INFECTION AND IMMUNITY, Dec. 1986, p. 728–734
0019-9567/86/120728-07$02.00/0

Vol. 54, No. 3

Influence of Endotoxin-Protein in Immunoglobulin G Isotype Responses of Mice to *Brucella abortus* Lipopolysaccharide

ROBIN S. KURTZ AND DAVID T. BERMAN*

Department of Veterinary Science, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received 10 June 1986/Accepted 27 August 1986

***Brucella abortus* endotoxin preparations, containing approximately 5 to 6% protein, induce strong immune and adjuvant immunoglobulin G (IgG) responses as compared with *Escherichia coli* endotoxin preparations, with equivalent amounts of protein, which induce responses in which IgM antibody predominates. Using an enzyme-linked immunoassay with isotype-specific conjugates, we found that antibody of all four subclasses of IgG were evoked during the course of the immune responses of C3H/HeAu mice to *B. abortus* endotoxin. Secondary responses of endotoxin-hyporesponsive C3H/HeJ mice were similar to those seen in C3H/HeAu mice, although lower levels of antibody were produced during their primary responses. The primary responses of BALB/c athymic mice consisted almost entirely of IgG3, and IgG1 appeared following a second injection. The effects of lipopolysaccharide (LPS)-associated protein on the immunogenic properties of *B. abortus* endotoxin were examined by comparing responses to endotoxin with those to a purified *B. abortus* LPS containing <1% protein. The endotoxin evoked strong primary and secondary responses in which antibody directed to LPS determinants consisted mainly of IgG3 and those to the protein determinants were largely IgG1 antibody. Primary and secondary responses to purified LPS consisted mainly of IgG3 antibody. The potential mechanism of the contribution of protein to the immunogenic properties of the endotoxin as well as possible immune mechanisms involved in these responses are discussed.**

We have shown previously that the immunogenic and adjuvant properties of preparations of *Brucella abortus* endotoxin were different from those of lipopolysaccharide (LPS) from *Escherichia coli* (13). Consistent with previous reports (16, 22), *E. coli* LPS did not stimulate significant primary or seondary specific antibody responses in LPS-hyporesponsive C3H/HeJ mice and induced the production of immunoglobulin M (IgM) and only low levels of IgG antibodies in congenic LPS-responsive C3H/HeAu mice, as well as in euthymic and athymic BALB/c mice. By contrast, *B. abortus* endotoxin stimulated primary and secondary responses in which IgM and high levels of IgG antibody were produced by both responsive and hyporesponsive C3H/He mice and by athymic mice as well. Although it has been established that IgG antibody production in response to LPS, as well as to other thymus-independent antigens, can be detected if sensitive specific methods are used (10, 17, 18), IgG levels of the magnitude induced by *B. abortus* endotoxin have not been reported. Furthermore, the IgG antibody responses of athymic mice to the *B. abortus* endotoxin were particularly noteworthy.

Karch et al. (5) and Karch and Nixdorff (6) reported that outer membrane components of *Proteus mirabilis* had a profound effect on primary or secondary splenic plaque-forming cell (PFC) responses of outbred NMRI mice to LPS from *P. mirabilis* or *E. coli*. Primary and secondary responses to LPS alone consisted principally of IgM PFC, with a small number of IgG PFC produced in the secondary response. LPS in complexes with bacterial membrane phospholipids, outer membrane proteins, or hydrophobic nonbacterial proteins stimulated production of increased numbers of IgG PFCs, with the relative proportions of IgG1, IgG2, and IgG3 isotypes varying, depending on the nature of the protein or phospholipid in the complex.

* Corresponding author.

We had pointed out that these findings, as well as those of many others on the immunomodulatory properties of outer membrane protein components, raise questions about the contribution made by the 5 to 6% protein content of our *B. abortus* endotoxin preparations to their unusual immunogenicity and adjuvanticity, although the *E. coli* preparation used also had 5% protein. Evidence was presented supporting the hypothesis that the unusual fatty acid composition of the lipid moiety of the *B. abortus* endotoxin, and not its protein component, was responsible for these biological activities (11, 12). The refractiveness of *B. abortus* endotoxin-protein to dissociation from LPS has made it impossible until now to determine whether the IgG antibody produced in primary and secondary responses is directed against O polysaccharide determinants, against protein determinants, or against both. In this report we describe the antibody responses induced by *B. abortus* endotoxin, containing 5 to 6% protein, and LPS, containing <1% protein, obtained after proteinase K digestion and gel filtration of the endotoxin. The IgG isotypes of antibody produced by hyporesponsive C3H/HeJ, responsive C3H/HeAu, and euthymic and athymic BALB/c mice were determined by means of an isotype-specific enzyme-linked immunoassay (ELISA).

