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Immunogenicity of Ribosomal Vaccines Isolated from Group A, Type 14 *Streptococcus pyogenes*

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A ribosomal preparation isolated from group A, type 14 *Streptococcus pyogenes* protected mice against a challenge of 1,000 mean lethal doses. Optimal immunization consisted of subcutaneous administration of 326 μg of protein with Freund incomplete adjuvant. Animals immunized with higher or lower antigen concentrations showed decreased survival. Freund incomplete adjuvant was necessary to insure optimum immunization. Immunochemical studies showed no M protein or C carbohydrate present in the ribosomal preparation. Mice optimally immunized with the type 14 ribosomal preparation were protected against heterologous challenge with M types 2, 5, 8, and 12. Protein-free ribonucleic acid extracts isolated from the type 14 ribosomal preparation did not protect mice against homologous challenge. But ribonucleic acid-free protein extracts showed 100% protection in mice after homologous challenge.

During the past 8 years, subcellular fractions from bacteria have been studied for their ability to induce immunity in experimental animals. Youmans and Youmans (28, 29) found that certain subcellular fractions of *Mycobacterium tuberculosis* protected mice against a lethal challenge of the homologous organism. Further work by Youmans and Youmans (30-34) concentrated on the isolation of ribosomes, ribosomal protein, and ribosomal ribonucleic acid (rRNA) to determine the role that each of these may play in inducing immunity. These investigations have led others to examine the immunogenicity of ribosomes isolated from several different bacteria. Venneman and Bigley (22) and Venneman et al. (24) have reported the immunogenicity of ribosomal vaccines prepared from *Salmonella typhimurium*. Winston and Berry (26, 27) isolated ribosomal vaccines from *Staphylococcus aureus*, and Thompson and Snyder (20) investigated vaccines prepared from pneumococcal ribosomes. Johnson (9, 10) has shown protection in mice immunized with ribosomes and ribosomal proteins isolated from *Salmonella typhimurium*.

The immunogenicity of ribosomes isolated from group A, type 14 *Streptococcus pyogenes* was investigated in this study. Experiments were directed toward elucidating the nature of the immunogenic moiety and to determine if the immunizing potential would protect against a challenge with the homologous and heterologous M types of *S. pyogenes*.

MATERIALS AND METHODS

Bacterial strain. Group A, type 14 *S. pyogenes* was obtained from Rebecca Lancefield (Rockefeller University, New York, N.Y.). M types 2, 5, 8, and 12 were obtained from the Center for Disease Control, Atlanta, Ga. All cultures were maintained on blood agar slants containing 5% sheep erthrocytes, and the virulence of each M type was maintained by constant mouse passage. Mean lethal dose (LD_{50}) values were: type 2, 1.05×10^3; type 5, 3×10^1; type 8, 3×10^3; type 12, 1×10^3; and type 14, 2×10^4, as determined by the method of Reed and Muench (15).

Preparation of cultures. Fernbach flasks containing 1.5 liters of Todd-Hewitt broth (Difco) supplemented with 1% neopeptone were inoculated with 100 ml of an exponential-phase broth culture of *S. pyogenes*. The flasks were incubated at 37 C for 8 h on a reciprocal shaker. Cells were harvested by centrifugation at 25,000 × *g* and washed three times in cold (4 C) 0.02 M phosphate buffer containing 0.01 M $MgCl_2$ (0.02 M PMB), pH 7.4.

Animals. A strain of randomly bred Swiss-Webster female mice, maintained by the Department of Microbiology, University of Iowa, was used in this study.

