INFECTION AND IMMUNITY, May 1979, p. 371-378
0019-9567/79/05-0371/08$02.00/0

Vol. 24, No. 2

Immune and Mitogenic Responses by BALB/c, C3H/HeJ, and Nude Mice to *Brucella abortus* Bacterin and Lipopolysaccharide

JOAN M. SPELLMAN AND NORMAN D. REED*

Department of Microbiology, Montana State University, Bozeman, Montana 59717

Received for publication 7 November 1978

The immunogenic and mitogenic properties of *Brucella abortus* 1119-3 bacterin (BA) and biologically active *B. abortus* lipopolysaccharide (BA-LPS) were studied using normal and athymic (nude) BALB/c and C3H/HeJ mice. Although BA stimulated 2-mercaptoethanol-sensitive (2-ME-S) primary and secondary antibody responses in all mice, nude mice, in contrast to normal BALB/c and C3H/HeJ mice, did not make substantial 2-mercaptoethanol-resistant (2-ME-R) antibody responses. Similarly, all mice injected with BA-LPS made 2-ME-S primary responses, and the secondary response of thymus-bearing mice contained a substantial 2-ME-R component. Collectively, these observations suggest that although both BA and BA-LPS can stimulate thymus-independent 2-ME-S antibody synthesis, thymus-derived cells are required for optimal immune responses containing a 2-ME-R component. The antibody responses of normal BALB/c and C3H/HeJ mice to BA and BA-LPS were qualitatively and quantitatively similar. Both BA and BA-LPS were mitogenic for spleen cells from normal and nude BALB/c and C3H/HeJ mice but not for thymus cells from normal BALB/c or C3H/HeJ mice, suggesting that both preparations are B-cell mitogens.

There are conflicting reports (5, 12, 16, 21, 37) about the ability of the brucellae to elicit the biological and immunological responses evoked by the *Enterobacteriaceae*. These early reports, based on various animal models, either confirmed or denied the cytotoxic, pyrogenic, and antigenic properties of killed *Brucella abortus* cells (BA) or *B. abortus* lipopolysaccharide (BA-LPS). The properties attributed to the BA-LPS were further clouded because different investigators have used products of various purification procedures as the endotoxin preparation. In 1960, Redfearn (Ph.D. thesis, Univ. of Wisconsin, Madison, Wisc.) reported that, unlike enterobacterial LPS which partitions into the aqueous-phase, BA-LPS extracted by the Westphal procedure partitions into the phenol phase. Leong et al. (16) thoroughly documented that this phenol-phase-extracted BA-LPS shares many of the physiological, structural, and chemical properties of aqueous-phase-extracted enterobacterial LPS. Their results also indicated homology between BA-LPS and *Escherichia coli* LPS.

Although many immunological responses by animals to endotoxin preparations from the *Enterobacteriaceae* have been well characterized (7, 9, 15, 24, 27, 35), investigation of the immunological properties of BA-LPS has been largely neglected. We have studied several aspects of the immunological response by mice and mitogenic responses of murine cell cultures to BA and BA-LPS in light of several known immunogenic characteristics of *E. coli* bacterin and LPS. Congenitally athymic (nude) mice were used to investigate the role of T lymphocytes in the response to both BA and BA-LPS. C3H/HeJ mice, shown to have a genetically controlled low response to the endotoxins of *E. coli* and *Salmonella* (27, 30, 32, 36) were included in these studies to determine if this genetic defect would also limit the C3H/HeJ response to BA or BA-LPS.

MATERIALS AND METHODS

Animals. Three strains of mice were used in these experiments: BALB/c, nude (*nu/nu*, 11th and 12th generations produced by successive cross-intercrossing onto a BALB/c background), and C3H/HeJ mice. The BALB/c and nude mice were derived from stock maintained in our laboratory. The C3H/HeJ mice were purchased from Jackson Laboratories (Bar Harbor, Me.). All mice received sterilized Purina Lab Chow 5010 and acidified-chlorinated water ad libitum. Experimental animals ranged in age from 6 to 16 weeks; those used in each experiment varied in age by less than 2 weeks.

Antigens and immunizations. The *B. abortus* 1119-3 antigen used was the U.S. Department of Agriculture tube agglutination test antigen. This stock bacterin (BA) was washed three times with sterile phosphate-buffered saline and resuspended to the concentrations indicated in Results.

BA-LPS, extracted from *B. abortus* 1119-3 by the phenol-water method of Redfearn (Ph.D. thesis, Univ. of Wisconsin, Madison, Wisc., 1960), was kindly supplied by Lois Jones, University of Wisconsin, Madison, Wisc. Analyses done at the University of Wisconsin (B. Wilson and L. Jones, unpublished results) provided the following information. This BA-LPS preparation contained 35.2% protein, 11.42% total sugars (anthrone), 6.57% hexose, and 0.31% 2-keto-3-deoxy-octulosonic acid. The 50% lethal dose in mice for this BA-LPS preparation was 500 to 1,000 μg. The BA-LPS also gave a positive *Limulus* lysate test at concentrations down to 0.5 ng of BA-LPS per ml.

