INFECTION AND IMMUNITY, July 1980, p. 132-139
0019-9567/80/07-0132/08$02.00/0

Vol. 29, No. 1

Influence of Carrier-Specific, Thymus-Derived Cells on the Immunoglobulin M Antibody Response to Staphylococcal Lipoteichoic Acid

PAUL R. BEINING,[1]* GERALDINE M. FLANNERY,[2] BENJAMIN PRESCOTT,[2] AND PHILLIP J. BAKER[3]

University of Scranton, Scranton, Pennsylvania 18510[1]; Biomedical Research Institute, Rockville, Maryland 20852[2]; and Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205[3]

The immunoglobulin M antibody response to the lipoteichoic acid (LTA) of *Staphylococcus aureus* ATCC 6538P was examined by a procedure in which erythrocytes sensitized with periodate-activated LTA were used for the detection of immunoglobulin M-producing plaque-forming cells. LTA-specific plaque-forming cells were first detected 2 days after immunization with heat-killed bacterial cells, and maximal numbers of plaque-forming cells, mostly of the immunoglobulin M class rather than the immunoglobulin G or immunoglobulin A class, were attained by day 4; specificity for LTA was affirmed by plaque inhibition tests. No plaque-forming cells were found in mice given isolated LTA over a 10,000-fold range of immunizing doses. Mice pretreated with a carrier known to activate thymus-derived helper lymphocytes produced a plaque-forming cell response to LTA only when immunized with LTA bound to the same carrier. This suggests that carrier-specific thymus-derived cells are needed to initiate an antibody response to poorly immunogenic LTA. Since an antibody response can be elicited in mice given heat-killed cells, other cell wall and/or cell membrane constituents may play an important role as immunologically active carriers for this antigen.

Lipoteichoic acids (LTA) of gram-positive bacteria are composed of a glycerol phosphate polymer covalently linked to a lipid moiety (19, 40) and substituted with glycosyl (3) and D-alanyl ester groups (38). It is hypothesized that this LTA is located mainly in the mesosome (26) or plasma membrane (39); however, it can function as a surface antigen as well (40).

The injection of rabbits with whole bacteria or disintegrated gram-positive organisms results in the production of several classes of circulating antibodies against LTA (30, 41); also, the administration of soluble streptococcal teichoic acid adsorbed to methylated bovine serum albumin stimulates the formation of serum antibody specific for teichoic acid (22). However, depending on the nature and extent of the extraction procedure used, as well as the amount of contaminating protein present (15, 29), LTA per se elicits a weak antibody response and is considered to be a poor immunogen.

Because of the presence of LTA in staphylococci, it is desirable to characterize more fully the antibody response to this antigen, especially at the cellular level, so that its potential role in the pathogenesis of staphylococcal infections can be assessed. In the present work, the immunoglobulin class of antibody made after immunization with LTA and the kinetics of immunoglobulin M (IgM) antibody production were examined by using a newly developed procedure for the detection of cells making antibody specific for this antigen. Also, the requirement for a carrier in order to obtain a significant IgM antibody response to this antigen was investigated. The results obtained show that the immunogenicity of LTA can be improved greatly by attaching it to a carrier capable of generating carrier-specific thymus-derived (T) helper cells.

MATERIALS AND METHODS

Animals. The selection of the strains of mice used in this work was based primarily on their availability. Female mice 8 to 10 weeks old were used in this work. BALB/cBy and C57BL/6By mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/cCum mice were obtained from Cumberland View Farms, Clinton, Tenn.

Preparation of heat-killed bacterial cells. *Staphylococcus aureus* ATCC 6538P was grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). The cells were harvested by centrifugation, washed with saline, heat killed, and then treated with Formalin, as described by Dubos (21) and Campbell et al. (17). The cell suspensions, which were adjusted in concentration by direct microscopic counts, were stored at 4°C in saline until used.

Preparation of LTA. The method employed for the isolation of LTA from *S. aureus* ATCC 6538P has been described in detail elsewhere (9). Two preparations of LTA were used in this work. The first was designated crude LTA (CLTA) and contained, in addition to polyglycerophosphate, 72.3% protein, 3.1% fatty acid, 7.0% hexose, and 5.5% alanine by dry weight (9, 11). The second preparation, for convenience designated purified LTA (PLTA), represented the protein-free and trichloroacetic acid-treated fraction of CLTA. The procedures used for isolating PLTA and the protein fraction of CLTA have also been described previously (9). Another preparation of PLTA, which was obtained from *Bacillus* sp. OSU 372, was also used and was kindly provided by Frank Chorpenning, Ohio State University, Columbus; its composition and immunological properties have been described previously (20).