Isolation of ribosomes. Ribosomes were isolated by a modification of the method used by Johnson (9). All procedures were conducted at 4 C. The washed, packed cells were suspended in 4 volumes of 0.02 M PMB. Lysozyme hydrochloride (Miles-Servac Co.) was added in an amount of 15 mg/g of packed cells. After incubation with lysozyme for 1 h at 37 C, 2.0 μg of deoxyribonuclease (Sigma Chemical Co.) per ml was added, and the cells were broken in a French pressure cell at 18,000 lb/in^2. An equal amount of 1% (wt/vol) sodium dodecyl sulfate (SDS) and 0.70 M NH_4Cl dissolved in 0.02 M PMB was added to the

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disrupted slurry with gentle agitation. The extract was centrifuged at 25,000 × g for 10 min to remove cellular debris and intact cells. A 42-g amount of dry $(NH_4)_2SO_4$ was added to 200 ml of the supernatant fluid with gentle stirring for 3 min. The slurry was centrifuged at 25,000 × g for 15 min, and the pellet was discarded. An additional 42 g of dry $(NH_4)_2SO_4$ was added to the supernatant fluid with gentle stirring for 3 min. The slurry was centrifuged at 25,000 × g for 15 min, and the supernatant fluid (SF-1) was saved. The precipitate was suspended in 1 volume of 0.02 M PMB and centrifuged for 15 min at 25,000 × g. The supernatant fluid (SF-2) from this last centrifugation and the previous supernatant fluid (SF-1) were combined and centrifuged at 117,000 × g for 90.0 min. The ribosomal pellet was suspended in 0.02 M PMB with the aid of a Teflon homogenizer and dialyzed against several changes of 0.02 M PMB to remove the remaining $(NH_4)_2SO_4$, NH_4Cl, and SDS. The dialysate was centrifuged at 105,000 × g for 2.5 h. The final ribosomal pellet was suspended on 0.02 M PMB.

Isolation of ribosomal protein and rRNA. Ribosomal protein was isolated by the method of Fogel and Sypherd (7). rRNA was isolated by a modified method of Venneman et al. (24). A 0.02 M PMB solution containing 1% SDS was saturated with redistilled phenol. This phenol-PMB-SDS solution was used in the first four steps of the extraction process.

Sedimentation analysis. Sedimentation analysis was performed using a Beckman model E analytical ultracentrifuge and ultraviolet absorption scanner. The starting optical density was 0.80 at 260 nm. Centrifugation was at 36,000 rpm for a total of 30 min. The sedimentation rate was calculated according to the Svedberg equation as described by Chervenka (3).

Sucrose density gradients. A 0.1-ml sample of the ribosomal suspension was layered on a 15 to 30% (wt/vol) sucrose gradient and centrifuged at 150,000 × g for 1 h. Fractions of 0.1 ml were collected on 0.9 ml of 0.02 M PMB and read at 260 nm.

Biochemical assay. Protein was determined by the method of Lowry et al. (12). RNA was measured by the orcinol method (4), and deoxyribonucleic acid was measured by the diphenylamine method (1). Methyl pentose was determined by colorimetric analysis (11). Bovine serum albumin (Sigma Chemical Co.), yeast RNA (Calbiochem), pancreatic deoxyribonucleic acid (Calbiocem), and L(+)-rhamnose (Sigma Chemical Co.) served as standards.

Polyacrylamide electrophoresis. rRNA was examined in polyacrylamide gels by a modified method of Savage and Kuchler (16). Protein was subjected to electrophoresis in acrylamide gels using the method of Weber and Osborn (25).

Immunodiffusion. The ribosomal preparations were analyzed for the presence of C carbohydrate and M protein by immunodiffusion (2). Preliminary experiments indicate that is was necessary to incubate the ribosomal preparations with SDS to facilitate migration through 2% agarose. However, by using 1% agarose, it was found that SDS was no longer needed.

Immunization. In initial experiments the route, concentration, and use of Freund incomplete adjuvant were determined. Injection routes were either subcutaneous or intraperitoneal, as indicated in the text.

