INFECTION AND IMMUNITY, June 1976, p. 1585-1590

Vol. 13, No. 6
Printed in U.S.A.

# Immunogenicity of a Purified and Carrier-Complexed Streptococcal Lipoteichoic Acid

BARRY A. FIEDEL[1] AND ROBERT W. JACKSON*

*Department of Biology, Syracuse University, Syracuse, New York 13210; and The School of Medicine, Southern Illinois University, Carbondale, Illinois 62901**

Received for publication 30 December 1975

Soluble teichoic acid could not stimulate the formation of antibodies either as an aqueous solution or in an emulsion with Freund incomplete adjuvant. However, when given as precipitates with methylated bovine serum albumin, it stimulated strong anti-teichoic acid responses. High levels of anti-teichoic acid antibodies usually resulted in allergic symptoms upon secondary challenge either with immunogenic teichoic acid-methylated bovine albumin complexes or with non-immunogenic soluble teichoic acid. These symptoms suggested anaphylactic shock, but probably also included the formation of complement-consuming immune complexes.

The teichoic acids (TAs) were first recognized as distinct chemical entities about 18 years ago (3, 16). Since then numerous studies describing the chemistry (17), biosynthesis (2), and regulation (4, 29) of these substances have been conducted. It is apparent that this diverse family of substances is characteristic of gram-positive bacteria and constitutes important surface antigens in a number of cases (22, 23, 25, 28). More recently it has been shown that the membrane-associated TAs exist in covalent complexes with lipids (24, 27); it is the lipid that confers the capacity that certain TAs have for binding to mammalian cells (12, 21). Furthermore, although most isolated, soluble TAs have been non-immunogenic, it has been suggested that the lipid confers immunogenicity on the isolated lipoteichoic acid of *Lactobacillus fermenti* NCTC 6991 (13).

The lipoteichoic acids bear some compositional resemblance to the gram-negative lipopolysaccharide endotoxins. Although lipoteichoic acids are generally less chemically complex than the lipopolysaccharides, it may be anticipated that the former share at least some of the many biological effects of the latter (15). Ne'eman and Ginsberg (20) have described the induction of a TA-immune complex arthritis in rabbits; in addition, this laboratory reported that a group A streptococcal teichoic acid could suppress the antibody response to sheep cells in mice (18).

We now wish to report on the results of a study on the immunogenicity in rabbits of a soluble group A streptococcal TA.

[1] Present address: Department of Immunology, Rush-Presbyterian-St. Lukes Medical Center, Chicago, Ill. 60612.

## MATERIALS AND METHODS

**Experimental animals.** All animals were commercially supplied New Zealand White rabbits of mixed sex and weighed approximately 2.5 kg at the start of the experiments.

**TA extracts.** These were prepared from lyophilized *Streptococcus pyogenes* 1-RP41 cells by the phenol extraction procedure of Moskowitz (19) and analyzed as described previously (18). The TAs were free of protein and nucleic acid contamination, and their chemical nature was consistent with an alanyl-polyglycerophosphate covalently attached with lipid and glucose (A. P. Rudczynski, C. F. Repetti, and R. W. Jackson, submitted for publication); these preparations contained 200 to 300 μg of dry material per ml and had an average chain length of 50 to 60 residues, similar to the polyglycerophosphate reported by McCarty (16). In the text, teichoic acid and lipoteichoic acid have been used synonomously, and the term soluble teichoic acid refers to the physical state of the teichoic acid in extracts and is meant to contrast with the physical state of the TA-methylated bovine serum albumin (mBSA) complexes.

**mBSA.** This was prepared by the method of Fraenkel-Conrat and Olcott (10), using fraction V powder obtained from Sigma Chemical Co., St. Louis, Mo.