Mice received 0.25 ml of the appropriate antigen preparation intravenously via the lateral tail vein.

Assay of immune responses in recipients. After immunization of mice with BA or BA-LPS, serum agglutinin titers were determined. The antigen used was the *Brucella* tube agglutination test antigen, and the procedure of Spink et al. (29) was followed. 2-Mercaptoethanol-sensitive (2-ME-S; presumably immunoglobulin M) and 2-mercaptoethanol-resistant (2-ME-R; presumably immunoglobulin G) antibodies were distinguished using the method of Adler (1). Sensitivity to 2-ME (Sigma Chemical Co., St. Louis, Mo.) was determined by reduction of agglutinin titer after incubation of 1:5 saline-diluted serum with an equal volume of 0.2 M 2-ME for 1 h at room temperature.

Mitogen assays. Cell cultures for mitogen assays were prepared essentially by the methods of Janossy and Greaves (11). Spleen and thymus cell suspensions were prepared in RPMI 1640 medium supplemented with 5% fetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) (all from Microbiological Associates, Los Angeles, Calif.), and 2-ME (5×10^{-5} M). The cell density was adjusted to 5×10^6 spleen or thymus cells per ml, and 0.2 ml of the suspension was added to each well of a Microtiter II tissue culture plate (Falcon Plastics). All cultures were done in triplicate.

BA and BA-LPS were tested for mitogenic activity. *E. coli* 0113 LPS (kindly prepared and donated by J. A. Rudbach, University of Montana, Missoula, Mont.) and concanavalin A (ConA) (Miles Labs., Inc., Kankakee, Ill.) were included as known mitogenic materials. The gross chemical composition of the *E. coli* 0113 LPS was: nitrogen, 3.4%; nucleic acid, 6%, hexose, 23.6%; total carbohydrate, 34.4%; hexosamine, 16.4%; fatty acid amide plus fatty acid ester, 35%; ketodeoxyoctulosonate, 3.9%; dideoxysugars, <0.1%; and heptose 2.4% (J. Rudbach, unpublished results). Substances used in the mitogen assays were dispersed or dissolved in phosphate-buffered saline and 25 μl was added to each well. The final concentration of each preparation per ml of culture was as follows: (i) BA: 5×10^6, 5×10^7, 5×10^8, 5×10^9, or 1×10^{10} cells/ml; (ii) BA-LPS: 0.5, 5, 50, 250, or 500 μg/ml; (iii) *E. coli* LPS: 0.5, 2.5, 5, 25, or 50 μg/ml; or (iv) ConA: 0.5, 1, 2, 5, or 10 μg/ml.

The cultures were incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 54 h, pulsed with 1 μCi of [^{3}H]thymidine (1.9 Ci/mmol specific activity, New England Nuclear, Boston, Mass.) delivered in 25 μl of RPMI, and incubated for an additional 18 h. The cell cultures were harvested by precipitating the labeled material with 5% aqueous trichloroacetic acid, dissolving the precipitate in 0.15 ml of 1 M NaOH, and transferring the samples into plastic scintillation vials containing 0.8 ml of 0.5 M acetic acid. After the addition of 10 ml of Aquasol, radioactivity in vials was quantified using a Beckman LS-100C liquid scintillation system. In all cases, background counts were low and were subtracted from the experimental values.

RESULTS

Antibody responses of mice to BA. A series of experiments was performed to characterize the magnitude and kinetics of the antibody response by BALB/c, C3H/HeJ, and nude mice to BA. We first examined the total and 2-ME-R antibody titers in these animals after primary and secondary immunizations with 2.5×10^8 BA. Animals were immunized on day 0 and bled 7 days later. On day 17, representative mice from each group were injected again and on day 23 all were bled (Table 1). The day 7 responses by the three groups of mice were low but compa-

TABLE 1. *Primary and secondary antibody responses by BALB/c, C3H/HeJ, and nude mice to BA*[a]

Expt	Mouse strain	Day 7, 1° titer[b]		Day 23, 1° titer[b]		Day 23, 2° titer[c]	
		Total	2-ME-R	Total	2-ME-R	Total	2-ME-R
I	BALB/c	26	<10	806	403	5120	1940
	Nude	49	<10	48	<10	905	61
II	BALB/c	25	<10	134	20	2560	640
	C3H/HeJ	25	<10	160	40	2560	640

[a] Total titers represent the geometric mean of the individual agglutination titers. 2-ME-R titers were determined on pooled sera from the 4 to 10 mice within each group.

[b] Total and 2-ME-R primary (1°) titers were determined on days 7 and 23 after injection of bacterin (2.5×10^8 cells) on day 0.