Bacterial clearance. At specific time intervals after intraperitoneal challenge with 1,000 LD_{50}, optimally immunized mice and control mice were sacrificed. The trunk was opened ventrally, blood was withdrawn from the heart, and the spleen was excised. Tenfold dilutions were made on each blood sample, and duplicate 0.1-ml aliquots of each dilution were streak plated. Spleens were homogenized, 10-fold dilutions were made of each homogenate, and duplicate 0.1-ml aliquots of each were also streak plated. All plates were incubated at 37 C, and plate counts were made at 24 h. Gram stains of blood and spleen showed an average of 7.4 cocci/chain.

Whole cell vaccines. A whole cell vaccine using M type 14 was prepared according to the method of Hirsh and Lancefield (8).

Statistical evaluation. The level of significance for protection was determined by the chi-square test according to Fisher and Yates (6).

RESULTS

Chemical studies. Chemical assays indicated an RNA-to-protein ratio of 2:1, which agrees with previously reported values (1, 13, 21). Methyl pentose and deoxyribonucleic acid were not detected in the ribosomal preparations. A methyl pentose determination was employed to insure that cell wall carbohydrate was not contaminating the ribosome preparation. Spectral analysis showed an average 260:280 ratio of 1.82.

Sedimentation analysis. Sedimentation analysis of the ribosomal preparation showed one sedimenting particle. The sedimentation constant of the particle was calculated from the Svedberg equation and the plot of $\log_{10}(x)$ versus time indicated an uncorrected sedimentation value of 70.76*S* for the ribosomal preparation. The corrected sedimentation value was $70.92s_{20,w}$.

Density gradient centrifugation. Sucrose density gradient analysis is shown in Fig. 1. Although no standards were employed, one peak was observed which corresponded to approximately 70*S*. The presence of this one peak suggested that the 70*S* integrity of the ribosomes had been maintained during the isolation procedures.

Electrophoretic studies. Polyacrylamide protein gels are shown in Fig. 2. Ribosomal preparations isolated at different times are labeled RIII and RIV for convenience. The same number of protein bands is found in both the RIII and RIV preparations. RNA acrylamide gels showed three distinct bands in all the ribosomal preparations.

Immunological studies. The immunodiffu-

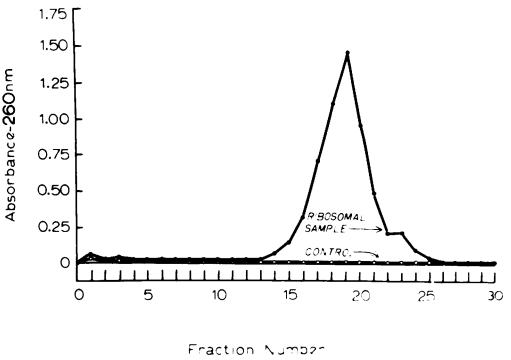

FIG. 1. *Sucrose density gradient analysis of the streptococcal ribosomal preparation. A 15 to 30% sucrose gradient with 0.1 ml of the ribosomal sample layered on top was centrifuged at 150,000 × g for 60 min. Fractions of 0.1 ml were collected in 0.9 ml of 0.02 M PMB and read at 260 nm.*

sion slide (Fig. 3) indicated that the ribosomal preparations did not contain M protein or C carbohydrate. M protein (M) in the center well showed a precipitin in reaction with its homologous antiserum (M-Ab). It did not show a precipitin reaction with antiserum (C-Ab) directed against C carbohydrate. Antiserum prepared against two ribosomal preparations (RIII-Ab and RIV-Ab) isolated at different times failed to show a precipitin reaction with the M protein. In the second Ouchterlony pattern of this figure, the C carbohydrate (C) in the center well does show a precipitin reaction with its homologous antiserum (C-Ab), but it does not react with antibody to the M proteins or antibody to the ribosomal preparations. In both diffusion patterns, a normal control serum (S-Ab) was used to demonstrate the specificity of the reactions. The Ouchterlony patterns on Fig. 4 show the two ribosomal preparations, isolated at different times, in the center wells. In the first pattern, the RIII preparation precipitates with antisera (RIII-Ab) to the RIII preparation and with antisera (RIV-Ab) to the RIV preparation. It does not react with antisera to the M protein, C carbohydrate, or the control serum. The RIV preparation in the center well of the second pattern shows a precipitin reaction with antisera to both the RIII and RIV preparations but does not react with antisera to the M protein or C carbohydrate, or to the control serum.