**Preparation and analysis of TA-mBSA precipitates.** These were made in acid-cleaned glassware by two alternate procedures. In procedure 1, 3 ml of TA extract containing 1.7 μmol of phosphate per ml was added dropwise to 1 ml of 1% mBSA. Each addition was followed by mixing. When addition was complete, the mixture was incubated at 37 C for 15 min and the precipitate was collected by centrifugation. In procedure 2, the order of addition was reversed and again each addition was followed by mixing. After final addition, the precipitate was collected by centrifugation.

For analysis of their composition (see Table 1),

TABLE 1. *Protein and phosphate content of TA-mBSA complexes*

| Prepn no. | Procedure 1[a] | | | Procedure 2[a] | | |
|---|---|---|---|---|---|---|
| | Protein (mg) | Phosphate (mg) | Protein:phosphate ratio | Protein (mg) | Phosphate (mg) | Protein:phosphate ratio |
| 1 | 0.913 | 0.0412 | 22.1:1 | 0.950 | 0.0334 | 28.5:1 |
| 2 | 0.912 | 0.0418 | 21.8:1 | 0.930 | 0.0307 | 30.3:1 |
| 3 | 0.913 | 0.0415 | 22.0:1 | 0.945 | 0.0320 | 29.5:1 |
| 4 | 0.912 | 0.0418 | 21.8:1 | 0.960 | 0.03 | 32.0:1 |
| 5 | 0.920 | 0.0415 | 22.2:1 | 0.940 | 0.0320 | 29.4:1 |
| Avg | 0.914 | 0.0416 | 22.0:1 | 0.944 | 0.0316 | 29.9:1 |

[a] See Materials and Methods for details of preparation.

the precipitates were desiccated to a constant weight. The protein content of the dry residue was determined by the procedure of Lowry et al. (14). A portion of the residue was twice subjected to a $Mg(NO_3)_2$ ashing and then analyzed for phosphate. The phosphate content was determined by the method of Ames and Dubin (1). DL-$\alpha$-Glycerophosphate (Sigma Chemical Co., St. Louis, Mo.) was used as the standard.

**Preparation of precipitates for injection.** Two milligrams (dry weight) of the respective precipitates was suspended in 1 ml of distilled water, and 1 ml of Freund incomplete adjuvant (Difco Laboratories, Detroit, Mich.) was added. The mixture was emulsified on a Vortex mixer, and then 2 ml of 2% Tween 80 (Fisher Scientific Co., Pittsburgh, Pa.) in physiological saline was added. The mixture was then vigorously stirred by a Vortex mixer to produce the double-emulsion preparation of Herbert (11). Mixing was repeated immediately before injection.

**Collection of serum.** Blood was obtained via cardiac puncture, using 10-ml red-stoppered Vacutainers (Becton-Dickinson) and 20-gauge 1.5-inch (about 3.7-cm) Vacutainer needles. The blood was allowed to clot in the collection tube for 1 h at room temperature. Then, depending on the experimental purpose, the clot was allowed to retract for either 1 h or overnight at 4 C. The clots were then removed and the serum was centrifuged to remove sediment.

**Serological analysis.** Passive hemagglutination (PHA) of sensitized erythrocytes was used to follow production of antibodies against TA and mBSA. Sensitization of rabbit erythrocytes by TA was achieved by adding 2 ml of TA extract and 8 ml of PBS-A (phosphate-buffered saline A containing 1.416 g of $Na_2HPO_4$, 0.018 g of $KH_2PO_4$, and 8.418 g of NaCl in 1 liter of solution, pH 7.4) to 0.2 ml of packed washed erythrocytes, resuspending the cells, and incubating the mixture for 30 min at 37 C. Sensitized cells were collected by centrifugation, washed twice with PBS-A, and finally suspended to a total volume of 10 ml in PBS-A. To sensitize sheep erythrocytes with mBSA, the cells were first tanned with a 0.0025% tannic acid solution by the method in Campbell et al. (8). Tanned cells were then sensitized by mixing 1 ml of a 2.5% (by volume) suspension, 4 ml of a PBS (prepared by mixing 0.15 M $Na_2PHO_4$, 0.15 M $KH_2PO_4$, and 0.85% [wt/vol] saline in the ratio of 32.2 ml:67.8 ml:100 ml, respectively; pH 6.4), and 1 ml of an aqueous mBSA solution containing 5 $\mu$g/ml. The mixture was incubated at room temperature for 4 min. The sensitized cells were collected by centrifugation and washed with 2% normal rabbit serum in PBS-A. The washed cells were then suspended in a final volume of 1.5 ml of 2% normal rabbit serum.