MATERIALS AND METHODS

Endotoxin and LPS. The extraction by phenol-water, purification, chemical analysis, and characterization of endotoxin from *B. abortus* 1119-3 have been described previously (14). To reduce protein content, 100 mg of endotoxin containing 6% protein was dissolved in 10 ml of 0.2 M Tris hydrochloride buffer (pH 8) with sonication. A 5-mg portion of proteinase K (Sigma Chemical Co., St. Louis, Mo.) was added, and the mixture was held at room temperature for 3 h and at 4°C for 12 h. Five volumes of methanol, containing 1% methanol saturated with sodium

acetate, was added, and the LPS was sedimented at 7,000 × *g*. The pellet was redissolved in 10 ml of 0.2 M Tris hydrochloride (pH 8) and the procedure was repeated. The precipitate was dissolved in distilled H_2O, dialyzed against distilled H_2O, and lyophilized. Portions, 50 mg, were dissolved in 5 ml of 6 M guanidine HCl in 0.2 M Tris hydrochloride (pH 8) and chromatographed in a Sephacryl S-200 column (80 by 2 cm) equilibrated with the same buffer. Samples, 5 ml, were collected, and the presence of LPS was monitored by methanol-sodium acetate precipitation of aliquots. The first peak, obtained in the void volume of the column, contained the LPS which was precipitated with 3 volumes of cold methanol-sodium acetate and sedimented at 10,000 × *g* and 4°C for 15 min. The LPS was finally dissolved in 5 ml of double-distilled H_2O, dialyzed against multiple changes of distilled H_2O at 4°C for 4 days, and finally lyophilized. The final yield was approximately 50% of the weight of the starting preparation. All preparations were analyzed for protein by the Lowry method (9) and for 2-keto-3-deoxyoctulosonic acid by the method described by Warren (21), as modified by Karkhanis et al. (7). Protein, as percentage of dry weight of four such preparations, ranged from 0.73 to 1.2. 2-Keto-3-deoxyoctulosonic acid was 0.84% in endotoxin and 0.85% in LPS. Values for protein concentration of the LPS were verified on the basis of amino acid values obtained by automated analysis of hydrolysates, using ninhydrin reagent, in a Durram D-500 (Dionex) amino acid analyzer as described previously (12). Only those preparations of *B. abortus* endotoxin with ≤6% protein and LPS with ≤1% protein were used for immune response experiments.

Mice. Male and female C3H/HeAu, C3H/HeJ, and BALB/c (*nu*/+) mice, were raised in our own colony (11). BALB/c (*nu*/*nu*) mice were bred from BALB/c (*nu*/+) females and BALB/c (*nu*/*nu*) thymus-reconstituted males. The nude mouse colony was kept in cages equipped with filter covers in laminar flow units and fed autoclaved mouse food (Purina Co., St. Louis, Mo.) and chlorinated water.

Immunizations. In previous experiments (14) mice injected intravenously with 20 μg of endotoxin made optimal antibody responses. Animals in groups of four to seven mice were injected intravenously with 0.2 ml of a solution containing 100 μg of either *B. abortus* endotoxin or LPS per ml. Mice were bled from the tail on day 0 and subsequently at 3- to 7-day intervals. Blood was collected in four heparinized capillary tubes per mouse; plasma was separated from cells by centrifugatigon (750 × *g*), diluted 1:50 in phosphate-buffered saline-Tween 20, and kept at 4°C until assayed. The primary antibody response was determined over a period of 60 days after a single injection of endotoxin or LPS. The secondary response was followed after a second injection given on day 60.

Control sera. Positive control anti-*B. abortus* endotoxin sera were obtained from C3H/HeAu mice immunized by three intraperitoneal injections of 0.2 ml each of emulsions containing 150 μg of endotoxin per ml of Freund complete adjuvant, during a one-month period. One week after the last injection, the sera from five animals were pooled and tested for antibody by ELISA (13). Negative control sera were obtained from unimmunized, age-matched C3H/HeAu or BALB/c (*nu*/+) mice.

Conjugates. Monospecific rabbit sera against mouse IgM and IgG (Miles Laboratories, Elkhardt, Ind.) showed only one line of precipitation by immunoelectrophoresis and immunodiffusion with mouse serum or the appropriate immunoglobulin-rich fraction. Affinity-purified anti-mouse IgG1, IgG2a, IgG2b, and IgG3 sera were purchased from Litton Bionetics (Kensington, Md.).

Anti-mouse immunoglobulin conjugates were prepared by dialyzing the antisera aginst 0.01 M carbonate buffer (pH 9.5) and conjugating them to horseradish peroxidase (type VI; Sigma) by the method of Nakane and Kawaoi (19).

ELISA. ELISA was performed as described by Engvall and Perlmann (3) and modified by Lamb et al. (8). Plates were coated with antigen by dispensing 0.2 ml of a solution of endotoxin (1 μg/ml) or LPS (10 μg/ml) in 0.06 M carbonate buffer (pH 9.6) into the wells of 96-well polystyrene Immulon I microtiter plates (Linbro; Flow Laboratories, Inc., McLean, Va.). Plates were incubated for 3 h at 37°C and then kept at 4°C until used. Mouse serum was diluted 1:250, and conjugates were also used at a 1:250 dilution. These dilutions were selected on the basis of experiments in which they produced maximal differences in final absorbance values between positive and negative sera while remaining in the linear range of the spectrophotometer. Negative control sera were included with each day's assays, and the optical density values were subtracted from those for the sera of immunized animals. *O*-Phenylenediamine (Eastman Kodak Co., Rochester, N.Y.) was used as a substrate. After 30 min the enzymatic reaction was stopped with 0.05 M H_2SO_4, and absorbance was read at 490 nm.