Other antigens. The immunological properties of the preparation of type III pneumococcal polysaccharide (SSS-III) used, as well as the method by which it was prepared, have been described previously (6, 7); SSS-III is a linear polymer composed solely of glucose-glucuronic acid repeating units (24). Dextran B-1355, a branched polyglucose polymer, was prepared by the method of Jeanes et al. (27).

Immunological methods. Numbers of antibody-producing plaque-forming cells (PFC) produced after immunization were detected by a slide version of the localized hemolysis-in-gel technique (8), using indicator sheep erythrocytes (SRBC) sensitized with CLTA by the $CrCl_3$ coupling method (8) or SRBC sensitized with periodate-activated CLTA (IO_4-CLTA). CLTA was used mainly because sufficient amounts of PLTA were not available for this purpose. When the $CrCl_3$ method was used, 1 mg of CLTA was used to sensitize 0.5 ml of packed SRBC, as described previously (8). In the other procedure, 250 μg of IO_4-CLTA was added to 5 ml of a 10% (vol/vol) suspension of washed SRBC, and the mixture was held at 37°C for 30 min; then 1 drop of 1% sodium borohydride in saline was added, and the sensitized cells were washed five times with saline and adjusted to a 10% (vol/vol) suspension for use in the PFC assay (9). IO_4-CLTA was prepared by a modification of a previously described method (37). A 20-mg amount of CLTA was dissolved in 10 ml of borate-saline buffer (pH 9.0), and the solution was placed in an ice bath. Then 0.1 ml of 0.1 M sodium metaperiodate was added, and the solution was stirred for 2 min, after which it was dialyzed against three changes of borate-saline buffer. The final product was stored frozen until used.

Although the results of the PFC assay generally were expressed as the mean number of direct, IgM-producing PFC detected per spleen for groups of similarly treated mice, facilitation experiments were conducted to determine the immunoglobulin class of antibody produced after immunization; the goat anti-IgM blocking method was used for this purpose. Details concerning this method, as well as the specificity of the antisera used, have been described elsewhere (36). Unless stated otherwise, all PFC values given represent the number of direct IgM-producing PFC found. Since the spleen is a major lymphoid organ in mice, as well as the main site of IgM synthesis, such values provide a reasonably good estimate of the magnitude of the total cellular IgM antibody response produced after immunization. The results obtained were corrected by subtraction for low numbers of background PFC specific for unsensitized SRBC and therefore represent the number of antigen-specific PFC actually detected. The specificity of the PFC detected was affirmed by a plaque inhibition assay, which has been described in detail previously (5).

In some experiments, mice were immunized with horse erythrocytes (HRBC) or turkey erythrocytes (TRBC) that were sensitized with IO_4-CLTA as described above. These cells were prepared as 10% (vol/vol) cell suspensions initially and were diluted to 0.005 and 1% suspensions in saline before being administered intravenously in 0.2 ml of saline for priming and immunization, respectively.

Statistical methods. Student's *t* test was used to evaluate the significance of the differences observed. Since values for the number of PFC per spleen are log normally distributed (23), the data expressed as $\log_{10}$ number of PFC per spleen for individual mice were used for these calculations. Differences were considered to be significant when probability values of <0.05 were obtained. Individual values derived from groups of similarly treated mice ($\log_{10}$ PFC per spleen) were used to calculate the mean ± standard error of the mean in all cases.

RESULTS

Kinetics of the PFC response of mice immunized with heat-killed *S. aureus*. BALB/cBy and C57BL/6By mice were given a single intraperitoneal (i.p.) injection of 10^9 heat-killed *S. aureus* cells. On different days after immunization, spleen cell suspensions from individual mice were prepared and assayed for numbers of PFC by using indicator cells (SRBC) sensitized with IO_4-CLTA or with CLTA coupled to SRBC by the $CrCl_3$ method.

Table 1 shows that appreciable numbers of PFC were detected in the spleens of mice immunized with heat-killed *S. aureus* when both types of indicator cells were used; the numbers found were much greater (about 13-fold) than those reported in previous studies (10), in which other types of indicator cells were used. For both strains of mice, regardless of the type of indicator cells used, PFC were first detected 2 days after immunization and increased in number until maximal numbers were found 4 days after immunization; then there was a rapid decline in PFC. Greater numbers of PFC were detected when indicator cells sensitized with IO_4-CLTA were used, and such PFC were much larger and better defined; this may reflect differences in the sensitivities of the two methods used (9). Periodate and $NaBH_4$ produced no obvious cellular change in erythrocytes, and IO_4-activated erythrocytes did not show lower serological reactivity in passive hemagglutination and hemolysis tests