[c] Secondary (2°) total and 2-ME-R titers were determined on day 23 after a primary immunization on day 0 (2.5×10^8 cells) and a secondary immunization (2.5×10^8 cells) on day 17.

rable. By day 23, however, marked differences were apparent. The day 23 primary responses by BALB/c and C3H/HeJ mice were at least fourfold greater than on day 7 and contained a 2-ME-R component. In contrast, in the nude mice, no increase in total antibody response was apparent between days 7 and 23, and no 2-ME-R antibody was detectable on day 23. When the animals were reimmunized on day 17, heightened antibody synthesis occurred. The day 23 C3H/HeJ and BALB/c secondary titers were at least 10-fold greater than those seen on day 7, and a large portion of these responses was 2-ME-R. Compared with BALB/c and C3H/HeJ mice, nude mice mounted a weaker secondary response which was primarily 2-ME-S with a small 2-ME-R antibody component.

Because of the differences seen by day 23 in the primary responses of BALB/c, C3H/HeJ, and nude mice, we decided to examine more closely the kinetics of the primary response of these mice to a single antigenic stimulation of BA. Accordingly, on day 0, groups of four mice were injected intravenously with one of three BA doses: 7.5×10^8, 7.5×10^6, or 2.5×10^4 cells. After the injection of BA, mice were bled on days 7, 14, 21, and 28, and total antibody and 2-ME-R antibody titers were determined (Fig. 1).

The most striking difference appeared in response to the high dose of 7.5×10^8 BA. Both BALB/c and C3H/HeJ animals responded with antibody synthesis which steadily increased to a titer of about 500. Both groups showed substantial synthesis of 2-ME-R antibody which first appeared after day 14 and 21 in C3H/HeJ and BALB/c mice, respectively. This pattern was conspicuously absent from the nude response in which a titer of 40 was reached by day 7 and did not increase throughout the remaining 21 days of the experiment. The total nude antibody response appeared to be 2-ME-S; at no point was 2-ME-R antibody detected.

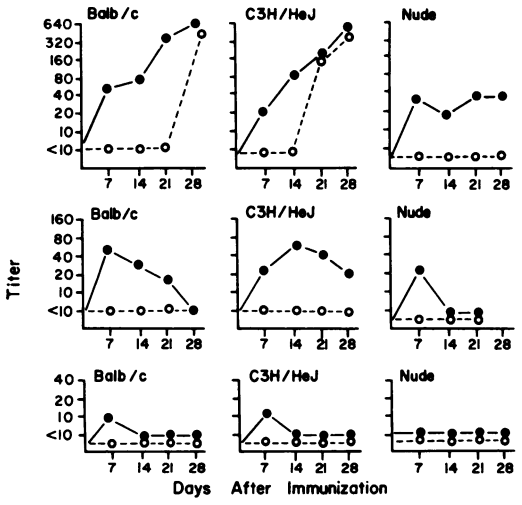

FIG. 1. *Total (●) and 2-ME-R (○) antibody responses of BALB/c, C3H/HeJ, and nude mice given a single injection of 7.5×10^8 (upper panels), 7.5×10^6 (middle panels), or 2.5×10^6 (lower panels) BA cells. Total antibody titers represent the geometric mean of the individual titers of 4 to 10 mice. 2-ME-R antibody titers represent the titer of a pool of sera of the mice in each group.*

In mice receiving 7.5×10^6 BA, a different trend was seen. In BALB/c mice, antibody production peaked at day 7 and decreased slowly until on day 28 no antibody could be detected. The C3H/HeJ response peaked on day 14 and subsided. In neither group was a 2-ME-R component detectable. The response of the nude mice peaked at day 7, and declined below the sensitivity of the assay on days 14 and 21. A 2-ME-R response by nude mice was not detected.

At the low dose of 2.5×10^4 BA, BALB/c and C3H/HeJ mice both produced antibody in quantities sufficient to be detected at day 7. The antibody appeared to be totally 2-ME-S. By day 14 the antibody responses had subsided. No response by nude mice was found at any time.

These experiments (Table 1 and Fig. 1) indicate that BA can elicit primary and secondary antibody responses in BALB/c, C3H/HeJ, and nude mice. The secondary responses are composed of both 2-ME-S and 2-ME-R antibody. The antibody responses of normal and nude mice to BA differ in timing, magnitude, and 2-ME sensitivity. If only a primary injection of BA bacterin is given, the peak titer, kinetics, and the type of antibody detected are dependent on the antigen dose.

Antibody response of mice to BA-LPS. We next performed a series of experiments to examine the antibody responses of BALB/c, C3H/HeJ, and nude mice to BA-LPS. Using selected regimens for injecting the antigen, we were specifically interested in observing the kinetics, antibody type, and antibody titer of the response to this endotoxin preparation.