Dose response. The effect of antigen concentration and the use of Freund incomplete adjuvant on immunity is shown in Table 1. All groups of mice were immunized intraperitoneally with varying ribosome concentrations based on protein content, with and without adjuvant. The immunization period consisted of an initial immunization, a booster of equal concentration after 2 weeks, and a final booster of equal concentration 1 week later (1-2-1). The challenge of 1,000 LD_{50} was given intraperitoneally 1 week after the last booster immunization. Antigen concentrations of 50 and 100 μg of protein induced only very low levels of protection. At these low concentrations of antigen, adjuvant does not appear to significantly increase the level of protection. When animals were immunized with 326 μg of protein administered with adjuvant, the survival was 90%, compared to 20% survival when this same protein concentration was administered without adjuvant. Animals immunized with 600 μg of protein administered with adjuvant showed 76.7% survival, but without adjuvant there was 20% survival. Animals immunized with 1,500 μg of protein administered with adjuvant showed 40% survival, whereas without adjuvant there was 23.3% survival. Controls receiving no

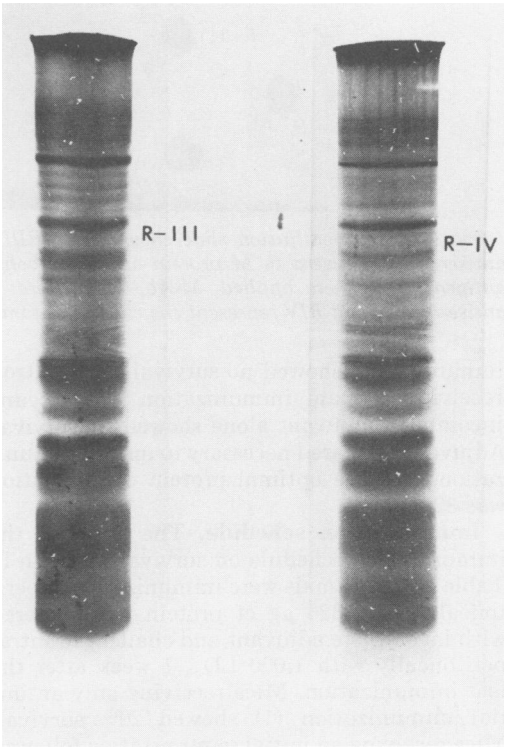

FIG. 2. *Electrophoresis of ribosomal preparations isolated at different times and labeled RIII and RIV. The polyacrylamide gels were subjected to electrophoresis for 180 min at 4 C at 8 mA/column. The gel columns were stained with Coomassie brilliant blue.*

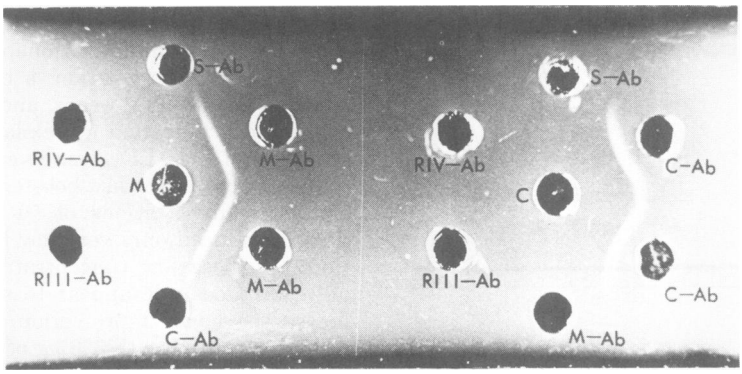

FIG. 3. *Immunodiffusion slide showing M protein and C carbohydrate with their homologous antisera and antisera prepared against the ribosomal preparations RIII and RIV. Antigens and antisera are marked with letters by the well in which they were placed: C, C carbohydrate; M, M protein; M-Ab, M antisera; C-Ab, C antisera; RIII-Ab, RIII antisera; RIV-Ab, RIV antisera; S-Ab, normal control sera.*