In both assays, 0.025 ml of serial dilutions of serum in buffered physiological saline was prepared in microtiter trays (Linbro Chemical Co., New Haven, Conn.) and 0.025 ml of washed, sensitized cells was added. The contents of the wells were mixed and the trays were placed at room temperature for 2 h. The titers were expressed as the reciprocal of the last dilution giving distinct agglutination.

The complement levels of certain sera were determined by the method in Campbell et al. (8). Anti-sheep erythrocyte hemolysin was obtained from Grand Island Biological Co., Grand Island, N.Y.

**Serum protein.** Serum protein levels were determined on hemolysis-free sera diluted 1,000-fold by the procedure of Lowry et al. (14).

**Criteria for anaphylactoid response (6).** Animals were monitored for the presence of anaphylactoid symptoms after injection. The symptoms included (i) rapid onset of labored breathing, accompanied by tremors and frequently followed by serous nasal discharges; (ii) widely dilated pupils and dark-colored eyes; (iii) characteristic carriage of head cocked back over shoulders; (iv) loss of muscle control and sense of balance; (v) polydipsea and/or convulsions; and (vi) death, often occurring at the water trough. Pleural cavities at autopsy were heavily edematous. Provided the first three conditions (i, ii, and iii) were simultaneously present, an allergic condition was considered to be present, although mild.

## RESULTS

**Immunogenicity of the TA.** In this laboratory, more than 100 rabbits and several thousand mice have received injections of soluble TA. These injections have been given by different routes (intravenous [i.v.], intradermal [i.d.], subcutaneous, and intraperitoneal) for varying periods up to 1 year and have been given as single doses, every-other-day doses, and weekly and monthly doses. The total dosages have ranged from the order of 100 $\mu$g to the order of 100 mg in rabbits. In neither species has there ever been any evidence of an antibody response to soluble TA. In addition,

rabbits were given i.v. injections of TA (approximately 700 μg per injection) in Freund incomplete adjuvant, using the double-emulsion technique of Herbert (11) under a variety of immunization schedules; none of these animals raised antibodies to TA as determined by passive hemagglutination of sensitized rabbit erythrocytes.

In distinct contrast, when rabbits were given i.v. injections of a double emulsion of Freund incomplete adjuvant containing TA precipitated with mBSA, antibody to the TA was detectable by passive hemagglutination by day 6 or 7. The data in Table 2 show the titers obtained from rabbits given serial injection of TA-mBSA complexes. Injections containing approximately 2 mg of the respective complexes were given every 7th day for 5 weeks. Blood samples were taken 1 day before each injection and the titers, both anti-TA and anti-mBSA, were determined by PHA. Although there was substantial variation in the maximum titers found, every animal gave a strong response to the TA and little quantitative difference existed between the groups. In contrast, antibody to mBSA was either undetectable or negligible (PHA titers, 0 to 128), and delayed-type skin reactions to mBSA were negative.

In the course of immunizing rabbits with TA-mBSA complexes, it became apparent that the animals underwent an anaphylactoid reaction after secondary injections. This reaction first occurred for both groups during the 3rd week and was concomitant with mortality during weeks 3 and 5; no significant difference in the incidence or the mortality was observed between the groups. The serum protein levels of the animals shown in Table 2 were determined as an indication of renal dysfunction attributable to a generalized systemic reaction. Significant reductions occurred in the 22:1 group (22.1 ± 16.5% decrease; $P = 0.001$) but not in the 30:1 group (1.3 ± 0.2% decrease; $P$ = not significant).