The kinetics of the primary response to BA-LPS were examined. The antibody titers were determined over a period of time after a single intravenous injection of BA-LPS. In addition to BALB/c and C3H/HeJ mice, nude mice were included in this experiment to determine if the response to BA-LPS, like that to *E. coli* LPS, could be considered to be independent of T cell cooperation. Mice were immunized on day 0 with 50 μg (high dose), 10 or 1 μg (intermediate dose), or 0.01 μg (low dose) of BA-LPS. Animals were bled on days 5, 11, and 30 after administra-

tion of antigen. At doses of 50, 10, and 1 μg of BA-LPS, the BALB/c response appeared to be slightly but consistently higher than that of either nude or C3H/HeJ mice (Table 2). In none of these three groups was 2-ME-R antibody demonstrable between days 0 and 17. Due to animal death in some groups, day 30 responses were not included in Table 2. However, the serum antibody titers of surviving mice were determined. Day 30 titers were diminished from day 17, and no 2-ME-R antibody was detected. Antibody was not detected in any mouse injected with 0.01 μg of BA-LPS.

Little difference in the primary responses of BALB/c C3H/HeJ, and nude mice could be detected over a wide range of BA-LPS. These data suggest that the primary response to BA-LPS, like that to *E. coli* LPS, is thymus independent. These results also indicate that, in contrast to the C3H/HeJ response to *E. coli* LPS, the C3H/HeJ response to BA-LPS does not appear to be aberrant.

TABLE 2. *Serum antibody responses of BALB/c, C3H/HeJ, and nude mice to various doses of BA-LPS*

Dose (μg)	Mouse strain	Serum antibody titers[a]		
		Day 5	Day 11	Day 17
50	BALB/c	48	28	40
	C3H/HeJ	17	12	34
	Nude	34	10	14
10	BALB/c	57	34	40
	C3H/HeJ	28	12	34
1	BALB/c	25	20	28
	C3H/HeJ	20	<10	10
	Nude	24	14	10
0.01	BALB/c	<10	<10	<10
	C3H/HeJ	<10	<10	<10
	Nude	<10	<10	<10

[a] Titers represent the geometric mean of the total serum antibody titers of four mice. 2-ME-R titers were determined on a pool of the sera of the mice in each group; all 2-ME-R titers were less than 10 at the three assay periods.

Next, the primary and secondary responses of BALB/c and C3H/HeJ mice to BA-LPS were determined. Prior experiments (data not shown) indicated that a priming dose of 100, 50, 10, or 1 μg of BA-LPS, when followed by a second immunization with 10 μg of BA-LPS, elicited comparable antibody responses in BALB/c mice. Therefore, on day 0, BALB/c and C3H/HeJ mice were immunized with 10 μg of BA-LPS; they were bled on day 5 (previously determined to be the day of peak antibody titer). On day 18, selected mice in each group were reimmunized with 10 μg of BA-LPS and on day 23 all animals were bled. Total and 2-ME-R antibody titers were determined. As shown in Table 3 (experiment I) the day 5 and day 23 primary responses and day 23 secondary responses by BALB/c and C3H/HeJ mice were identical. 2-ME-R antibody was not detected after a primary immunization with BA-LPS, but the secondary responses elicited by BA-LPS in both BALB/c and C3H/HeJ mice had a strong 2-ME-R antibody component. In a separate experiment (Table 3, experiment II), nude mice also were shown to make a heightened antibody response to a second injection of BA-LPS although the magnitude of the response was less than that of BALB/c mice.

Mitogenic responses of murine cell cultures to BA and BA-LPS. At least two groups of investigators have reported that *Brucella* LPS is not a mitogen (13, 23). However, Kreutzer et al. (13) did not mention whether their preparation was toxic or antigenic. Renoux et al. (23) used a preparation which was nontoxic; the antigenicity of this preparation was not discussed. We decided to examine the mitogenicity of BA-LPS using a preparation which has been shown to be both toxic and antigenic.

Mitogen studies on BA, BA-LPS, and *E. coli* LPS were carried out on cultures of spleen cells derived from BALB/c, C3H/HeJ, and nude

TABLE 3. *Primary and secondary antibody responses by BALB/c, C3H/HeJ, and nude mice to BA-LPS*[a]

Expt	Mouse strain	Day 5, 1° titer[b]		Day 23, 1° titer[b]		Day 23, 2° titer[c]	
		Total	2-ME-R	Total	2-ME-R	Total	2-ME-R
I	BALB/c	28	<10	13	<10	278	160
	C3H/HeJ	25	<10	15	<10	278	160
II	BALB/c	25	ND[d]	ND	ND	368	ND
	Nude	22	ND	ND	ND	106	ND

[a] Total titers represent the geometric mean of the individual agglutination titers. 2-ME-R titers were determined on pooled sera from the 4 to 10 mice within each group.

[b] Total and 2-ME-R primary (1°) titers were determined on days 5 and 23 after injection on day 0 of 10 μg of BA-LPS.

[c] Secondary (2°) total and 2-ME-R titers were determined on day 23 after a primary immunization (10 μg) on day 0 and a secondary injection (10 μg) on day 18.

[d] ND, Not done.

mice, and on thymocyte cultures from BALB/c and C3H/HeJ mice. The primary emphasis of the studies was to determine if both BA and BA-LPS are mitogenic. ConA, a potent stimulator of deoxyribonucleic acid synthesis in T cells of spleen and thymus, was always included as a positive control on the cultures. In all experiments with spleen and thymus cells from BALB/c and C3H/HeJ mice, the ConA responses were within normal stimulation values reported by other investigators; spleen cells from nude mice were unresponsive to ConA.