TABLE 1. *Numbers of PFC detected on different days after mice were immunized with 10^9 heat-killed S. aureus cells: results obtained with two types of indicator SRBC*

Mouse strain	Method for sensitizing indicator SRBC	No. of PFC detected on the following days after immunization:[a]							
		2	3	4	5	6	7	9	
BALB/cBy	IO_4-CLTA	1.610 ± 0.439 (40)[b]	3.582 ± 0.068 (3,822)	4.015 ± 0.171 (10,361)	3.313 ± 0.039 (2,055)	2.424 ± 0.229 (265)	2.869 ± 0.096 (739)		
	$CrCl_3$ + CTLA	1.388 ± 0.574 (25)	3.237 ± 0.112 (1,726)	3.839 ± 0.119 (6,910)	0.909 ± 0.563 (8.1)	1.175 ± 0.486 (15)	1.150 ± 0.640 (35)		
C57BL/6By	IO_4-CLTA	0.340 ± 0.340 (2)	3.068 ± 0.136 (1,168)	3.783 ± 0.051 (6,072)	3.613 ± 0.158 (4,097)	2.702 ± 0.685 (503)		2.057 ± 0.517 (112)	
	$CrCl_3$ + CTLA	0.740 ± 0.456 (6)	1.536 ± 0.628 (34)	3.422 ± 0.151 (2,644)	2.095 ± 0.148 (125)	2.280 ± 0.588 (191)		0.740 ± 0.456 (5)	

[a] Mice were given a single i.p. injection of 10^9 heat-killed *S. aureus* cells in 0.2 ml of saline. Individual mice were assayed on different days after immunization for numbers of PFC present.

[b] Log_{10} number of PFC per spleen ± standard error of the mean for five mice. Numbers in parentheses are geometric means.

than that seen with erythrocytes spontaneously sensitized with CLTA (9). In view of these findings, indicator cells sensitized with IO_4-CLTA were used in the remaining experiments. PFC versus indicator cells sensitized with CLTA were not found in unimmunized mice, and immunization did not result in an increase in background PFC specific for SRBC (polyclonal activation). The magnitude of the PFC response of BALB/cBy mice was greater than that of C57BL/6By mice at most of the time intervals considered.

Immunoglobulin class of antibody produced. Groups of BALB/cCum mice were immunized i.p. with either 10^9 heat-killed *S. aureus* cells or 0.2 ml of 10% SRBC. After 4 days, pooled spleen cell suspensions were prepared and assayed for the presence of class-specific PFC by the IgM-blocking facilitation procedure (36). Table 2 shows that most (86%) of the total number of PFC detected in mice immunized with *S. aureus* represent direct PFC making antibody of the IgM class; all direct PFC could be inhibited by the addition of goat anti-IgM-blocking antiserum (36), indicating that they indeed represent PFC making antibody of the IgM class. PFC making antibodies of the IgA and IgG classes comprise only 10 and 4%, respectively, of the total number of PFC found on day 4 after immunization. The data obtained for mice immunized with SRBC (positive controls) are typical of the data obtained in other studies with this antigen and are shown merely to indicate that both the facilitating antisera and the procedure used were capable of detecting PFC making antibodies of the IgG and IgA classes, if they were present in mice immunized with *S. aureus*.

Specificity of PFC detected. The specificity of the PFC found in mice immunized with heat-killed *S. aureus* was examined by a plaque inhibition assay in which different amounts of various test inhibitors were added to the reaction mixture before incubation and the development of PFC (5). The inhibitors tested included CLTA, PLTA preparations made in our labora-

TABLE 2. *Immunoglobulin class of antibody made by PFC after immunization with 10^9 heat-killed S. aureus cells or SRBC*

Immunogen	No. of class-specific PFC/spleen		
	Direct IgM	IgG	IgA
S. aureus	10,200[a]	537	1,235
SRBC	300,000	138,667	45,300

[a] Number of PFC per spleen for pooled spleen cell suspensions from five similarly treated BALB/cCum mice 4 days after i.p. immunization with either 10^9 heat-killed *S. aureus* cells or 0.2 ml of 10% SRBC.

tory and in the laboratory of F. Chorpenning, a protein fraction extracted from CLTA, SSS-III, and dextran B-1355. Pooled spleen cell suspensions from BALB/cBy mice immunized with 10^9 heat-killed *S. aureus* cells and indicator erythrocytes sensitized with IO_4-CLTA were used in the assay. The mean number of PFC detected for each concentration of inhibitor added was compared with the mean PFC count for assay samples to which no inhibitor was added to calculate the percent inhibition of PFC for each concentration of inhibitor examined; these values were based on the means of triplicate determinations.