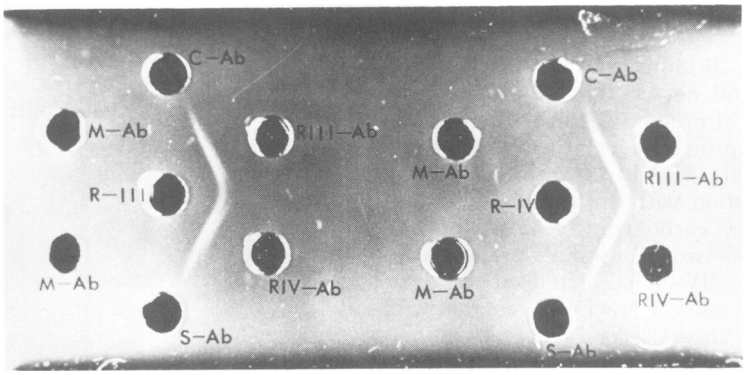

FIG. 4. *Immunodiffusion slide showing the RIII and RIV ribosomal preparations with their homologous antisera and antisera to M protein and C carbohydrate. The letters marking each well correspond to the antigens or antisera applied. M-Ab, M antisera; C-Ab, C antisera; RIII-Ab, RIII antisera; RIV-Ab, RIV antisera; RIII and RIV represent the ribosomal samples in each center well; S-Ab, normal control sera.*

immunization showed no survival, and controls receiving a sham immunization with Freund incomplete adjuvant alone showed no survival. Adjuvant appeared necessary to insure immunization, and the optimal protein concentration was 326 μg.

Immunization schedule. The effect of the immunization schedule on survival is shown in Table 2. All animals were immunized intraperitoneally with 326 μg of protein administered with incomplete adjuvant and challenged intraperitoneally with 1,000 LD_{50} 1 week after the last immunization. Mice receiving only an initial immunization (1) showed 20% survival. Mice receiving an initial immunization followed by a booster of equal antigen concentration at 2 weeks (1-2) showed a 75% survival, whereas mice receiving an initial immunization followed by a booster after 2 weeks and a final booster 1 week later (1-2-1) showed a 95% survival. All control mice not immunized died. The optimal immunization schedule was the 1-2-1-week immunization sequence.

Immunization route. The effect of the immunization route on survival is shown in Table 3. All animals were immunized with 326 μg of protein administered with adjuvant on a 1-2-1 immunization schedule and challenged intraperitoneally 1 week after the last immunization. When animals were immunized intraperitoneally and challenged with 10 LD_{50}, there was 85.5% survival. Animals receiving subcutaneous immunization and a 10 LD_{50} challenge showed 100% survival. When the challenge was increased to 1,000 LD_{50}, intraperitoneally immunized animals showed 90.5% survival and subcutaneously immunized animals showed 100% survival. From these results a subcutaneous administration of 326 μg of protein, with adjuvant, on a 1-2-1 weekly immunization schedule was chosen as optimal.