It was evident that inoculation of animals with either 22:1 or 30:1 TA-mBSA complex led to anaphylactoid responses resulting in a high rate of mortality and, further, that inoculation of the 22:1 complex resulted in a more generalized and dramatic involvement. Although the immune response to mBSA remained negligible during the course of immunization, it remained unclear whether immune responsiveness to TA could result in a hypersensitive state responsible for the observed complications. This was determined in the next group of experiments using the 22:1 complex.

**TA-induced hypersensitivity.** The presence of cytotropic antibody directed towards TA was demonstrated as described by Brocklehurst (5). Normal rabbits were administered i.d. injections of serial dilutions of pooled immune sera obtained from immunized rabbits before the onset of anaphylactoid symptoms; pooled

TABLE 2. *Anti-TA titers (PHA) and allergic responses after serial injections of TA-mBSA complexes*[a]

| Rabbit | Preimmune ↓ | Week 1 ↓ | Week 2 ↓ | Week 3 ↓ | Week 4 ↓ | Week 5 |
|---|---|---|---|---|---|---|
| 22:1 group | | | | | | |
| B3 | 0 | 50 | 1,000 | 5,000 (+) | 10,000 (+) | 14,000 |
| B4 | 0 | 10 | 1,000 | 4,000 (+) | 8,000 (+) | 15,000 |
| B5 | 0 | 0 | 1,000 | 1,000 (+) (D) | | |
| B6 | 0 | 0 | 1,000 | 4,000 (+) | 7,000 (+) | 11,000 (+) (D) |
| B19 | 0 | 10 | 1,000 | 1,000 (+) (D) | | |
| B20 | 0 | 100 | 1,000 | 6,000 | 7,000 (+) | 10,000 |
| B26 | 4 | 128 | 512 | 4,096 (+) (D) | | |
| B27 | 4 | 128 | 512 | 4,096 (+) | 16,384 (+) | 32,768 (+) (D) |
| B28 | 2 | 64 | 2,048 | 4,096 (+) | 16,384 (+) | 32,768 (+) |
| B38 | 2 | 64 | 128 | 4,096 (+) | 16,384 (+ | 32,768 (+) (D) |
| 30:1 group | | | | | | |
| B21 | 4 | 128 | 256 | 1,024 (+) | 2,048 (+) | 8,192 |
| B23 | 4 | 256 | 512 | 1,024 | 4,096 | 16,384 |
| B29 | 4 | 64 | 128 | 4,096 (+) (D) | | |
| B30 | 4 | 64 | 512 | 4,096 (+) | 16,384 (+) | 16,384 |
| B31 | 4 | 64 | 2,048 | 4,096 (+) (D) | | |
| B37 | 4 | 32 | 128 | 2,048 (+) | 4,096 (+) | 16,384 (+) |
| B39 | 4 | 64 | 512 | 2,048 (+) | 4,096 (+) | 4,096 (+) (D) |
| A5 | 0 | 4 | 2,048 | 2,048 (+) | 16,384 (+) | 16,384 (+) (D) |
| A15 | 0 | 0 | 16 | 64 | 128 | 512 |
| A16 | 0 | 0 | 16 | ND (+) (D) | | |

[a] Point of injection is indicated by ↓. Allergic response is indicated by (+). Death ensuing from allergic response is indicated by (D). ND, Not determined.

preimmune sera from these same animals were used as the negative control. Sufficient time was allowed to elapse to permit non-tissue-fixing competitive antibody to leak from the extravascular area (5), and all animals were subsequently given an i.v. injection of Evans blue (10 mg per kg of body weight) and TA (0.7 μmol of P per kg of body weight); skin reactive sites were measured at periodic intervals for 1 h (Table 3). All test animals gave a positive passive cutaneous anaphylaxis (PCA) (up to 45 min) which decreased with increasing serum dilution. Control sites were consistently negative.