Validation of method. The specificity of anti-isotype conjugates was confirmed in an ELISA, using purified myeloma proteins of each isotype (Litton Bionetics) as test antigens. These proteins were adsorbed on wells of microtiter plates at a concentration of 10 μg/ml, incubated at 37°C for 3 h, and kept at 4°C until used. Conjugates (1:250 dilution) were added directly to the plates and incubated for 5 h at room temperture. Plates were subsequently washed three times with phosphate-buffered saline-Tween, substrate was added, and standard ELISA procedures were followed.

Contribution of endotoxin-protein to immunogenic properties of *B. abortus* LPS. The contribution of endotoxin-protein to the immunogenic properties of *B. abortus* LPS was determined in two experiments. In the first, BALB/c athymic mice were given two intravenous injections, 60 days apart, of 20 μg of *B. abortus* endotoxin containing 5% protein, as described above. Serum samples obtained from individual mice 10, 15, 20, and 25 days following the second injection were then assayed by ELISA, using both crude endotoxin, containing ca. 15% protein, and purified LPS, containing <1% protein, as test antigens. Crude endotoxin was used as test antigen, as it had been found that ELISA values were nearly identical in tests with it and a partially purified preparation, designated f5p, which contained approximately 6% protein (13). Absorbance values for each test antigen were plotted together against time to facilitate comparison. In the second experiment, four BALB/c euthymic mice were immunized with purified *B. abortus* LPS containing <1% protein on the same schedule. Serum samples were then assayed by ELISA, using the purified LPS as test antigen.

RESULTS

Validation of method. Moreno et al. (13) used an ELISA with IgM- and IgG-specific cojugates to characterize the magnitude, kinetics, and immunoglobulin classes of antibody produced by individual mice of different strains immunized with various preparations of *B. abortus* endotoxin. Use of this system was justified by the fact that comparable results were obtained with the immunoglobulin class-specific

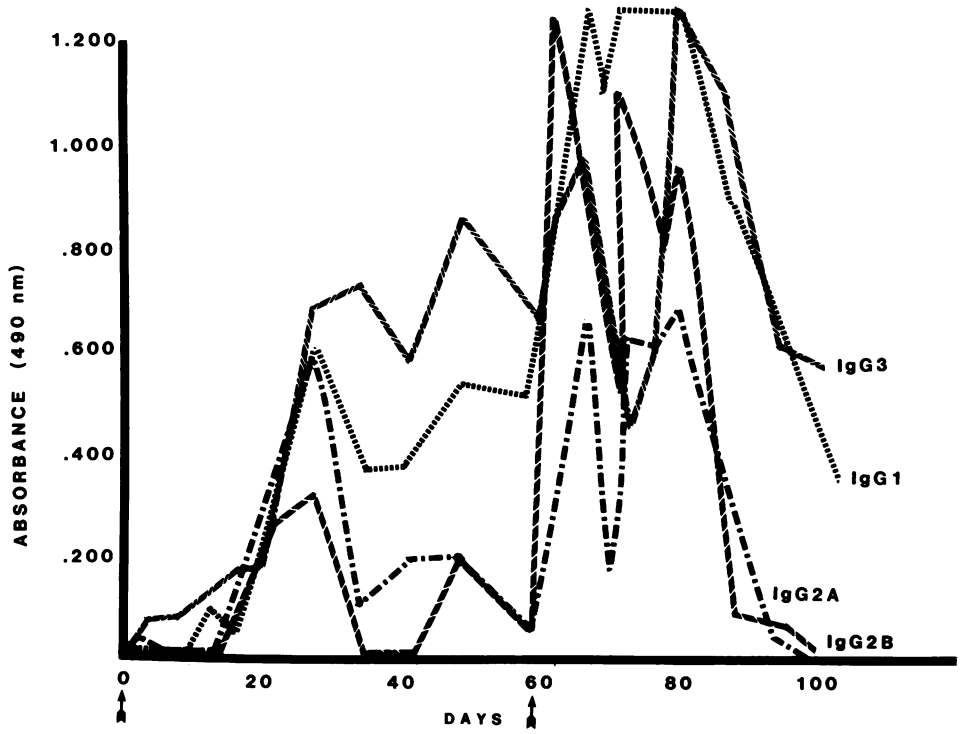

FIG. 1. Primary and secondary isotype-specific antibody responses of a C3H/HeAu mouse to *B. abortus* endotoxin (5% protein) as determined by ELISA, with crude endotoxin (15% protein) as test antigen. Arrows indicate the times of the first and second injections of endotoxin.

ELISA and agglutination tests performed with and without 2-mercaptoethanol. A micro-ELISA was used in the experiments described here so that assays could be made with each plasma sample for antibody of all four isotypes of IgG, as well as total IgG and IgM. To establish the specificity of these conjugates, they were assayed with myeloma protein test antigens. Although some cross-reactivity was apparent, the absorbance value for each conjugate was greatest with its respective myeloma protein (Table 1). Since the concentration of each myeloma protein in the ELISA at 10 μg/ml was greater than its physiological concentration in a 1:250 dilution of whole mouse serum, cross-reactivity among immunoglobulin subclasses should not constitute a major source of error. Although these values permit a good approximation of the relative concentration of each immunoglobulin subclass in the test serum or plasma, an exact correlation cannot be obtained with this system because of differences in affinity of antibody over the course of the response (20).