Chemical composition and immunogenic-

TABLE 1. *Effect of antigen concentration and Freund incomplete adjuvant on immunity induced by intraperitoneal immunization*[a]

Dosage (μg of protein)	Use of Freund incomplete adjuvant	No. of survivors/ total	Survival (%)	
50	Yes	2/30	6.6	
50	No	2/30	6.6	
100	Yes	3/30	10	
100	No	4/30	13	
326	Yes	27/30	90	$P < 0.01$
326	No	6/30	20	
600	Yes	23/30	76.6	$P < 0.01$
600	No	6/30	20	
1,500	Yes	12/30	40	$P < 0.01$
1,500	No	7/30	23.3	
Controls, no immunization		0/30	0	
Controls, sham immunization with adjuvant alone		0/20	0	

[a] All animals were immunized by the intraperitoneal route on a 1-2-1 immunization schedule and challenged intraperitoneally with 1,000 LD_{50} 1 week after the last booster immunization.

TABLE 2. *Effect of immunization schedule on survival*[a]

Immunization period (weeks)	No. of survivors/ total	Survival (%)	
1	4/20	20.0	$P < 0.01$
1-2	15/20	75.0	NS
1-2-1	19/20	95.0	
Controls, no immunization	0/20	0	

[a] All animals were immunized intraperitoneally with 326 μg of protein administered with Freund incomplete adjuvant and challenged intraperitoneally with 1,000 LD_{50} 1 week after the last booster immunization.

ity. The effect of immunization with ribosomal protein and rRNA is shown in Table 4. Mice immunized with 300 and 150 μg of protein showed 91 and 100% survival, respectively, against homologous challenge of 1,000 LD_{50}. Animals immunized with 100 μg of protein showed lower, but significant, levels of protection. Mice immunized with rRNA were not protected against challenge with 1,000 LD_{50}. Controls immunized with intact ribosomes showed 91% survival, whereas nonimmunized controls all died.

Heterologous challenge. The results of the heterologous challenge in mice immunized with a type 14 streptococcal ribosomal preparation are shown in Table 5. The immunized mice were protected against a challenge of 100 LD_{50} with M types 2, 5, 8, and 12. Type 14-challenged mice (positive control) showed 90% survival and nonimmunized control mice all died. A comparison of the protective ability of whole cells and ribosomes of M type 14 is also shown in Table 5. Mice immunized with whole cells from type 14 showed 96% survival against homologous challenge and insignificant protection against heterologous challenge with M types 2, 5, 8, or 12. In contrast, mice immunized with ribosomes from M type 14 showed significant levels of protection against challenge with the homologous as well as heterologous M types.

Bacterial clearance. The results of the bacterial clearance are shown in Fig. 5. The number of colony-forming units (CFU) of type 14 *S. pyogenes* recovered from the blood of immunized animals was less than the number recovered from nonimmunized animals. The duration that numbers of CFU could be recovered from the blood and spleen of immunized ani-

TABLE 3. *Effect of immunization route on survival*[a]

Immunization route	Amt of challenge (LD_{50})	No. of survivors/ total	Survival[b] (%)
Intraperitoneal	10	25/30	85.5
Subcutaneous	10	20/20	100
Intraperitoneal	1,000	27/30	90.5
Subcutaneous	1,000	20/20	100
Controls, no immunization	10	0/20	0
Controls, no immunization	1,000	0/20	0

[a] All animals were immunized with 326 μg of protein administered with adjuvant on a 1-2-1 immunization schedule and challenged intraperitoneally 1 week after the last booster immunization.

[b] Statistical analysis yielded no significant differences in immunized animals.

TABLE 4. *Effect of immunization with ribosomal protein and rRNA*[a]

Amt of antigen (μg)	Antigen type	Survivors/ total	Survival (%)
50	Protein	0/24	0
100	Protein	8/24	33
150	Protein	24/24	100
300	Protein	22/24	91
300	RNA	0/24	0
600	RNA	1/24	4
Controls, 300	Intact ribosomes	22/24	91
Controls, no immunization		0/0	0

[a] All animals were immunized subcutaneously with Freund incomplete adjuvant on a 1-2-1 immunization schedule and challenged intraperitoneally with 1,000 LD_{50} 1 week after the last booster immunization.