Figure 1 shows that virtually complete inhibition (>95%) of PFC produced after immunization with heat-killed *S. aureus* was achieved by the addition of CLTA, as well as by the addition of both preparations of PLTA tested; the degree of inhibition observed depended upon the amount of each inhibitor added. Although 25 μg of CLTA was required for complete inhibition of PFC, complete inhibition was attained with 6 to 8 μg of the two preparations of PLTA made in different laboratories. The plaque inhibition curves for CLTA and the preparation of PLTA made by us (PLTA, Beining) are reasonably parallel, which would be the case if the same immunodominant groups were present in both preparations isolated from *S. aureus* ATCC 6538P and they differed only in the densities of the immunodominant groups present. However, the PLTA preparation provided by F. Chorpenning (PLTA, Chorpenning), which was isolated from *Bacillus* sp., also gave complete inhibition, but it showed a plaque inhibition curve with a different slope. No inhibition was noted with the addition of much larger amounts (50 μg) of the protein fraction extracted from CLTA; this suggests that few, if any, of the PFC produced represented cells making antibody specific for this protein. Since both SSS-III and dextran B-1355 are known to be structurally different from LTA, no plaque inhibition was expected with the addition of 50 μg of these antigens; this was found to be the case. On the basis of these findings, it is reasonable to conclude that most, if not all, of the PFC detected after immunization with *S. aureus* represent cells making antibody specific for LTA, despite the fact that CLTA was used to sensitize indicator erythrocytes.

PFC response of mice immunized with LTA. Mice were given a single i.p. injection of different amounts (0.005 to 50 μg) of CLTA; 5 days later, pooled spleen cell suspensions from similarly treated mice were assayed for numbers of PFC by using indicator erythrocytes sensitized with IO_4-CLTA. Compared with the numbers of PFC detected in mice immunized with heat-killed *S. aureus* (Table 1), extremely small numbers of PFC (<500 cells per spleen) were found, regardless of the dose of CLTA used for immunization. In another experiment, mice were immunized i.p. with 10^7 or 10^9 heat-killed *S. aureus* cells; 7 days later, groups of mice were immunized i.p. with different amounts (0.005 to 0.5 μg) of CLTA, and the magnitude of the PFC response produced was determined 4 days after immunization with CLTA. The numbers of PFC detected were no greater ($P > 0.05$) than the numbers found in mice given only CLTA (<500 PFC per spleen). Thus, although CLTA-specific IgM-producing PFC are made in mice immu-

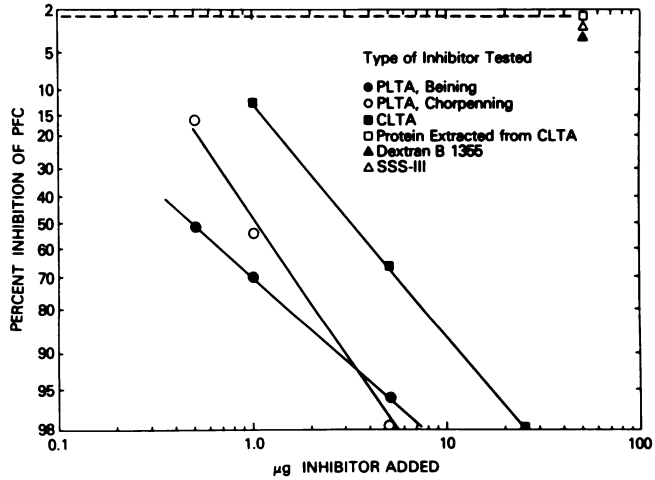

FIG. 1. *Inhibition of PFC produced in BALB/cCum mice immunized with heat-killed S. aureus by different preparations of LTA and other test materials.*

nized with heat-killed *S. aureus*, they are produced only in extremely small numbers in mice immunized with isolated CLTA, regardless of the dose of CLTA given or whether mice had been immunized previously with heat-killed *S. aureus*.