In other experiments, animals were given two injections of the 22:1 complex and allowed to rest until the 34th day after the initial challenge. Serum TA titers had declined from their maxima but were still comparatively high. The animals were divided into four groups. Group one received 1 ml of soluble TA containing 1.4 μM P via the ear vein; group two received the same dosage distributed at two i.d. sites; group three received mBSA i.v. (1 ml containing 2 mg of protein); and group four received 1 mg of mBSA in each of two i.d. sites. Unimmunized controls were included in each group, and the animals were monitored for anaphylactoid reactions, serum complement levels, and anti-TA and anti-mBSA titers at 4 h and then at 24-h intervals for 1 week.

There were no positive skin reactions or anaphylactoid responses in either unimmunized or mBSA-treated animals. TA-treated animals exhibited rapid onset of anaphylactoid symptoms, although less severe and prolonged than those seen after serial injections of TA-mBSA complexes, and those i.d.-inoculated animals developed classical wheal-and-flare reactivity. These skin reactions intensified during the following 4 h and gradually changed character in the following week. By 24 h, the skin sites had demarcated pallid centers; necrosis was apparent by day 3, and sloughing of scabs with weeping sores was apparent by day 6.

TABLE 3. *Passive cutaneous anaphylaxis in normal rabbits sensitized with pooled rabbit anti-TA antiserum*[a]

| Sensitizing serum | Test site size after i.v. challenge with TA[b] | | | |
|---|---|---|---|---|
| | At 15 min | At 30 min | At 45 min | At 60 min |
| 1:2 anti-TA | 64[c] | 100 | 110 | 90 |
| | 64 | 99 | 110 | 81 |
| | 56 | 99 | 110 | 80 |
| 1:4 anti-TA | 20 | 70 | 72 | 42 |
| | 25 | 63 | 64 | 49 |
| | 25 | 72 | 72 | 56 |
| 1:8 anti-TA | 6 | 20 | 25 | 20 |
| | 9 | 25 | 20 | 16 |
| | 12 | 25 | 25 | 25 |
| Preimmune control serum | 4 | 6 | 9 | 9 |
| | 6 | 9 | 9 | 6 |
| | 12 | 12 | 9 | 9 |

[a] Anti-TA sera were raised against 22:1 TA-mBSA complexes and were collected on day 13 after two stimulations seven days apart.

[b] TA was given 30 to 60 min after sensitizing serum.

[c] The product, in square millimeters, of two diameters measured at right angles to each other.

Immunized animals challenged with soluble TA showed rapid loss of circulating antibody by 4 h after TA inoculation (Table 4). These TA titers remained low for 7 days with the possible exception of rabbit B85, which unfortunately died. Prechallenge anti-mBSA titers were so low that further decline after injections of soluble mBSA could not be said to be significant. In contrast to the TA challenges, in at least two cases there was an increase in anti-mBSA titers far beyond those observed after sequential inoculation of the TA-mBSA complexes. No significant reduction in complement was observed in unimmunized or mBSA-treated animals. In immunized animals administered TA, significant amounts of complement were consumed; additional experiments revealed that the extent of complement consumed was dependent upon the dosage of TA used. In animals challenged i.d. with 1.4, 2.8, or 5.6 μmol of TA phosphate, complement titers (as total hemolytic complement) were reduced by 20, 43, and 73%, respectively, 4 h after challenge and remained depressed in the 2.8- and 5.6-μmol groups for at least 3 days.

These data suggested that immune responsiveness directed solely towards TA could result in a systemic anaphylactoid reaction that most probably involves both antibody demonstrable by PCA and antibody capable of forming complement-consuming immune complexes.