Shown in Fig. 2 are the data of a typical experiment investigating the effects of BA and BA-LPS on spleen cells from BALB/c, C3H/HeJ, and nude mice. It appears that both the *Brucella* bacterin and the LPS preparation are indeed mitogens. Peak responses to BA by spleen cell cultures from BALB/c and C3H/HeJ mice occurred at 5×10^8 BA cells per ml of culture (10^8 cells per well) and for nude mice at 10^9 cells per well. The peak response to BA-LPS occurred at a dose of 250 μg per ml of culture (50 μg per well). At all doses of BA-LPS, the C3H/HeJ and BALB/c mitogenic responses were very similar to the response by nude spleen cells, thus crediting the idea that BA-LPS is a mitogen for B cells. It also appears that the *Brucella* bacterin is a B cell mitogen.

Kruger and Gershon (14) have suggested that BA is a stimulator of deoxyribonucleic acid synthesis in thymocytes in vivo. Therefore, mitogen assays were performed on cultures of thymus cells from BALB/c and C3H/HeJ mice to determine if their in vivo observations were reflected in an in vitro system. The data in Fig. 3 represent the mitogenic responses by thymocytes from the two mouse strains to ConA, *E. coli* LPS, BA, and BA-LPS. The slight stimulation observed in response to the *Brucella* bacterin and endotoxin might be indicative of modest stimulation of T cells; however, we suggest that the stimulation might be attributed to the presence of B cells trafficking through the thymus or to contamination of the thymocyte preparation with parathymic lymph node cells, rather than to a direct mitogenic effect of BA or BA-LPS on thymocytes.

These experiments (Fig. 2 and 3) suggest that BA and BA-LPS are B cell mitogens. Not only are they stimulatory for spleen cells from BALB/c and nude mice, but they are also mitogenic for C3H/HeJ spleen cells which are refractory to the mitogenic effects of *E. coli* LPS.

DISCUSSION

In the experiments reported here we have examined the antibody responses by mice to BA and BA-LPS extracted by the Redfearn technique from *B. abortus* 1119-3. The emphasis in these studies was to determine the kinetics of the primary and secondary antibody responses to both antigens, the requirement for thymus-derived cells to participate in the antibody and mitogenic responses, and genetic requirements for antibody and mitogenic responses.

Our data indicate that BA is antigenic and that both the antibody titer and type of antibody synthesized are dose dependent (Table 1 and

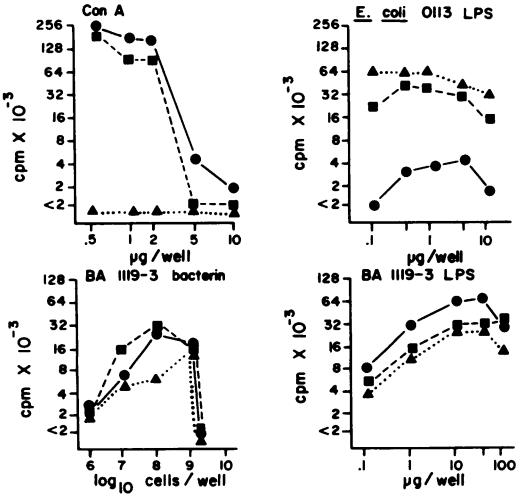

FIG. 2. *[³H]thymidine incorporation by BALB/c (■), C3H/HeJ (●), or nude (▲) mouse spleen cell cultures containing ConA, E. coli 0113 LPS, BA-LPS, or BA.*

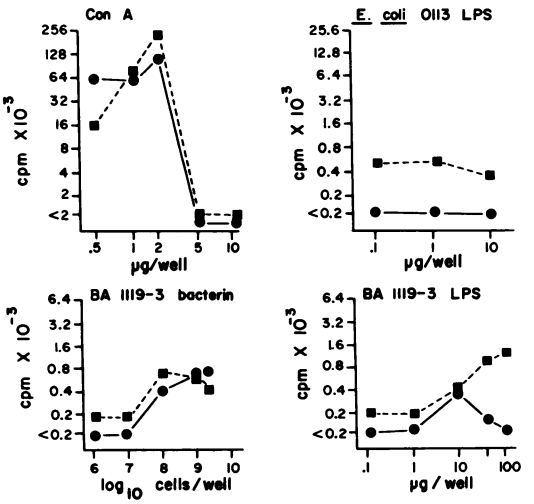

FIG. 3. *[³H]thymidine incorporation by BALB/c (■) or C3H/HeJ (●) thymus cell cultures containing ConA, E. coli LPS, BA, or BA-LPS.*

Fig. 1). Low doses of BA injected into BALB/c or C3H/HeJ mice resulted in a transient 2-ME-S response which peaked on day 7 and rapidly diminished. Nude mice did not respond to the low dose of BA. Intermediate doses of BA elicited 2-ME-S antibody responses in BALB/c, C3H/HeJ, and nude mice; the response peaked between day 7 and day 14 and diminished thereafter. Larger doses of BA elicited both 2-ME-S and 2-ME-R antibody in BALB/c and C3H/HeJ mice. In these mice, antibody titers continued to increase at least until day 28. Nude mice generated only 2-ME-S antibody to this high dose of BA.