Profiles of anti-*B. abortus* endotoxin production. The response profile of each individual mouse was plotted, and a representative profile, neither the highest nor the lowest, was chosen for presentation. The profile of anti-endotoxin antibody production by a C3H/HeAu mouse, representative of the four immunized, is shown in Fig. 1. All four subclasses of IgG were evoked during the course of both primary and secondary responses. IgG3 was the predominant immunoglobulin expressed during the primary response, and it remained at a relatively high level throughout the secondary response. IgG1 antibody was present in the primary response and increased to levels comparable to those of IgG3 in the secondary response. IgG2b antibody was present at low levels during the primary response, and it increased dramatically within 3 days after the second injection. IgG2a antibody was present throughout both responses but never was predominant.

The isotype pattern of a C3H/HeJ mouse representative of the four immunized is shown in Fig. 2. The secondary response to *B. abortus* endotoxin was nearly identical to that seen with C3H/HeAu mice, but there were significant differences in primary responses. Although all four isotypes were present, C3H/HeJ mice produced less antibody of each isotype and IgG3 was not predominant. Furthermore, IgG1 peaked at day 45 but never reached levels as high as those in the congenic endotoxin-responsive mice.

The profile of the response of a BALB/c (*nu/nu*) mouse, representative of the seven athymic mice immunized, is shown in Fig. 3, in which the most striking feature was the relatively low levels of IgG1, IgG2a, and IgG2b in the primary anti-*B. abortus* endotoxin response. By comparison, IgG3 antibody was present at high levels during both the

TABLE 1. ELISA of isotype-specific conjugates with mouse myeloma proteins of each IgG isotype

Conjugate[a]	A_{490} of myeloma protein[b] of:			
	IgG1	IgG2a	IgG2b	IgG3
Anti-IgG1	**1.862**[c]	0.222	0.225	0.196
Anti-IgG2a	0.157	**0.629**	0.227	0.157
Anti-IgG2b	0.323	0.464	**1.433**	0.275
Anti-IgG3	0.327	0.464	0.398	**1.759**
Anti-IgG	**2.308**	**2.563**	**3.371**	**2.344**
Anti-IgM	0.141	0.129	0.143	0.123

[a] Conjugates used at a concentration of 1:250.
[b] Myeloma proteins coated onto microtiter plates at a concentration of 10 μg/ml.
[c] Boldface values indicate homologous sets.

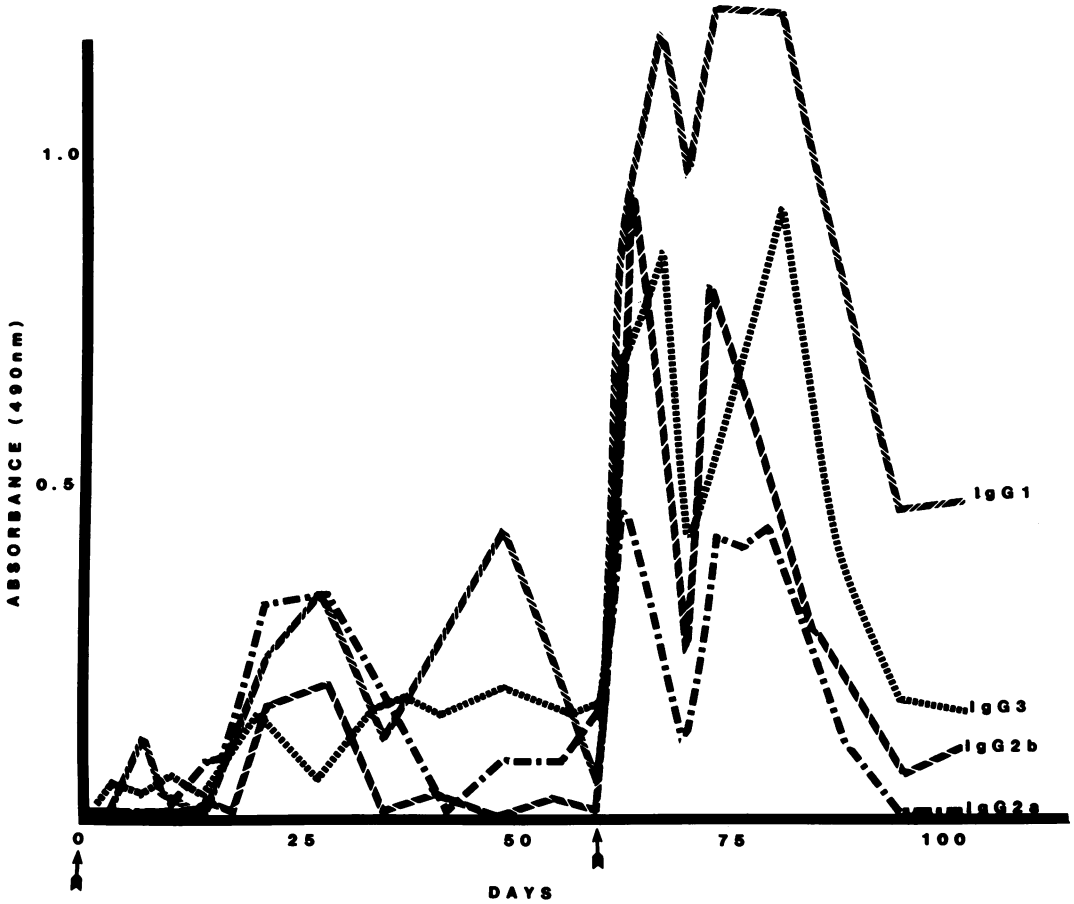

FIG. 2. Primary and secondary isotype-specific antibody responses of a C3H/HeJ mouse to *B. abortus* endotoxin (5% protein) as determined by ELISA, with crude endotoxin (15% protein) as test antigen. Arrows indicate the times of first and second injections of endotoxin.