## DISCUSSION

Isolated, soluble teichoic acids have been considered to be nonimmunogenic unless complexed with a carrier or in a precipitated state (7) or contaminated with protein (13). Since antibodies to these substances may be elicited by inoculations of whole cells or cell fragments but not against preparations that are free of major contamination (16), it appears that they should be regarded as complex haptens.

It has been reported that the phenol-extracted soluble lipoteichoic acid from *L. fermenti* was immunogenic in the soluble form and that Freund complete adjuvant strongly enhanced antibody production but incomplete adjuvant gave only a slight enhancement. Knox et al. (13) have suggested that the lipid

TABLE 4. *Serum anti-TA and anti-mBSA titers after challenge by soluble TA or mBSA*[a]

| Group | Animal | Prechallenge titer | Titers at time after challenge | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 4 h | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| Intravenous TA | B70 | 2,048 (anti-TA) | 64 | 32 | 32 | 16 | 128 | 64 | 32 | 64 |
| | B71 | 4,096 (anti-TA) | 512 | 512 | 512 | 16 | 128 | 64 | 64 | 64 |
| | B72 | 8,192 (anti-TA) | 256 | 256 | 256 | 512 | 1,024 | 256 | 128 | 32 |
| Intradermal TA | B74 | 2,048 (anti-TA) | 32 | 256 | 256 | 128 | 512 | 128 | 256 | 64 |
| | B77 | 16,384 (anti-TA) | 512 | 512 | 512 | 1,024 | 2,048 | 1,024 | 1,024 | 512 |
| | B85 | 4,096 (anti-TA) | 256 | 256 | 256 | 32 | 1,024 | Animal died | | |
| Intravenous mBSA | B73 | 8 (anti-mBSA) | 4 | 4 | 8 | 16 | 32 | 64 | 32 | 32 |
| | B86 | 2 (anti-mBSA) | 4 | 2 | 0 | 64 | 128 | 256 | 256 | 1,024 |
| | B87 | 2 (anti-mBSA) | 4 | 4 | 2 | 32 | 256 | 512 | 512 | 1,024 |
| Intradermal mBSA | B76 | 128 (anti-mBSA) | 32 | 16 | 32 | 128 | 64 | 128 | 128 | 128 |
| | B82 | 16 (anti-mBSA) | 0 | 0 | Animal died on day 2 | | | | | |
| | B83 | 4 (anti-mBSA) | 4 | 4 | 4 | 2 | 0 | 2 | 0 | 0 |

[a] Titers are the reciprocals of the highest serum dilution showing definite agglutination of sensitized erythrocytes. Those animals that received TA got a total dose of 1.4 μmol of TA phosphate (approximately 375 μg of TA); those that received mBSA got a total dose of 2 mg of protein.

may provide a suitable state of aggregation to confer immunogenicity on the lactobacillus lipoteichoic acid. However, the streptococcal lipoteichoic acid reported on herein is quite free of protein or nucleic acid contamination and behaves very similarly on Sephadex, Bio-Gel, or Agarose columns, sensitizes erythrocytes, and appears chemically similar except for a somewhat lower lipid content (Rudczynski et al., submitted for publication). In no case, however, have we been able to induce antibodies to either the soluble TA or to an emulsion in Freund incomplete adjuvant. Freund complete adjuvant was not used since it was considered possible that TA would bind to the mycobacterial surfaces, which could then function as immunogenic carriers.

Miller and Jackson (18) have previously reported that the soluble TA suppressed the antibody response to sheep erythrocytes in mice. It is not known whether a similar suppressive effect occurs in rabbits and whether this may be related to its lack of immunogenicity in the soluble state. In a recent report, Wicken et al. (26) found that the immunogenicity of lactobacillus lipoteichoic acid-protein complexes isolated from the organism was directly related to the protein content of the complexes. As is clear from the results in Table 2, TA complexed with a suitable carrier became strongly immunogenic.

