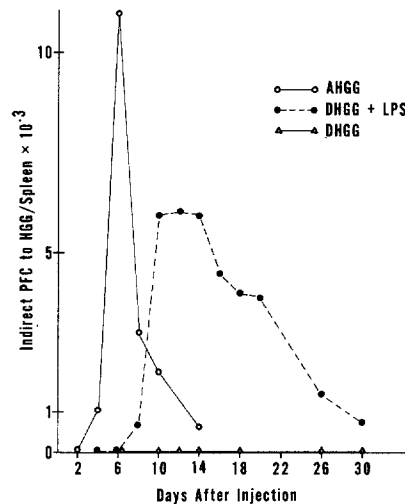


FIG. 4. Kinetics of the cellular response (PFC) of mice injected with either AHGG (immunogen), DHGG (tolerogen), or tolerogen and LPS. On day 0 mice were injected with either 400 μ g AHGG i.v., 1.0 mg DHGG i.p., or 1.0 mg DHGG i.p. and 50 μ g LPS i.v. 3 h later. At various times after these injections, groups of mice were sacrificed and their spleens assayed individually for PFC specific to HGG. Each point represents the arithmetic average of responses of five individual mice.

induce the formation of specific PFC. It should be pointed out that in those responses which induced antibody formation, i.e. DHGG and LPS or AHGG, the PFC response observed was virtually that of indirect PFC.

Effect of LPS on the Induction of an Unresponsive State of the T-Cell Level.— Since LPS minimally interfered with the induction of tolerance at the level of those cells which produced specific antibody, i.e. B cells, it was relevant to determine whether T cells were or were not similarly affected. The following experiment was designed to determine the effect of LPS on the induction of tolerance to DHGG in thymus cells. Mice were injected with one of the following schemes: (a) 0.2 ml saline i.v.; (b) 1.0 mg DHGG i.p.; (c) 50 μ g LPS i.v.; (d) 1.0 mg DHGG i.p. and 50 μ g LPS i.v. 3 h later. 4 days later thymus cells obtained from the various groups and 30×10^6 normal bone marrow cells were transferred to lethally irradiated syngeneic recipients. On the day of transfer and again 10 days later, recipient mice received 400 μ g AHGG. 5 days after the last injection, their spleens were individually assayed for detection of PFC specific to HGG. As can be seen in Table I, none of the recipients reconstituted with thymus cells obtained from animals previously injected with DHGG was able to mount an immune response to HGG whether or not the donors of thymus cells had been injected with LPS. In contrast, donors treated only with saline or LPS had thymus cells which were capable of cooperation. These data support the conclusion that thymus cells from animals injected with tolerogen and subsequently with LPS are in fact as unresponsive as those from animals injected with tolerogen alone. It should be pointed out that the use of 5×10^6 thymocytes in some of the reconstitution experiments was made necessary by the fact that treatment of mice with LPS markedly reduced the yield of thymus cells obtained from these organs. However, as can be seen in Table I, the im-

TABLE I
Effect of LPS on the Induction of Unresponsiveness in Thymocytes

Thymus cells transferred		No. of mice‡	Indirect PFC to HGG/ spleen§
Treatment*	No. $\times 10^6$		
Saline	90	7	862
Saline	5	7	0
DHGG	90	5	0
LPS¶	5	10	550
DHGG + LPS	5	9	0

* Treatment of donors of thymus cells 4 days before transfer.

‡ The recipient mice were reconstituted with the indicated number of thymus cells and 30×10^6 normal bone marrow cells.

§ Each experiment represents the arithmetic mean of the response in individually assayed mice, expressed as indirect plaque-forming cells per spleen.

|| 1.0 mg of deaggregated HGG (tolerogen) given i.p.

¶ 50 μ g of LPS given i.v.

munological effect of thymic atrophy is functionally similar to that previously observed to occur after hydrocortisone treatment (21, 22). That is, the number of thymocytes remaining after LPS treatment, approximately 5–10% of that found in a normal thymus, is capable of participating in cellular cooperation to a degree comparable to that obtained with a much higher number of normal thymocytes. Therefore, mice appropriately treated with tolerogen and with LPS demonstrate a state of immunocompetence whose cellular elements can be immunologically defined as unresponsive T cells and immune B cells.