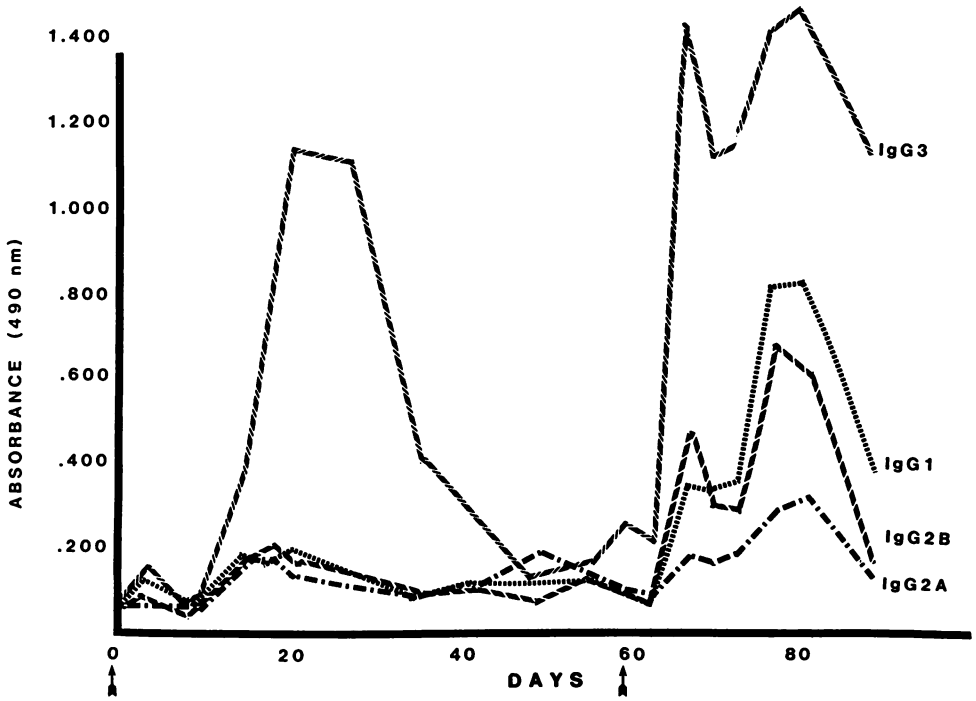

FIG. 3. Primary and secondary isotype-specific antibody responses of a BALB/c athymic mouse to *B. abortus* endotoxin (5% protein) as determined by ELISA, with crude endotoxin (15% protein) as test antigen. Arrows indicate times of first and second injections of endotoxin.