TABLE 5. *Comparison of the protective ability of whole cells and ribosomes of M type 14 S. pyogenes*[a]

Challenge organism[b]	% Survival when immunized with	
	Type 14 whole cells	Type 14 ribosomes[c]
Type 2	0	80
Type 5	2	60
Type 8	0	60
Type 12	3	80
Type 14	96	90

[a] Vaccines were prepared as described in Materials and Methods.

[b] All animals were challenged with 100 LD_{50} of the appropriate strain of *S. pyogenes*. All nonimmunized controls died.

[c] Mice were immunized with 326 μg of protein.

mals was less than for nonimmunized animals. After the number of CFU decreased in both blood and spleen of immunized animals, the nonimmunized animals still showed high numbers of CFU in both areas.

DISCUSSION

The results of these studies show that ribosomes isolated from *S. pyogenes* M type 14 are capable of eliciting an immune response which protects mice against challenge with homologous and heterologous M serotypes. Although ribosomal vaccines have been isolated from various bacteria (19, 22, 26, 27, 29), the ribosomal immunogen from *S. pyogenes* is unique since it requires multiple injections of antigen to elicit optimum immunity. Previous studies with ribosomes isolated from *Yersinia pestis* (9) failed to show any protective response in animals receiving multiple injections of *Y. pestis* ribosomes. The requirement for adjuvant for optimal activity is consistant with previous reports that mycobacterial (28) and pneumococcal (20) ribosome preparations are effective only when injected with adjuvant. However, ribosomes isolated from *Salmonella typhimurium* (9, 22), *Neisseria meningitidis* (19), and *Staphylococcus aureus* (27) have been shown to induce good immunity without adjuvant. The reasons for these differences have not yet been elucidated.

Since the first report of the isolation of immunogenic ribosomal fractions (28), there have been conflicting reports regarding the exact chemical nature of the immunogenic moiety. Youmans and Youmans (29-32) have established that the immunogenic fraction isolated from mycobacterial ribosomes is a heat-labile, ribonuclease-sensitive, double-stranded RNA which is only effective when administered with adjuvant. Initially, the ribosomal immunogen isolated from *S. typhimurium* was also reported to be RNA (22), but recent investigations (9, 10, 17, 18) have shown that ribosomal protein plays a major role in the induction of immunity elicited by *Salmonella* ribosomal vaccines. The ribosomal immunogens isolated from *N. meningitidis* (19), *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (26, 27) also appear to be protein. Feit and Tewari (5) have recently suggested that the immunogenic moiety of *Histoplasma capsulatum* ribosomes is also protein. The results of our current experiments suggest that ribosomal protein is the immunogenic moiety. Mice immunized with 150 μg of *S. pyogenes* riboomal protein showed 100% protection against challenge, whereas mice immunized with as much as 600 μg of purified rRNA failed to show any significant levels of protection.

Although the ribosomal protein from *S. pyogenes* induced effective resistance to challenge, there are several quantitative differences between the streptococcal and *Salmonella* ribosomal immunogens. The *Salmonella* ribosomal immunogen induces good levels of immunity at doses as low as 25 μg of protein (9), whereas it requires over four times this amount to induce equivalent levels of protection using the streptococcal immunogen. In addition, it has been shown that 25 μg of purified ribosomal protein from *Salmonella typhimurium* will induce approximately the same level of protection as 25 μg protein of whole ribosomes (9). In contrast, our current studies have shown that on the basis of protein content over twice as much whole ribosomal protein (326 μg of protein) is required to give the same level of protection as 150 μg of