In general, the symptoms of the allergic responders in Table 2 were those of anaphylactic shock. This was further suggested by the biphasic nature in the incidence of mortality. According to some definitions, anaphylaxis involves only reaginic antibodies (9) and the positive PCA obtained may be regarded as a demonstration of homocytotropic antibodies. However, the responses cannot be considered as being symptomatic solely of a type 1, anaphylactoid hypersensitivity (9). The consumption of complement and the rapid decline of serum anti-TA after both i.v. and i.d. challenge also suggested the possibility of immune complex formation. The immune complex and complement-mediated release of platelet pharmacological agents in the rabbit has been well documented and may play some role in the observed anaphylactoid hypersensitivity. The progressive changes in the dermal lesion during the first 24 h after challenge with soluble TA would also support the formation of immune complexes, as would the significant decline in serum protein levels after hyperimmunization with 22:1 complexes.

That the TA–anti-TA reactions were sufficient to produce allergic responses in the hyperimmunized animals was indicated by the fact that the responses were elicited solely by soluble TA and not by soluble mBSA. Thus, the essential role of mBSA was merely to confer immunogenicity on the TA. Nevertheless, the allergic responses were significantly less severe with soluble TA than with the complexes. It is not unreasonable to suppose that the physical state of the antigen affected its reactions with antibody and subsequently the allergic responses.

It is of interest that the antibody response to the mBSA in the complexes was so low. When hyperimmunized animals were challenged with soluble mBSA (see Table 4), at least two of them showed increased anti-mBSA titers by

day 3, and by day 7 they had titers an order of magnitude higher than previously after immunization by TA-mBSA complexes. It is therefore possible that the TA in the complexes allowed priming to the mBSA but suppressed anti-mBSA production. The possibility needs further examination.

On comparing antibody titers by PHA, the time of onset of the antibody responses, and the incidence of allergic responders and of mortality, it does not appear that the 22:1 complexes differed significantly from the 30:1 complexes. However, significant complement consumptions and lowered levels of serum protein were found only in those animals hyperimmunized with 22:1 complexes, and therefore the two complexes are not pathologically equivalent. In subsequent reports, it will be shown that they are immunogenically distinct although antigenically similar.