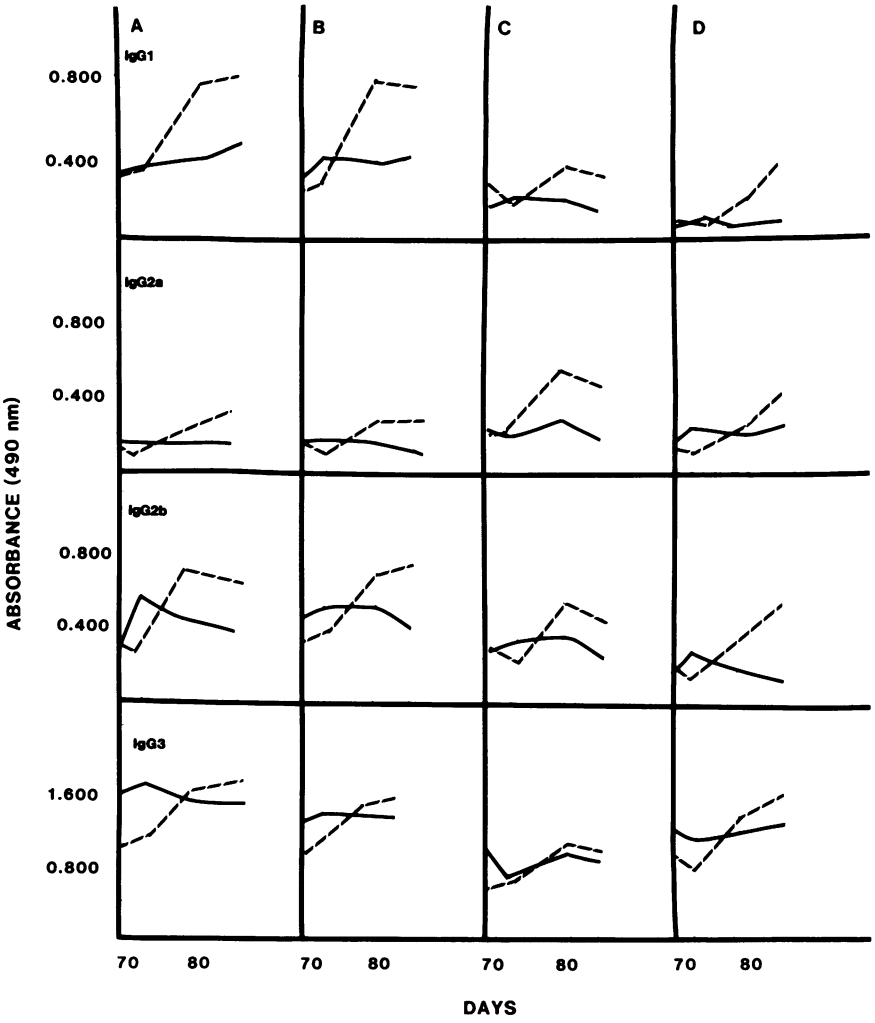

FIG. 4. Contribution of antibody specific for protein determinants to the total anti-*B. abortus* endotoxin responses. Athymic BALB/c mice received two intravenous injections of 20 μg of endotoxin 60 days apart. Sera obtained on days 70, 73, 77, and 81 were assayed with crude endotoxin (15% protein) and purified LPS (<1% protein) as test antigens by ELISA. Parts A, B, C, and D represent values for individual mice. Note difference in scale for IgG3. Symbols: Solid line, absorbance values with LPS as test antigen; broken line, abosrbance values with crude endotoxin as test antigen.

primary and secondary responses. IgG1 and IgG2b became apparent by 10 days following the second injection but never reached levels as high as those in euthymic mice of all strains. Although there was considerable variation in the amounts of IgG1, IgG2a, and IgG2b produced by the individual athymic mice during the primary response, all responses were similar in the low levels of these isotypes. These low levels do not reflect a mouse strain difference because the antibody isotype profiles of four BALB/c (*nu*/+) mice immunized with endotoxin (not shown) were not different from those of the euthymic C3H/He mice.

Contribution of endotoxin-protein to immunogenic properties of LPS. The antibody isotype response profiles to *B. abortus* endotoxin with endotoxin or LPS as test antigens were compared with serum samples from four athymic mice obtained 10, 15, 20, and 25 days following a secondary injection of *B. abortus* endotoxin. These were the time intervals at which all four IgG isotypes were present at maximal levels (Fig. 3). Differences observed in the profiles with the two test antigens could be attributed only to a contribution of antibody directed against protein determinants. A substantial proportion of the anti-endotoxin IgG1, IgG2a, and IgG2b antibodies was directed against protein determinants present on endotoxin but not on purified LPS (Fig. 4). By contrast, the levels of IgG3 antibody were substantially higher than those of the other isotypes and were similar with both endotoxin and LPS as test antigens.

Isotype profiles of the responses of four euthymic BALB/c (*nu*/+) mice to purified LPS were determined with LPS as test antigen, and one for a representative mouse is shown in Fig. 5. IgG3 was the predominant isotype of antibody produced during the course of the primary and secondary anti-*B. abortus* LPS responses. Although IgG1 was present at low levels, IgG2a and IgG2b were barely detectable.

DISCUSSION

The earliest views that thymus-independent antigens induce little or no IgG antibody in mice have been modified by the demonstration that polysaccharide antigens, as well as LPS, can induce substantial amounts of IgG3 and lesser amounts of IgG1, IgG2a, and IgG2b antibody (17). Although