T-Cell Independency of the Secondary Response to HGG in Mice Primed with DHGG and with LPS.—The demonstration that LPS was able to induce antibody formation in animals treated with DHGG in spite of T-cell tolerance predicted that this response, as well as the previously described secondary responses (Figs. 1 and 2), was occurring without specific T-cell helper function. To determine whether T-cell cooperation was required in the present system, mice were injected with one of the following regimen: (a) 0.2 ml of saline i.v.; (b) 50 μ g LPS i.v.; (c) 2.5 mg DHGG i.p. and 50 μ g LPS i.v. 3 h later; (d) 400 μ g AHGG i.v. 30 days later, the spleens of mice from each group were removed, made into a single cell suspension, and treated with either anti- θ serum and C, or with normal AKR serum and C as described in Materials and Methods. From each suspension 50×10^6 spleen cells were transferred to lethally irradiated syngeneic recipients. The recipient mice were injected with AHGG on the day of the transfer and again 10 days later. 5 days after the last injection, their spleens were assayed for PFC to HGG. It can be seen from Table II that anti- θ and C treatment effected more than a 90% reduction in the adoptive response of spleen cells obtained from animals injected 30 days earlier with either saline, LPS, or AHGG. In contrast, anti- θ treatment and C had no significant effect on the adoptive response to HGG of spleen cells from mice previously injected with DHGG and with LPS. It appears therefore that once B cells have been primed as the result of treatment with tolerogen and with LPS, the normal requirement of a helper T cell is not necessary for an immune response to a subsequent challenge.

DISCUSSION

The present studies demonstrate that in mice, LPS is able to interfere with the establishment of an unresponsive state to HGG by converting the induction of tolerance to a state of immunity. Three different experimental observations support such an interpretation: first, mice injected with DHGG and with LPS respond to a subsequent challenge of immunogenic HGG to a degree which is 25 times greater than that observed in mice treated with saline or LPS alone and receiving the same challenge; second, the priming effect observed when LPS was given after the administration of DHGG could be expressed in a transfer system, suggesting that as in the case of priming with immunogen, an irreversible cellular commitment of immunity could be maintained in a neutral environ-

TABLE II
Thymus Independence of the Response Obtained with Adoptively Transferred Spleen Cells from Mice Previously Injected with Tolerogen and LPS

Treatment of spleen cell donors*	% reduction of the adoptive indirect PFC response to HGG of recipients of anti- θ serum and C-treated spleen cells†
Saline	93
AHGG‡	91
LPS	95
DHGG¶ + LPS	4

* 30 days before transfer.

† The spleen cell suspensions from each group of donors were treated with either anti- θ serum and C or with normal AKR serum and C. From each suspension, 50×10^6 cells were transferred to lethally irradiated syngeneic recipients (eight mice per group). The recipients were injected with 400 μ g AHGG the day of the transfer and again 10 days later. 5 days after the last injection, their spleens were assayed for PFC to HGG. The percent reduction in the PFC response of recipients of anti- θ serum and C treated spleen cells was calculated by the following formula:

$$100 - \left[\frac{\text{Response of spleen + anti-}\theta \text{ serum and C}}{\text{Response of spleen + normal AKR serum and C}} \times 100 \right].$$

‡ 400 μ g of aggregated HGG i.v.

|| 50 μ g of LPS i.v.

¶ 2.5 mg of deaggregated HGG i.p.

ment; third, treatment of mice with DHGG and with LPS not only sensitizes the immune system but in fact leads to a specific primary immune response to HGG. These data support and extend the observations initially described by Claman (12) which showed that mice challenged after receiving LPS and a tolerogenic form of BGG were not tolerant, and, in fact, appeared to respond better than normal controls. Using the antigen pneumococcal polysaccharide, Brooke (23) reached a similar conclusion from experiments which showed that the injection of LPS after a tolerogenic regimen of pneumococcal polysaccharide resulted in immunity rather than tolerance.