Attempts to improve the immunogenicity of LTA. Mice were given a single intravenous injection of carrier (0.005% HRBC) 3 days before intravenous immunization with CLTA bound to the same carrier by the periodate oxidation method (1% CLTA-HRBC). The numbers of LTA-specific PFC produced were determined at or near peak (3 days later), and the results obtained were compared with those for unprimed mice given 1% CLTA-HRBC. Table 3 shows that considerable numbers of LTA-specific PFC were detected in BALB/cCum mice immunized with CLTA-HRBC after priming with carrier (HRBC); the mean values obtained (9,621 PFC per spleen) were similar to the maximal numbers found in mice immunized with heat-killed *S. aureus* (Table 1) and were about five times greater than those found in mice not pretreated with carrier ($P < 0.05$). Furthermore, the numbers of PFC found in unprimed mice given 1% CLTA-HRBC were much greater than those found in mice given only CLTA (<500 PFC per spleen). Thus, priming with carrier significantly increased the capacity of mice to make an antibody response to LTA, providing mice were immunized with CLTA bound to the same carrier. We have no information on the precise amount of LTA present in the volume (0.2 ml) of 1% CLTA-HRBC given for immunization. In practice, 250 μg of IO_4-CLTA is used to sensitize 5 ml of a 10% (vol/vol) suspension of HRBC. If one assumes that all of the IO_4-CLTA added is bound to HRBC, which is probably not the case, then the maximum amount of LTA that could be administered would be 0.5 μg. As noted above, this dose, as well as much larger doses, of unbound CLTA did not elicit a convincing PFC response. Therefore, the response produced in carrier-primed mice to LTA bound to carrier represents a considerable improvement in immunogenicity. Mice immunized with an optimal dose of 10% HRBC produced about 25,400 HRBC-specific PFC per spleen; however, no LTA-specific PFC could be detected in such animals. Thus, the additional numbers of PFC detected in carrier-primed mice given 1% CLTA-HRBC cannot be attributed to cross-reactivity between antibodies specific for HRBC and LTA.

In another experiment, mice were primed with a different carrier (TRBC) and then immunized with CLTA bound to the same carrier (1% CLTA-TRBC), as described in the preceding experiment; the numbers of LTA-specific PFC produced were determined 3 days later and were compared with those for unprimed mice given 1% CLTA-TRBC. The results obtained (Table 3) were in complete agreement with those of the preceding experiment and indicate that the effect observed is not restricted to only one type of carrier. About 11,000 PFC per spleen were detected in mice immunized with 1% CLTA-TRBC after priming with TRBC; this represents about a 10-fold increase in the magnitude of the PFC response produced as a result of priming ($P < 0.05$).

TABLE 3. *Effect of priming with carrier on the magnitude of the LTA-specific PFC response of mice immunized with CLTA bound to carrier*

Treatment[a]		No. of LTA-specific PFC/spleen[b]
Carrier priming	Immunization	
	1% CLTA-HRBC	3.295 ± 0.096 (1,971)
0.005% HRBC	1% CLTA-HRBC	3.983 ± 0.241 (9,621)
	1% CLTA-TRBC	2.981 ± 0.128 (957)
0.005% TRBC	1% CLTA-TRBC	4.046 ± 0.086 (11,120)

[a] BALB/cCum mice were given 0.005% HRBC or TRBC intravenously in 0.2 ml of saline; 3 days later, they were given 1% CLTA-HRBC or 1% CLTA-TRBC intravenously in 0.2 ml of saline.

[b] Log_{10} number of LTA-specific PFC per spleen ± standard error of the mean for five to eight mice 3 days after immunization with CLTA bound to carrier. Numbers in parentheses are geometric means.

Capacity of LTA to alter responsiveness. BALB/cCum mice were given a single i.p. injection of different amounts (0.005 to 0.5 μg) of CLTA 7 days before immunization with 2×10^9 heat-killed *S. aureus* cells; the magnitude of the PFC response produced 5 days after immunization was no different ($P > 0.05$) than that of mice not given CLTA before immunization with *S. aureus*. Also, mice were given a single i.p. injection of 5 μg of CLTA at the time of intravenous priming with 0.005% TRBC; 3 days later the mice were immunized with 1% CLTA-TRBC. The magnitude of the PFC response produced 3 days after immunization was no different ($P > 0.05$) than that of mice not given LTA at the time of priming with carrier (TRBC). Thus, prior exposure to isolated LTA does not reduce or suppress (34) the capacity of mice to respond to this antigen when it is given in a more immunogenic form.

DISCUSSION

Although the results of studies on the biosynthesis, structure, function, and antigenicity of teichoic acids have been reviewed extensively (1, 2, 4, 31), the immunological properties of these bacterial antigens, especially the LTA de-

rived from staphylococci, have not been well characterized. Antibody can be detected in the serum of rabbits immunized with either whole bacterial cells or teichoic acid bound to methylated bovine serum albumin (14); however, it is difficult to generalize from the results of these studies because of differences in (i) the sources and chemical compositions of the materials used for immunization, (ii) the methods used for extracting teichoic acids, (iii) the immunization protocols used to elicit a measurable antibody response, and (iv) the variability and sensitivity of the serological techniques used for the detection of antibody. Antibody-producing PFC making IgM and IgG antibodies specific for polyglycerophosphate have been found in rats immunized with whole bacterial cells when indicator erythrocytes sensitized with spontaneously adsorbed polyglycerophosphate were used (10); however, the numbers of PFC detected have been low and quite variable, perhaps due to the rather weak binding and ready elution at the temperatures employed of antigen from the surfaces of such indicator erythrocytes (9, 18). Consequently, even though isolated teichoic acids are generally considered to be poor immunogens, several issues pertaining to the induction and subsequent development of an antibody response to these antigens need to be reexamined or investigated more fully at the cellular level so that their potential roles in the pathogenesis of staphylococcal infections can be evaluated.