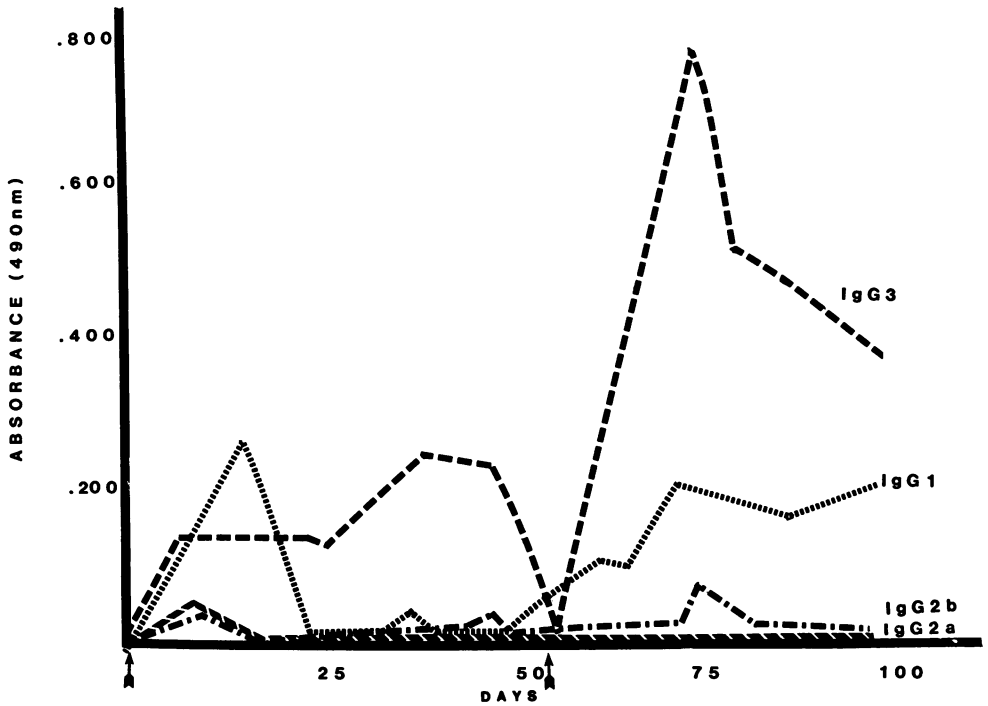

FIG. 5. Primary and secondary isotype-specific antibody responses of a euthymic BALB/c mouse to *B. abortus* LPS (<1% protein) as determined by ELISA. Note expanded scale of ordinate. LPS was used as immunogen and test antigen. Arrows indicate times of primary and secondary injections of LPS.

some investigators (2) found that enterobacterial LPS induced little or no IgG1 antibody, Karch et al. (5) described small but significant increases in numbers of specific IgG1, IgG2, and IgG3 splenic PFCs 5 days after a secondary injection of *P. mirabilis* LPS. Clearly, it was important to determine which isotypes were contributing to the unusually high IgG responses to *B. abortus* endotoxin by mice of each strain. All four subclasses of IgG were produced by C3H/HeAu mice during the course of their primary and secondary responses to *B. abortus* endotoxin. The antibody responses by C3H/HeJ mice were not idential in that, although all four isotypes of IgG were evoked during their primary response, the levels were lower than those in C3H/HeAu mice. The secondary responses of mice of both strains were similar and included high levels of all isotypes. These responses by euthymic mice suggest participation of T cells, as we had proposed, for total IgG responses to endotoxin (13).

The isotype profile of athymic mice was different from that seen with euthymic mice in that the primary and secondary response was largely IgG3, although substantial amounts of IgG1, IgG2a, and IgG2b were also expressed during the secondary response. These data clarify the apparently anomalous primary IgG antibody responses by nude mice immunized with *B. abortus* endotoxin that we had reported earlier (13). That is, the IgM to IgG3 switch is known not to require T-cell participation. On the other hand, the sharp peak of IgG1 antibody after the second injection does imply T-cell help. We have been unable to detect significant differences in the numbers of Thy-1^+ cells by flow cytometry of splenocyte suspensions obtained at various times after secondary stimulation of nude mice with *B. abortus* endotoxin, as compared with sham-treated, age-matched nude mice (data not shown). It remains unclear whether this immunization regimen induces maturation of small numbers of specific T helper cells at levels undetectable by flow cytometry or whether the isotype switch is mediated entirely by B cells alone or in concert with macrophages and their products.

B. abortus endotoxin preparations used in the first series of experiments (Fig. 1 to 3) were prepared by a method with which it had not been possible to reduce their protein content below 5 to 6% (14). The development of a procedure which resulted in preparations of *B. abortus* LPS with ≤1% protein made it possible to define the influence of the protein. In experiments with endotoxin as immunizing antigen (Fig. 4), it is clear that most of the antibody of IgG1, IgG2a, and IgG2b isotypes was directed against protein determinants present on the endotoxin preparation used as test antigen. IgG3 directed against LPS determinants was the predominant IgG isotype produced with either endotoxin or LPS as immunizing antigen. With LPS as immunizing antigen, although several peaks of IgG1 were observed, their levels were lower than those found when endotoxin was used as immunogen and IgG2a and IgG2b antibodies were at barely detectable levels.

There are two unique aspects of these results. First, an unusually high level of IgG3 antibody with only small amounts of IgM antibody was produced in response to primary and secondary injections of *B. abortus* LPS. Second, *B. abortus* endotoxin induced high levels of all IgG isotypes, again with only a small IgM response. This is unusual, as many other investigators, using commercial preparations of enterobacterial LPS which probably contained endotoxin protein in amounts comparable to those present in our *B. abortus* endotoxin preparations, have described antibody responses typical of thymus-independent antigens.

It remains to be determined whether the unusual immunogenic properties of the *B. abortus* endotoxin complex are a contribution of the endotoxin protein alone or are con-

ferred by the hydrophilic-hydrophobic nature or state of aggregation of the preparations, as suggested by Karch et al. (5). It would be possible to distinguish among these alternatives by comparing the immunogenicity of *B. abortus* endotoxin protein-*E. coli* LPS complexes and *E. coli* endotoxin protein-*B. abortus* LPS complexes. So far, we have been unable to dissociate *B. abortus* LPS from its endotoxin protein without destruction of the protein. Similarly, preparations of major outer membrane proteins of *B. abortus* are strongly bound to LPS (15).

The cyclic primary and secondary antibody responses of all euthymic mice immunized with endotoxin bear a superficial resemblance to the oscillatory IgM PFC responses to *E. coli* LPS (1, 4). We had not previously (13) observed significant oscillations in circulating IgM antibody levels by these methods, nor did we see it in the experiments reported here (data not shown). It is clear (Fig. 3 and 5) that there was not detectable oscillation of primary IgG responses either of athymic mice immunized with endotoxin or in primary or secondary responses of euthymic mice immunized with LPS. Studies with various priming and booster doses of antigen, as well as kinetic studies of isotype-specific PFCs, would be necessary for interpretation of this aspect of regulation.