When the animals were given a primary injection of 2.5×10^8 BA cells on day 0 and a second injection of 2.5×10^8 BA cells on day 17, the secondary antibody response was composed of both 2-ME-S and 2-ME-R antibody in mice of all three genetic types (Table 1). However, the total nude antibody response was much less than that detected in the BALB/c or C3H/HeJ mice, and the nude 2-ME-R component was proportionately much less than the 2-ME-R component found in the other two mouse groups.

We conclude from these experiments that the antibody response to BA requires T cells for optimum primary and secondary antibody production. This confirms the works of Tingle and Shuster (34), Crewther and Warner (8), and Jacobson et al. (10). However, these results are in conflict with the works of Mond et al. (18) and Thorbecke et al. (33) who, using cell transfers and in vivo systems, claim that BA is a thymus-independent antigen. It appears that BA is immunologically different from *E. coli* bacterin which is clearly a thymus-independent antigen and elicits only IgM production (2). Further, C3H/HeJ mice appear to respond as well as BALB/c mice to primary and secondary challenges with *Brucella* cells. Although data on the antibody response to *E. coli* 0113 bacterin were not shown in this report, Rudbach and Reed (25) have reported that *E. coli* bacterin elicits normal primary responses in both BALB/c and C3H/HeJ mice and that a secondary immunization with this antigen elicits a secondary response in the BALB/c but not in the C3H/HeJ mice (25).

Although Leong et al. (16) have demonstrated structural and physiological similarities between the endotoxins from *Brucella* and the *Enterobacteriaceae*, confusion has persisted concerning the immunological properties of *Brucella* LPS. Divergent reports have arisen from the use of different fractions of phenol-water purifications as the endotoxin-containing portion. In 1960 Redfearn reported that BA-LPS could be recovered from the phenol phase of the Westphal extraction procedure; endotoxin extracted from the *Enterobacteriaceae* has long been known to partition into the aqueous phase. Thus, experiments based on extracted materials incorrectly thought to be the *Brucella* endotoxin indicated that BA-LPS was not cytotoxic, pyrogenic, or antigenic (5, 12, 21, 37). We have, therefore, reexamined the immunogenic and mitogenic properties of biologically active BA-LPS.

Redfearn-extracted BA-LPS, like *E. coli* LPS, appears to be a thymus-independent antigen, at least in its elicitation of a primary antibody response which is composed of 2-ME-S antibody. We base this conclusion on the observation of equivalent antibody titers by BALB/c normal and athymic nude mice (Table 2). Primary injections of BA-LPS, covering a range of 1 to 100 μg of BA-LPS, indicate that the optimum immunological response occurs over a broad dose range. The same phenomenon is observed with *E. coli* LPS (36). However, unlike *E. coli* LPS, which elicits an antibody response in mice detectable by day 3 and waning by days 10 to 14 (17), BA-LPS elicits antibody detectable by day 4 (data not shown) and persisting to at least day 30.

Although BA-LPS and *E. coli* LPS appear similar with respect to evoking T-independent 2-ME-S primary responses in mice, secondary responses by BALB/c and C3H/HeJ mice to BA-LPS suggest that BA-LPS differs immunologically from *E. coli* LPS. BA-LPS elicits both 2-ME-S and 2-ME-R antibody in mice upon a secondary challenge (Table 2) whereas *E. coli* LPS elicits only a heightened 2-ME-S response (17). Although nude mice make secondary responses to BA-LPS, T-cells are apparently required for an optimum secondary response (Table 3). *Brucella* endotoxin appears to differ from *E. coli* endotoxin in at least one other respect. The C3H/HeJ mouse strain is well known for its unusually low response to most LPS preparations of *E. coli* or *Salmonella* spp. (27, 30, 32, 37). However, this strain yielded antibody responses comparable to BALB/c responses to BA-LPS (Tables 2 and 3).

Lastly, both BA and BA-LPS were found to be potent mitogens for spleen cells of BALB/c, C3H/HeJ, and nude mice. The data obtained from spleen cell cultures of nude mice suggest that BA and BA-LPS are B cell mitogens (Fig. 2). These antigens were weakly or perhaps non-mitogenic for thymus cell cultures (Fig. 3). Our demonstration that BA-LPS is a mitogen is in direct conflict with the report by Renoux et al. (23) who claim that BA-LPS is non-mitogenic. However, their preparation lacked toxicity, and

it is unclear if the BA-LPS they used was immunogenic. Recently, Kruetzer et al. (13) and Scheffel et al. (26) have reported that cell wall preparations from BA are mitogenic for rabbit and mouse spleen cell cultures, but they were unable to demonstrate mitogenic activity of phenol- or aqueous-phase BA lipopolysaccharide preparations for mouse spleen cells (13). However, these investigators do not indicate whether or not the BA-LPS preparations were toxic or immunogenic.