The experiments presently reported show that the antibody response resulting from the injection of DHGG and LPS did not appear to require specific T cell-B cell cooperation, since mice so treated could be experimentally defined as a cellular composite of specifically unresponsive T cells and specifically immune B cells. The mechanism by which the observed antibody response occurred would seem to be best explained by the ability of LPS to bypass the normal T-cell helper function. Such an effect in the antibody production to T-dependent antigens has been previously demonstrated both in vivo or in vitro with antigens such as heterologous erythrocytes (6-8), haptens (9, 10), and with the antigen used in the present studies, namely HGG (11). In this regard, perhaps

the most convincing data is that obtained by Watson et al. (10), who showed that the spleens of congenitally athymic mice (B cells only) could be induced to form antibody *in vitro* to a hapten free of any carrier molecule when the culture was supplemented with LPS. Recently, a correlation between the mitogenic effect of LPS on B cells and its adjuvant property has been suggested (24). The transformation of the induction of tolerance into an immunogenic stimulus could be viewed as a most stringent manifestation of this adjuvant effect (25) specifically occurring on B cells. In this light, the inability of LPS to interfere with the induction of unresponsiveness in specific T cells could be related to the inability of LPS to mitogenically stimulate such cells.

The mechanism by which LPS in adjunct with a tolerogenic form of an antigen can drive specific B cells to antibody production is not presently understood. It may be that LPS stimulation of B cells can alter the obligate tolerant state which otherwise results from the binding of tolerogen to specific B cells. In this regard, the phenomenon could be integrated within the two signal model of immunity proposed by Bretscher and Cohn (26) in that specific B-cell stimulation would occur after binding of tolerogen (signal 1) and LPS (signal 2). A similar interpretation (27) has recently been advanced to explain the observation that polymerized flagellin (signal 2) can modulate antibody formation to an otherwise tolerogenic form of deaggregated fowl gamma globulin (signal 1). Yet, another signal 2 may be provided by the allogeneic effect and would account for the observation by Osborne and Katz (28) that the induction of a mild graft-vs.-host reaction can modulate an obligate state of tolerance induced with DNP-D-G-L to the formation of antibody to the hapten. An alternative mechanism by which LPS could mediate its action would be that *in vivo* it is capable of aggregating the tolerogen, transforming it to an immunogen, and, in spite of the establishment of T-cell tolerance, immunity would result from the T-cell bypass function attributable to the adjuvant property of LPS. Although it has been previously demonstrated that a monomeric preparation of HGG is not physically altered *in vitro* by LPS (13), the possibility that such a phenomenon could be occurring in the animal cannot be ruled out.

Whatever mechanism is operational to explain the effect of LPS on converting *in vivo* tolerance to immunity, a critical requirement appears to be the temporal relationship between the injection of tolerogen and that of LPS. Thus, as previously demonstrated by Golub and Weigle (13) and confirmed in the present studies, LPS must be given within 48 h after the injection of DHGG and is totally ineffective if given before tolerogen. Such a transience of effectiveness is interesting when viewed in the light of two previous cellular observations dealing with the induction process of tolerance to HGG. First, the fact that as early as 12 h after the injection of tolerogen, there is a disappearance of specific antigen-binding B cells in lymphoid tissues (29) suggests that an early event in the process of unresponsiveness *in vivo* is the binding of antigen to specific cells. Second, the finding that irreversible tolerance in B cells does not occur until some 48–72 h after tolerogen injection (30) emphasizes that the functional state of B-cell tolerance occurs at a time subsequent to that marked for the binding of tolerogen to specific cells. Taken together, these data support a previously suggested two-step induction process for the state of B-cell tolerance in

mice to HGG (31). The first step would involve binding of tolerogen to specific cells, would occur rapidly, i.e. within 24 h, and would have the cell in a state of reversible tolerance, that is, still capable of being committed to immunity if provided with an appropriate signal 2. The second step would involve some process of either specific cellular repression or death, would require some 48–72 h of time, and would be irreversible in that any form of signal 2 would be incapable of inducing specific immunity. This model would be compatible with the present observations that LPS can provide an alternative form of the putative signal 2 to substitute for that usually furnished by sensitized T cells. In addition, the inability of LPS to modulate tolerance to immunity when given 48 h after tolerogen strongly suggests that a state of B-cell tolerance can exist which is irreversible even when exposed to a specific T-cell bypass mechanism. In the animal, this state of tolerance could be expected to be maintained only as long as a sufficient concentration of tolerogen persists after which the spontaneous loss of B-cell tolerance would again provide a cellular state composed of tolerant T cells and nontolerant B cells. The previous observation that LPS can at this late time circumvent the specific block of unresponsive T cells (11) provides experimental support for this suggestion. Previous data also demonstrate the experimental reversibility in the induction of B-cell tolerance including that of Diener and Feldmann (32) and Katz et al. (33), who have shown that trypsinization of tolerant cells can render such cells once again responsive to immunogen but that this enzymatic resuscitation is only effective within 48 h after the initial exposure of cells to tolerogen.