The demonstration of the unusual ability of *B. abortus* LPS to stimulate strong and predominant IgG3 antibody responses also makes it an attractive probe with which to study mechanisms of the IgM to IgG class switch. Experiments in which the interactions of *B. abortus* LPS with purified B-cell, T-cell, and macrophage populations have been investigated will be presented elsewhere (R. S Kurtz and D. T. Berman, manuscript in preparation).

ACKNOWLEDGMENTS

We thank E. Moreno for advice on the proteinase K digestion procedure and other aspects of the experiments, D. Omilianowski for the amino acid analysis, R. Auerbach and L. W. Morrissey for help with the flow cytometry, and Louise Boettcher-Troxel for expert technical assistance in all experiments.

This investigation was supported by the College of Agricultural and Life Sciences, by cooperative agreement 58-6125-6-1 with the Agricultural Research Service, U.S. Department of Agriculture, and by a grant from the Research Committee of the Graduate School.

LITERATURE CITED

1. **Baker, P. J., J. R. Heirnaux, P. W. Stashak, and J. A. Rudbach.** 1985. Cyclic development of immunological memory to bacterial lipopolysaccharide. Infect. Immun. **48**:1–6.
2. **Danneman, P. J., and J. G. Michael.** 1976. Adjuvant and immunogenic properties of bacterial lipopolysaccharide in IgE and IgG1 antibody formation in mice. Cell. Immunol. **22**:128–139.
3. **Engvall, E., and P. Perlmann.** 1971. Enzyme-linked immunosorbent assay (ELISA): quantitative assay of immunoglobulin. Immunochemistry **8**:871–874.
4. **Hiernaux, J. R., P. J. Baker, and C. DeLisi.** 1982. Oscillatory immune response to lipopolysaccharide, p. 121–136. *In* C. DeLisi and J. R. Hiernaux (ed.), Regulation of immune response dynamics. CRC Press, Inc., Boca Raton, Fla.
5. **Karch, H., J. Gmeiner, and K. Nixdorff.** 1983. Alteration of the immunoglobulin G subclass responses to lipopolysaccharide: effects of nonbacterial proteins and bacterial membrane phospholipids or outer membrane proteins of *Proteus mirabilis*. Infect. Immun. **40**:157–165.
6. **Karch, H., and K. Nixdorff.** 1981. Antibody-producing cell responses to an isolated outer membrane protein and to complexes of this antigen with lipopolysaccharide or with vesicles of phospholipids from *Proteus mirabilis*. Infect. Immun. **31**:862–867.
7. **Karkhanis, Y. D., J. Y. Zeltner, J. J. Jackson, and D. J. Carlo.** 1978. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysacharide. Anal. Biochem. **85**:595–601.
8. **Lamb, V. L., L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Enzyme-linked immunosorbent assay for bovine immunoglobulin subclass-specific response to *Brucella abortus* lipopolysaccharide. Infect. Immun. **26**:240–247.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
10. **Mongini, P. K., K. E. Stein, and W. E. Paul.** 1981. T cell regulation of IgG subclass antibody production in response to T-independent antigen. J. Exp. Med. **153**:1–11.
11. **Moreno, E., and D. T. Berman.** 1979. *Brucella abortus* lipopolysaccharide is mitogenic for spleen cells of endotoxin resistant C3H/HeJ mice. J. Immunol. **123**:2915–2919.
12. **Moreno, E., D. T. Berman, and L. A. Boettcher.** 1981. Biological activities of *Brucella abortus* lipopolysaccharide. Infect. Immun. **31**:362–369.
13. **Moreno, E., R. S. Kurtz, and D. T. Berman.** 1984. Induction of immune and adjuvant immunoglobulin G responses in mice by *Brucella* lipopolysaccharide. Infect. Immun. **46**:74–80.
14. **Moreno, E., M. W. Pitt, L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Purification and characterization of smooth and rough lipopolysaccharide from *Brucella abortus*. J. Bacteriol. **138**:361–369.
15. **Moriyon, I., and D. T. Berman.** 1983. Isolation, purification, and partial characterization of *Brucella abortus* matrix protein. Infect. Immun. **39**:394–402.
16. **Morrison, D. C., and J. L. Ryan.** 1979. Bacterial endotoxins and host immune responses. Adv. Immunol. **28**:377–390.
17. **Mosier, D. E., and B. Subbarao.** 1982. Thymus-independent antigens: complexity of B lymphocyte activation revealed. Immunol. Today **3**:217–222.
18. **Motta, I., D. Portnoi, and P. Truffa-Bachi.** 1981. Induction and differentiation of B memory cells by a thymus-independent antigen, trinitrophenylated-lipopolysaccharide. Cell. Immunol. **57**:327–338.
19. **Nakane, P. K., and A. Kawaoi.** 1974. Peroxidase labelled antibody, a new method of conjugation. J. Histochem. Cytochem. **22**:1084–1091.
20. **Steward, M. W., and A. M. Lew.** 1985. The importance of antibody affinity in the performance of immunoassays for antibody. J. Immunol. Methods **78**:173–190.
21. **Warren, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**:1971–1975.
22. **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 LPS. J. Exp. Med. **140**:1147–1161.