One might expect BA-LPS to be a mitogen. BA-LPS contains a lipid A-like component. The lipid A component of enterobacterial lipopolysaccharides has been shown to be mitogenic for B cells (3, 6, 20). However, the high protein content of our BA-LPS preparation may be important in the mitogenic capacity of the endotoxin. It is possible that the mitogenic moiety may be a polypeptide which is retained with the endotoxin during the phenol-water extraction, because BA-LPS contains considerably more protein than does *E. coli* endotoxin. Perhaps the mitogenic response by spleen cell cultures from C3H/HeJ mice to BA-LPS also suggests that a component other than lipid A is responsible for the induced DNA synthesis. In particular, most phenol-water-extracted *E. coli* LPS preparations or purified lipid A are non-mitogenic for C3H/HeJ spleen cells (28). However, Morrison et al. (19) and Sultzer and Goodman (31) have reported that *E. coli* LPS, which contains a polypeptide of about 10^4 daltons, is mitogenic for C3H/HeJ spleen cells.

ACKNOWLEDGMENTS

We thank Lois M. Jones, University of Wisconsin, Madison, Wisc., for valuable advice.

This work was supported by Public Health Service Research Grant CA-24443 from the National Cancer Institute, Research Career Development Award AI-70208 (to N.D.R.) from the National Institute of Allergy and Infectious Diseases, and by a grant from the USDA Agricultural Research Service.

LITERATURE CITED

1. **Adler, F. L.** 1965. Studies on mouse antibodies. I. The response to sheep red blood cells. J. Immunol. **95**:26–38.
2. **Andersson, B., and H. Blomgren.** 1971. Evidence for thymus independent humoral antibody production in mice against polyvinylpyrrolidone and *E. coli* lipopolysaccharide. Cell. Immunol. **2**:411–424.
3. **Andersson, J., F. Melchers, C. Galanos, and O. Luderitz.** 1973. The mitogenic effect of lipopolysaccharide on bone marrow-derived mouse lymphocytes. Lipid A as the mitogenic part of the molecule. J. Exp. Med. **137**: 943–953.
4. **Bauer, D. C., M. J. Mathies, and A. B. Stavitsky.** 1963. Sequences of synthesis of γ-1 macromolecules and γ-2 globulin antibodies during primary and secondary responses to proteins, salmonella antigens and phage. J. Exp. Med. **117**:889–907.
5. **Berger, F. M., G. M. Fukui, B. J. Ludwig, and J. P. Rosselet.** 1969. Increased host resistance to infection elicited by lipopolysaccharides from *Brucella abortus*. Proc. Soc. Exp. Biol. Med. **131**:1376–1381.
6. **Chiller, J. M., B. J. Skidmore, D. C. Morrison, and W. O. Weigle.** 1973. Relationship of the structure of bacterial lipopolysaccharides to its function in mitogenesis and adjuvanticity. Proc. Natl. Acad. Sci. U.S.A. **70**: 2129–2133.
7. **Coutinho, A., and G. Moller.** 1974. Immune activation of B. cells: evidence for "one nonspecific triggering signal" not delivered by the Ig receptors. Scand. J. Immunol. **3**:133–146.
8. **Crewther, P., and N. L. Warner.** 1972. Serum immunoglobulins and antibodies in congenitally athymic (nude) mice. Aust. J. Exp. Biol. Med. Sci. **50**:625–635.
9. **Golub, E. S., and W. O. Weigle.** 1967. Studies on the induction of immunological unresponsiveness. I. Effects of endotoxin and phytohemagglutinin. J. Immunol. **98**: 1241–1247.
10. **Jacobson, E. B., L. H. Caporale, and G. J. Thorbecke.** 1974. Effect of thymus cell injections on germinal center formation in lymphoid tissues of nude (thymusless) mice. Cell. Immunol. **13**:416–430.
11. **Janossy, G., and M. F. Greaves.** 1971. Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. Clin. Exp. Immunol. **9**:483–498.
12. **Kessel, R. W. I., W. Braun, and O. J. Plescia.** 1966. Endotoxin cytotoxicity: Role of cell associated antibody. Proc. Soc. Exp. Biol. Med. **121**:449–452.
13. **Kreutzer, D. L., J. W. Scheffel, L. R. Draper, and D. C. Robertson.** 1977. Mitogenic activity of cell wall components from smooth and rough strains of *Brucella abortus*. Infect. Immun. **15**:842–845.
14. **Kruger, J., and R. K. Gershon.** 1972. DNA synthesis of thymocytes to a variety of antigens. J. Immunol. **108**: 581–585.
15. **Landy, M., and P. J. Baker.** 1966. Cytodynamics of the distinctive immune response produced in regional lymph nodes by *Salmonella* somatic polysaccharide. J. Immunol. **97**:670–679.
16. **Leong, D., R. Diaz, K. Milner, J. Rudbach, and J. B. Wilson.** 1970. Some structural and biological properties of *Brucella* endotoxin. Infect. Immun. **1**:174–182.
17. **Moller, G.** 1975. 19S antibody production against soluble lipopolysaccharide antigens by individual lymphoid cells *in vitro*. Nature (London) **207**:1166–1168.
18. **Mond, J. J., L. H. Caporale, and G. J. Thorbecke.** 1974. Kinetics of B cell memory development during a thymus independent immune response. Cell Immunol. **10**:105–116.
19. **Morrison, D. C., S. J. Betz, and D. M. Jacobs.** 1976. Isolation of a lipid A bound polypeptide responsible for "LPS-initiated" mitogenesis of C3H/HeJ spleen cells. J. Exp. Med. **144**:840–846.
20. **Peavy, D. L., J. W. Shands, Jr., W. H. Adler, and R. T. Smith.** 1973. Mitogenicity of bacterial endotoxins: characterization of the mitogenic principle. J. Immunol. **111**:352–357.
21. **Rank, W. R., R. DiPauli, and U. Flugge-Rank.** 1972. The lipid A immunity system. I. Induction of heterophile antibodies by enterobacterial lipopolysaccharides and their lipid A component. Eur. J. Immunol. **2**:517–522.
22. **Renoux, G., M. Renoux, and R. Tinelli.** 1973. Phenol-water fractions from smooth *Brucella abortus* and *Brucella melitensis*: immunochemical analysis and biologic behavior. J. Infect. Dis. **127**:139–148.
23. **Renoux, M., G. Renoux, A. Palat, and C. Nehemie.** 1976. Activite mitogenique des fractions de Brucella sur les lymphocytes en culture. Dev. Biol. Stand. **31**:230–234.