ACKNOWLEDGMENT

This investigation was supported in part by Public Health Service Research grant AI 07361 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Ames, B., and D. Dubin.** 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. 235:769-775.
2. **Archibald, A. R., J. Baddiley, and N. Blumson.** 1968. The teichoic acids. Adv. Enzymol. 30:223-253.
3. **Armstrong, J. J., J. Baddiley, J. G. Buchanan, and B. Carss.** 1958. Nucleotides and the bacterial cell wall. Nature (London) 181:1692-1693.
4. **Boylan, R. J., N. H. Mendelson, D. Brooks, and F. E. Young.** 1972. Regulation of the cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. J. Bacteriol. 110:281-290.
5. **Brocklehurst, W. E.** 1967. Passive cutaneous anaphylaxis (PCA), p. 268-325. *In* D. M. Weir (ed.), Handbook of experimental immunology. F. A. Davis Co., Philadelphia.
6. **Broder, I.** 1971. Anaphylaxis, p. 334-388. *In* H. Z. Movat (ed.), Inflammation immunity and hypersensitivity, 1st ed. Harper and Row Publishers, New York.
7. **Burger, 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.
8. **Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf.** 1970. Methods in immunology, 2nd ed., p 279-283, 305-307. W. A. Benjamin, Inc., New York.
9. **Coombs, R. R. A., and P. G. H. Gell.** 1968. Classification of allergic reactions responsible for clinical hypersensitivity and disease, p. 575-596. *In* R. R. A. Coombs and P. G. H. Gell (ed.), Clinical aspects of immunology, 2nd ed. Blackwell Scientific Publications, Oxford.
10. **Fraenkel-Conrat, H., and H. S. Olcott.** 1945. Esterification of proteins with alcohols of low molecular weight. J. Biol. Chem. 161:259-268.
11. **Herbert, W. J.** 1967. Methods for the preparation of water-in-oil, and multiple emulsions, for use as antigen adjuvants; and notes on their use in immunization procedures, p. 1207-1214. *In* D. M. Weir (ed.), Handbook of experimental immunology. F. A. Davis, Philadelphia.
12. **Hewett, M. J., K. W. Knox, and A. J. Wicken.** 1970. Studies on the group F antigen of lactobacilli: detection of antibodies by hemagglutination. J. Gen. Microbiol. 60:315-322.
13. **Knox, K. W., M. J. Hewett, and A. J. Wicken.** 1970. Studies on the group F antigen of lactobacilli: antigenicity and serological specificity of teichoic acid preparations. J. Gen. Microbiol. 60:303-313.
14. **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.
15. **Luderitz, O., O. Westphal, A. M. Staub, and H. Nikaido.** 1971. Isolation and chemical and immunological characterization of bacterial lipopolysaccharodes, p. 145-233. *In* G. W. Weinbaum, S. Kadis, and A. J. Ajl (ed.), Microbial toxins, vol. 4. Academic Press Inc., New York.
16. **McCarty, M.** 1959. The occurrence of polyglycerophosphate as an antigenic component of various Gram-positive bacterial species. J. Exp. Med. 109:361-378.
17. **McCarty, M., and S. I. Morse.** 1964. Cell wall antigens of Gram-positive bacteria. Adv. Immunol. 4:249-286.
18. **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.
19. **Moskowitz, M.** 1966. Separation and properties of a red cell sensitizing substance from streptococci. J. Bacteriol. 91:2200-2204.
20. **Ne'eman, N., and I. Ginsberg.** 1972. Red cell sensitizing antigen of group A streptococci. I. Immunological and immunopathological properties. Isr. J. Med. Sci. 8:1807-1816.
21. **Pavlovskis, O., and H. D. Slade.** 1969. Absorption of $^{3}$H-fatty acid esters of streptococcal groups A and E cell wall polysaccharide antigens by red blood cells and their effect on hemagglutination. J. Bacteriol. 100:641-646.
22. **Sanderson, A. R., J. L. Strominger, and S. G. Nathenson.** 1962. Chemical structure of teichoic acid from *Staphyloccus aureus*, strain Copenhagen. J. Biol. Chem. 237:3603-3613.
23. **Sharp, M. E., A. J. Davison, and J. Baddiley.** 1964. Teichoic acids and group antigens in lactobacilli. J. Gen. Microbiol. 34:333-340.
24. **Toon, P., P. E. Brown, and J. Baddiley.** 1972. The lipid-teichoic acid complex in the cytoplasmic membrane of *Streptococcus faecalis* NCIB 8191. Biochem. J. 127:399-409.
25. **Wicken, A. J., S. D. Elliot, and J. Baddiley.** 1960. The identity of streptococcal group D antigen with teichoic acid. J. Gen. Microbiol. 31:231-239.
26. **Wicken, A. J., J. W. Gibbens, and K. W. Knox.** 1973. Comparative studies on the isolation of membrane lipoteichoic acids from *Lactobacillus fermenti*. J. Bacteriol. 113:365-372.
27. **Wicken, A. J., and K. W. Knox.** 1970. Studies on the group F antigen of lactobacilli: isolation of a teichoic acid-lipid complex from *Lactobacillus fermenti* NCTC 6991. J. Gen. Microbiol. 60:293-302.
28. **Young, F. E.** 1967. Requirement of glucosylated teichoic acid for absorption of phage in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. 58:2377-2384.
29. **Young, F. E., C. Smith, and B. E. Reilly.** 1969. Chromosomal location of genes regulating resistance to bacteriophage in *Bacillus subtilis*. J. Bacteriol. 98:1087-1097.