In the present work, the kinetics for the development of an IgM antibody response to LTA of *S. aureus* was examined in detail by using a recently developed procedure in which erythrocytes sensitized with IO_4-CLTA were used for the detection of PFC (9). Since IO_4-CLTA binds covalently to amino groups present on the surfaces of erythrocytes (37), such indicator cells are stable, give reproducible results among experiments, and enable the detection of numbers of PFC in immunized animals larger than the numbers found when indicator erythrocytes sensitized by the $CrCl_3$ method are used (Table 1) (9). The kinetics for the IgM PFC response of mice immunized with heat-killed *S. aureus* (Table 1) were similar to those reported for both SSS-III (6) and dextran B-1355 (25). The specificity of PFC was affirmed by plaque inhibition assays in which two different preparations of PLTA, prepared independently in separate laboratories, completely inhibited the development of all PFC found (Fig. 1). Therefore, even though mice were immunized with heat-killed *S. aureus*, virtually all PFC detected by using indicator erythrocytes sensitized with IO_4-CLTA represent cells making antibody specific for LTA, and the predominant antibody produced by these PFC is of the IgM class (Table 2). Although the immunodominant component of CLTA seems to be polyglycerophosphate because of the complete inhibition obtained with the PLTA used (Fig. 1), plaque inhibition studies have not yet been conducted using purified carbohydrate or D-alanyl groups as inhibitors. Only rarely have antibodies specific for D-alanine been reported after immunization with extracted LTA (28, 33).

An attempt was made to elicit an IgM antibody response to isolated CLTA by using a wide range of immunizing doses (0.005 to 50 μg); only extremely small numbers of PFC (<500 cells per spleen) were detected. Furthermore, prior immunization with heat-killed *S. aureus*, to which mice usually give a good LTA-specific PFC response (Table 1), did not improve the capacity of mice to respond to isolated CLTA. These findings attest to the relatively poor immunogenicity of isolated CLTA with respect to the development of an IgM antibody response (14), as well as to the inability of this antigen to elicit an anamnestic response in sensitized mice. However, it remains to be determined whether PFC making antibody of the IgG class can be detected after reimmunization.

The inability to obtain a convincing antibody response to isolated CLTA prompted us to examine whether a means could be found to increase its immunogenicity. It has been reported that rabbits make a reasonably good serum antibody response to isolated LTA (normally nonimmunogenic) when it is bound to an insoluble basic carrier, such as methylated bovine serum albumin (14). We, however, elected to try another experimental approach, which also enables mice to make an excellent antibody response after immunization with doses of SSS-III known to be too small to stimulate antibody formation (12, 13, 16, 32). This procedure involved the use of a carrier (e.g., HRBC or TRBC) to generate carrier-specific helper T cells; then the mice were immunized with a subimmunogenic dose of antigen bound to the same carrier, and the magnitude of the antibody (PFC) response produced was assessed and compared with that of unprimed mice given antigen likewise bound to the same carrier. Thus, this procedure is not dependent simply on the presentation of antigen in a particulate form. In previous studies conducted with SSS-III (13, 32), a substantial antibody response was obtained, which was both specific for SSS-III and dependent upon the generation of carrier-specific helper T cells. When this same experimental approach was applied in studies with LTA, a significant PFC response likewise was produced when two differ-

ent types of carrier erythrocytes were used (Table 3). The magnitude of the response obtained was similar to that produced in mice immunized with heat-killed *S. aureus* (Table 1). Thus, the antibody response to LTA, a poor immunogen, can be increased substantially, providing mice are immunized with LTA bound to an appropriate carrier, (i.e., a carrier capable of generating helper T-cell activity). This is consistent with the following unpublished observations. (i) Such a carrier effect could not be produced in athymic nude mice. (ii) Treatment with a dose of antilymphocyte serum, which was known to eliminate the carrier-specific helper T cells required for an antibody response to SSS-III-HRBC, also greatly reduced the PFC response of mice immunized with heat-killed *S. aureus*. And (iii) a substantial antibody response can be elicited to the hapten trinitrophenol conjugated to *S. aureus*. Since mice immunized with heat-killed *S. aureus* produce a PFC response of the same magnitude and since this response is specific for LTA, this suggests that in intact bacterial cells LTA may be associated with a cell wall or cell membrane component that is needed for the generation of this helper T-cell activity in order to obtain a significant antibody response to LTA.