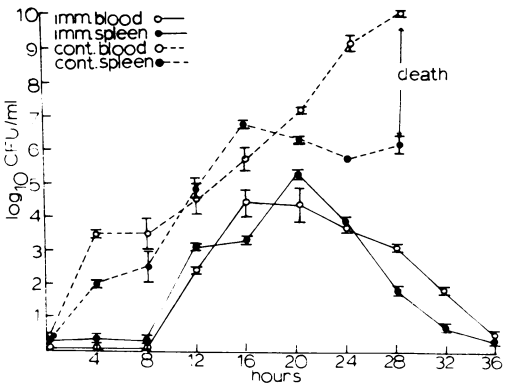

FIG. 5. *Bacterial clearance study of type 14 S. pyogenes in nonimmunized mice and mice immunized with a type 14 ribosomal preparation.*

purified ribosomal protein. One possible explanation for these results is that some of the antigenic determinants associated with immunity are "hidden" in the whole ribosome. Isolation and purification of the proteins could then make available more of these determinants and thus provide significant levels of protection at lower doses than are obtained with whole ribosomes.

Another explanation is that the immunity observed is due to the presence on the ribosome of some as yet undefined cell surface antigen which may be co-purified and concentrated by the techniques used for isolation of the protein. If the immunity is due to contamination of the ribosome, it is unlikely that C carbohydrate or M protein is involved. No C carbohydrate could be detected by chemical or immunological analysis of whole ribosomes or purified ribosomal protein. M protein also could not be detected by immunological analysis. In addition, the fact that whole ribosomes protected mice against heterologous challenge with M types 2, 5, 8, and 12 while the whole cell vaccine did not suggest that M protein is not involved in the immunity induced by these ribosomal preparations. The ribosome preparations could also be contaminated with one or several of the many toxins produced by *S. pyogenes* and therefore induce immunity based on the induction of antitoxins. However, the preparations were not toxic to mice when administered by the intraperitoneal or subcutaneous route. Also, the bacterial clearance study clearly shows that after challenge, there is a decrease in CFU per milliliter in both the blood and spleen of immunized animals when compared to nonimmunized controls. These results suggest that the protection induced in the immunized mice is probably directed against the streptococcal cell and not against streptococcal toxins. Although the mechanism by which streptococcal ribosomes induce protection has not yet been elucidated, we have shown that a circulating precipitating antibody which does not react with M protein or C carbohydrate is produced against the ribosomes. Preliminary experiments suggest that this antibody may enhance phagocytosis during the early critical stages of infection. This is in contrast to previous investigations (14, 23) which have suggested that ribosomal vaccines may induce a cell-mediated immune response. The immunity is not due to a local nonspecific cellular response to the intraperitoneal administration of adjuvant, since equally good protection was observed in animals immunized by the intraperitoneal and subcutaneous routes.

When mice were immunized with antigen concentrations above 326 μg, there was a decrease in survival. This is probably not the high zone tolerance typically observed in a dose response curve, since it is difficult to induce high zone tolerance when an antigen is administered with adjuvant. The decrease in immunity at higher antigen concentrations has been observed by other investigators (5, 19, 20). At present we are investigating the possibility that the decrease in immunity observed at high antigen concentrations is due to a shift in the type of immune response elicited by these preparations.

Thompson and Snyder (20) first reported that mice immunized with type 3 pneumococcal ribosomes were protected against challenge with pneumococcal types 1, 2, and 7. Thomas and Weiss (19) have shown that mice immunized with ribosomes from group B *N. meningtidis* are protected against challenge with serotypes A and C. Our study supports the findings of Thompson and Snyder (20) and Thomas and Weiss (19), since mice immunized with ribosomes from group A, type 14 *S. pyogenes* are protected against heterologous challenge with M types 2, 5, 8, and 12, although optimum protection was observed in mice immunized with ribosomes isolated from type 14 and challenged with type 14. In contrast, Johnson (10) has reported that immunity induced by ribosomes from *Salmonella typhimurium* appears to be type specific. This difference in specificity of protection induced by ribosomes of different bacterial species is still unexplained.

Experiments are currently in progress to isolate and purify the streptococcal ribosomal immunogen and to elucidate the mechanism by which it induces immunity.

ACKNOWLEDGMENTS

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