The present data also indicate that spleen cells primed by a combination of tolerogen and LPS do not seem to require T-cell cooperation when challenged after adoptive transfer into lethally irradiated recipients. This is based on two observations; namely, that specific T cells are in fact tolerant and that the elimination of nonspecific T cells by treatment with anti- θ serum and C does not diminish the ability to mount a secondary response to HGG. These findings are contrary to the previously reported demonstration for a T-cell requirement in the secondary response to a variety of antigens (34–36), and, in fact, to the present data which show that the secondary response of spleen cells from mice given a single immunogenic dose of AHGG is sensitive to treatment with anti- θ serum and C. It may be, therefore, that LPS affects the differentiation of specifically primed B cells to a stage where T-cell help is not necessary for subsequent stimulation. This conclusion is, in fact, supported by more recent data which demonstrate that LPS given with a single injection of immunogen, or immunogen given repeatedly over the course of 3 mo can each provide a state of sensitization which is resistant to anti- θ serum and C when assessed by a subsequent response in an adoptive transfer system (unpublished observations).

A final point which merits emphasis is the fact that the response observed after the injection of tolerogen and LPS is one which unexpectedly appears to be composed of PFC's producing antibody of the IgG class. Although the possibility that such cells are making antibody of the IgM class has not been completely ruled out, preliminary evidence using reduction and alkylation with

dithiothreitol and iodoacetamide (37) suggest that such plaques are not produced by IgM, but rather by IgG (Skidmore, B. J., and J. Louis, unpublished observation). Inasmuch as this response occurs without the requirement for functional specific T cells, the necessity of T cells to switch from IgM to IgG immunoglobulin classes in the formation of antibody to the antigen HGG is questionable. On face value, these data would support the hypothesis that rather than mediating a shift in the class of immunoglobulin produced (38), the function of T-cell help is to lower the threshold of antigen required to trigger terminal differentiation of B cells whose class specificity has already been determined (39).

SUMMARY

Studies were performed to define the cellular parameters involved in the interference with the induction of immunologic unresponsiveness to human gamma globulin (HGG) by bacterial lipopolysaccharide (LPS). Mice which were injected with deaggregated HGG (tolerogen) and with LPS did not become tolerant to that antigen, but rather became primed to a subsequent challenge with immunogen. The ability to prime with tolerogen and LPS was also demonstrated in an adoptive transfer system. The temporal relationship between the injection of tolerogen and that of LPS was critical for priming to occur. The injection of tolerogen and LPS not only primed mice to HGG, but also resulted in a primary antibody response to HGG. The capacity of LPS to interfere with the induction of tolerance was restricted to B cells and did not affect the ability to induce unresponsiveness in T cells. The secondary response to HGG in mice primed by tolerogen and LPS was found to be T-cell independent. These observations are interpreted and discussed from the standpoint of the ability of LPS to circumvent required T-cell cooperation and to modulate to tolerogenic stimulus into an immunogenic signal.

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Note Added in Proof: Recent data by Hamaoka and Katz (*J. Immunol.* In press) and Armerding and Katz (*J. Exp. Med.* In press) emphasize that the adjuvant effect of LPS assessed in a hapten-carrier system requires specific T cells, a conclusion similar to that previously reported by Allison and Davies (40). Applied to the present observation that LPS can provide an adjuvant effect in spite of specific T cell tolerance, these data force consideration of the possibility that LPS can exert its effect on tolerant T cells or on peripheral T cells which have escaped to tolerance induction. The latter is made unlikely by recent results which demonstrate peripheral T cell tolerance while the former possibility has not been ruled out experimentally.

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