In this work, we have shown that the generation of carrier-specific helper T-cell activity can greatly augment the magnitude of the IgM PFC response to LTA, a weak immunogen (Table 3); further studies will show whether this carrier effect also contributes to the formation of antibodies of other isotypes (IgG or IgA). The experimental approach described may be an aid in the development and detection of antibodies produced against relatively weak antigens and thereby permit us to evaluate the significance of such antibodies to the development of immunity after chronic infection. The cell wall and/or cell membrane constituents that may act as immunologically active carriers for the LTA of staphylococcal cells remain to be identified. However, it should be noted that because of its capacity to adsorb to erythrocytes and other mammalian cells, staphylococcal LTA may have access to particular host tissues and either directly or indirectly play a role in the pathogenesis of disease; such a mechanism has been suggested for streptococcal antigen in the pathogenesis of rheumatic fever (35).

ACKNOWLEDGMENT

This work was supported in part by Office of Naval Research contract N00014-76-C-0053.

LITERATURE CITED

1. **Archibald, A. R., and J. Baddiley.** 1966. The teichoic acids. Adv. Carbohydr. Chem. **21**:323-375.
2. **Archibald, A. R., J. Baddiley, and N. L. Blumson.** 1968. The teichoic acids. Adv. Enzymol. **30**:223-253.
3. **Archibald, A. R., J. Baddiley, and D. Button.** 1968. The glycerol teichoic acid of walls of *Staphylococcus lactis* 13. Biochem. J. **110**:543-558.
4. **Baddiley, J.** 1970. Structure, biosynthesis, and function of teichoic acids. Acc. Chem. Res. **3**:98-105.
5. **Baker, P. J., B. Prescott, P. W. Stashak, and D. F. Amsbaugh.** 1971. Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. III. Studies on the average avidity of the antibody produced by specific plaque-forming cells. J. Immunol. **107**:719-724.
6. **Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott.** 1971. Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. I. Dose-response studies and the effect of prior immunization on the magnitude of the antibody response. Immunology **20**:469-480.
7. **Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott.** 1971. Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. II. Studies on the relative rate of antibody synthesis and release by antibody-producing cells. Immunology **20**:481-492.
8. **Baker, P. J., P. W. Stashak, and B. Prescott.** 1960. Use of erythrocytes sensitized with purified pneumococcal polysaccharide for the assay of antibody and antibody-producing cells. Appl. Microbiol. **17**:422-426.
9. **Beining, P. R., G. M. Flannery, G. Caldes, B. Prescott, and P. J. Baker.** 1980. Use of the periodate oxidation coupling method for the detection of antibody and antibody-producing cells specific for staphylococcal lipoteichoic acid. J. Immunol. Methods **32**:167-175.
10. **Bolton, R. W., H. Rozmiarek, and F. W. Chorpenning.** 1977. Cyclic antibody formation to polyglycerophosphate in normal and injected rats. J. Immunol. **118**:1154-1158.
11. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248-254.
12. **Braley-Mullen, H.** 1974. Regulatory role of T cells in IgG antibody formation and immune memory to type III pneumococcal polysaccharide. J. Immunol. **113**:1909-1920.
13. **Braley-Mullen, H., and G. C. Sharp.** 1974. Conversion of type III pneumococcal polysaccharide low responders to high responders by immunization with a thymus-dependent form of antigen. Cell. Immunol. **12**:49-60.
14. **Burger, M. M.** 1966. Teichoic acids: antigenic determinants, chain separation and their location in the cell wall. Proc. Natl. Acad. Sci. U.S.A. **56**:910-917.
15. **Burger, M. M., and L. Glaser.** 1964. The synthesis of teichoic acids. I. Polyglycerophosphate. J. Biol. Chem. **239**:3168-3177.
16. **Byfield, P.** 1972. Increased plaque-forming cell response to pneumococcal polysaccharide type III-coated sheep erythrocytes after priming with erythrocytes but not with polysaccharide. Cell. Immunol. **3**:316-321.
17. **Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf.** 1963. Methods in immunology. W. A. Benjamin, Inc., New York.
18. **Chorpenning, F. W., and H. B. Stamper.** 1973. Spontaneous adsorption of teichoic acids to erythrocytes. Immunochemistry **10**:15-20.
19. **Coley, J., M. Duckworth, and J. Baddiley.** 1975. Extraction and purification of lipoteichoic acids from gram-positive bacteria. Carbohydr. Res. **40**:41-52.
20. **Decker, G. P., F. W. Chorpenning, and G. T. Frederick.** 1972. Naturally occurring antibodies to bacillary