24. **Rudbach, J. A.** 1971. Molecular immunogenicity of bacterial lipopolysaccharide antigens: establishing a quantitative system. J. Immunol. **106**:993–1001.
25. **Rudbach, J. A., and N. D. Reed.** 1977. Immunological responses of mice to lipopolysaccharide: lack of secondary responsiveness by C3H/HeJ mice. Infect. Immun. **16**:513–517.
26. **Scheffel, J. W., D. L. Kreutzer, D. C. Robertson, and L. R. Draper.** 1977. Responsiveness of rabbit spleen and appendix cells to bacterial mitogens. Infect. Immun. **16**:493–499.
27. **Skidmore, B. J., J. M. Chiller, D. C. Morrison, and W. O. Weigle.** 1975. Immunologic properties of bacterial lipopolysaccharide (LPS): correlation between the mitogenic, adjuvant, and immunologic activities. J. Immunol. **114**:770–775.
28. **Skidmore, B. J., D. C. Morrison, J. M. Chiller, and W. O. Weigle.** 1975. Immunologic properties of bacterial lipopolysaccharide (LPS). II. The unresponsiveness of C3H/HeJ mouse spleen cells to LPS-induced mitogenesis is dependent on the method used to extract LPS. J. Exp. Med. **142**:1488–1508.
29. **Spink, W. W., N. B. McCullough, L. M. Hutchings, and C. K. Mingle.** 1954. A standardized antigen and agglutination technique for human brucellosis. Am. J. Clin. Pathol. **24**:496–498.
30. **Sultzer, B. M.** 1968. Genetic control of leukocyte responses to endotoxin. Nature (London) **219**:1253–1254.
31. **Sultzer, B. M., and G. W. Goodman.** 1976. Endotoxin protein: B-cell mitogen and polyclonal activator of C3H/HeJ lymphocytes. J. Exp. Med. **144**:821–827.
32. **Sultzer, B. M., and B. S. Nilsson.** 1972. PPD tuberculin: B-cell mitogen. Nature (London) New Biol. **240**:198–200.
33. **Thorbecke, G. J., T. Takahashi, and W. P. McArthur.** 1971. Surface antigens of immunocompetent cells, p. 467–479. *In* K. Lindahl-Kiessling, G. Alm, and M. G. Hanna, Jr. (ed.), Morphological and functional aspects of immunity. Plenum Press, New York.
34. **Tingle, A. J., and J. Shuster.** 1974. The role of T lymphocytes in the primary humoral antibody response to brucellin. J. Immunol. **112**:716–721.
35. **Watson, J., R. Epstein, I. Nakoinz, and P. Ralph.** 1973. The role of humoral factors in the initiation of *in vivo* immune responses. II. Effect of lymphocyte mitogens. J. Immunol. **110**:43–52.
36. **Watson, J., and R. Riblet.** 1974. Genetic control of responses to bacterial lipopolysaccharide in mice. I. Evidence for a single gene that influences mitogenic and immunogenic responses to lipopolysaccharides. J. Exp. Med. **140**:1147–1161.
37. **Wilson, J. B., S. Kolbye, and P. J. Baker.** 1964. Role of immunity in sensitivity of mice to Brucella endotoxin, p. 230–246. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Rutgers University Press, New Brunswick, N. J.