teichoic acids. J. Immunol. **108**:214-222.
21. **Dubos, R. J.** 1938. The effect of formaldehyde on pneumococci. J. Exp. Med. **67**:389-398.
22. **Fiedel, B. A., and R. W. Jackson.** 1976. Immunogenicity of a purified and carrier-complexed streptococcal lipoteichoic acid. Infect. Immun. **13**:1585-1590.
23. **Gottlieb, C. F.** 1974. Application of transformations to normalize the distribution of plaque-forming cells. J. Immunol. **113**:51-57.
24. **How, M. J., J. S. Brimacombe, and M. Stacey.** 1964. The pneumococcal polysaccharides. Adv. Carbohydr. Chem. **19**:303-358.
25. **Howard, J. G., and B. M. Courtenay.** 1975. Influence of molecular structure on the tolerogenicity of bacterial dextrans. II. The α1-3-linked epitope of dextran B-1355. Immunology **29**:599-610.
26. **Huff, E., R. M. Cole, and T. S. Theodore.** 1974. Lipoteichoic acid localization in mesosomal vesicles of *Staphylococcus aureus*. J. Bacteriol. **120**:273-281.
27. **Jeanes, A., W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya, and C. E. Rist.** 1954. Characterization and classification of dextrans from ninety-six strains of bacteria. J. Am. Chem. Soc. **76**:5041-5052.
28. **Karakawa, W. W., and J. A. Kane.** 1971. Immunochemical analysis of a galactosamine-rich teichoic acid of *Staphylococcus salvarius*, phage type 187. J. Immunol. **106**:900-906.
29. **Knox, K. W., M. J. Hewett, and A. J. Wicken.** 1970. Studies on the group F antigen of lactobacilli: detection of antibodies by hemagglutination. J. Gen. Microbiol. **60**:315-322.
30. **Knox, K. W., and A. J. Wicken.** 1972. Serological studies on the teichoic acids of *Lactobacillus plantarum*. Infect. Immun. **6**:43-49.
31. **Knox, K. W., and A. J. Wicken.** 1973. Immunological properties of teichoic acids. Bacteriol. Rev. **37**:215-257.
32. **Markham, R. B., P. W. Stashak, B. Prescott, D. F. Amsbaugh, and P. J. Baker.** 1978. Selective sensitivity to hydrocortisone of regulatory functions that determine the magnitude of the antibody response to type III pneumococcal polysaccharide. J. Immunol. **121**:829-834.
33. **Matsuno, T., and H. D. Slade.** 1971. Group A streptococcal polysaccharides. Infect. Immun. **3**:385-389.
34. **Miller, G. A., and R. W. Jackson.** 1973. The effect of a *Streptococcus pyogenes* teichoic acid on the immune response of mice. J. Immunol. **110**:148-156.
35. **Moskowitz, M.** 1966. Separation and properties of a red cell-sensitizing substance from streptococci. J. Bacteriol. **91**:2200-2204.
36. **Pasanen, V. J., R. Asofsky, and P. J. Baker.** 1979. Synthesis of two classes of antibody, γM and γG, or γM and γA, by identical cells. Amplification of the antibody response to pneumococcal polysaccharide type III. J. Exp. Med. **149**:1227-1237.
37. **Sanderson, C. J., and D. V. Wilson.** 1971. Methods for coupling protein or polysaccharide to red cells by periodate oxidation. Immunochemistry **8**:163-168.
38. **Toon, P., P. E. Brown, and J. Baddiley.** 1972. The lipid-teichoic acid complex in the cytoplasmic membrane of *Streptococcus faecalis* N.C.I.B. 8191. Biochem. J. **127**:399-409.
39. **Van Driel, D., A. J. Wicken, M. R. Dickson, and K. W. Knox.** 1973. Cellular location of the lipoteichoic acids of *Lactobacillus fermenti* NCTC 6991 and *Lactobacillus casei* NCTC 6375. J. Ultrastruct. Res. **43**:483-497.
40. **Wicken, A. J., and K. W. Knox.** 1975. Lipoteichoic acids: a new class of bacterial antigen. Science **187**:1161-1167.
41. **Yoshida, K., and R. D. Ekstedt.** 1968. Antibody response to *Staphylococcus aureus* in rabbits: sequence of immunoglobulin synthesis and its correlation with passive protection in mice. J. Bacteriol. **96**:1540-1545.