ENHANCED RESISTANCE TO STREPTOCOCCAL INFECTION INDUCED IN MICE BY CELL WALL MUCOPEPTIDE*

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Stetson has reported that disrupted hemolytic streptococci contain an active material with properties similar to those of endotoxin (1). In these experiments the cell walls were removed from the disrupted bacterial suspension by centrifugation, and the endotoxin properties were associated with the supernatant. Raska and Rotta have reported that mice injected with Group A cell walls exhibited enhanced resistance to subsequent challenge with a homologous type of Group A streptococci (2). One of the characteristic properties of endotoxin is the capacity to induce enhanced non-specific resistance following injection into mice (3). In view of the fact that these two experiments employed different fractions of disrupted streptococci, these biological properties may be dependent upon more than one streptococcal substance. On the other hand, a single cellular material may have been distributed in both the cytoplasmic and cell wall fractions. The present studies were undertaken to clarify the status of streptococcal material which possesses endotoxin properties. Particular attention has been directed to the identification of a cell wall constituent which enhances the resistance of mice to subsequent challenge with streptococci.

Materials and Methods

Streptococcal Strains.—Group A streptococci Type 6 (Strain S43/100/13) and Type 14 (Strain PY) were employed for mouse inoculations. Strain S43/100/13, Type 6, was obtained

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from Dr. R. C. Lancefield, the Rockefeller University, New York, and strain PY from Dr. T. Mortimer, Western Reserve University, Cleveland.

Mice.—Male mice, Webster strain, weighing 18 to 22 gm were employed. The mice were fed a diet of Purina chow and water.

Preparation of Streptococcal Challenge Dose.—One ml aliquots of a stock culture of the streptococcal strains were stored at −70°C. On the day of challenge the aliquot was thawed, added to 50 ml of Difco brain heart infusion, and incubated at 37°C for 6 hours. Colony counts on these cultures were 2 to 5 × 10^8 per ml. An appropriate dilution of the culture was employed as the inoculum for mice.

The Cultivation of Mouse Specimens for Streptococci and L Forms.—The following specimens from infected mice were cultured on sheep blood agar: mouse blood collected prior to death by means of a retroorbital puncture with a sterile heparin capillary tube; at autopsy, hearts' blood, kidney, spleen, liver, and peritoneal fluid.

In addition to regular bacteriologic examination, the autopsy materials were cultured on an L form agar medium consisting of 3.7 per cent Difco brain heart infusion, 2 per cent NaCl, 1.1 per cent agar, 10 per cent horse serum, and 0.5 per cent yeast extract. The horse serum was added to the melted agar just prior to use. Penicillin was added to the melted agar with the final concentration being 1000 units/ml.

Preparation of Cell Walls.—Streptococcal cell walls were prepared as previously described (4). For most purposes the walls underwent additional purification by treatment with proteolytic enzymes and RNAse and DNAse. Throughout this procedure, care was taken to avoid contamination by Gram-negative organisms. All glassware, including the Mickle cups and the Ballotini glass beads were sterilized by dry heat at 180°C for 2 hours. Sterile pyrogen-free water, prepared for intravenous use in patients, was used for washing the cell walls and for the preparation of the buffers for the enzyme treatment. The final cell wall preparations were stored in the lyophilized state.

Preparation of Cell Wall Mucopeptide and Carbohydrate.—The group-specific carbohydrate and the mucopeptide residue were obtained from cell walls by the formamide extraction procedure (4). The separation of the carbohydrate from the mucopeptide residue was demonstrated by the fact that the residue contained less than 1 per cent rhamnose.

Preparative Injections.—The cell walls, carbohydrate and mucopeptide residue as well as other control substances, were suspended in sterile saline just prior to injection into mice. The volume of all preparative injections was 0.25 ml.

In some instances the mucopeptide employed in the preparative injection was solubilized by disintegration for 15 minutes in an MSE ultrasonic disintegrator, 20 kc. For this process the concentration of mucopeptide was 1 mg/ml. After ultrasonic treatment residual particulate material was removed by centrifugation, and the supernatant containing the solubilized mucopeptide was used for injection.

Detection of Pyrogenic Effect of Mucopeptide.—Albino rabbits, 1.5 to 1.7 kg, maintained on an antibiotic-free diet, were employed in the fever studies. Temperature was recorded from the rectum. All rabbits had a normal temperature for at least 24 hours prior to experimental injection.

Serological Identification of Streptococci and L Forms.—Streptococci were identified by the capillary precipitin technique of Swift, Wilson, and Lancefield (5). L forms were identified by the immunofluorescent technique of Karakawa *et al.* (6).

EXPERIMENTAL

Enhanced Resistance.—Enhanced resistance to a subsequent intraperitoneal challenge with virulent Group A streptococci commences 4 hours after an intraperitoneal injection of Group A cell walls and persists for at least 72

hours (2). It is apparent from the following experiment that the serologic type of the preparative dose of cell walls need not be identical to that of the challenge dose of streptococci. Two groups of mice were given an intraperitoneal preparative dose of 250 μg of trypsinized Type 6 cell walls and two groups of control mice were injected with saline. 24 hours later one group receiving the preparative dose and one group receiving the saline injection were challenged intraperitoneally with Type 6 streptococci. The other two similar groups of mice were challenged with Type 14 streptococci. Recorded in Table I are the number of mice in each group and the number of mice sur-

TABLE I

Increased Survival of Mice Which Received an Intraperitoneal Preparative Injection of Type 6 Cell Walls Prior to Intraperitoneal Challenge with Homologous and Heterologous Streptococci

Preparative injection* intraperitoneal	Challenge‡ intraperitoneal	No. of mice injected	Survivors		
			Days after challenge		
			2	4	7
Type 6 cell walls	Type 6	8	8	8	7
Saline	Type 6	8	7	2	1
Type 6 cell walls	Type 14	12	12	11	10
Saline	Type 14	8	5	2	1

* Preparative injection: 24 hours prior to challenge each mouse received intraperitoneally 250 μg of enzymatically treated Type 6 cell walls suspended in 0.25 ml saline.

‡ Challenge was administered 24 hours after the preparative injection: Type 6 streptococci, 0.2 ml of a 10^{-4} dilution of a standard culture of S43/100; Type 14 streptococci, 0.2 ml of a 10^{-3} dilution of a standard culture of T14 (PY).

viving 2, 4, and 7 days after challenge. It is apparent that resistance to the heterologous Type 14 challenge is equal to that of the homologous Type 6 challenge.

Enhanced Resistance Stimulated by Cell Wall Mucopeptide.—Group A cell walls contain three major types of constituents; protein, including the M, T, and R antigens, the group-specific carbohydrate, and the mucopeptide. Because a preparative injection of the Group A cell walls, devoid of the proteins and adherent nucleic acids, was as effective in stimulating enhanced resistance as was a preparative injection of cell walls which had not been treated with enzymes, experiments were designed to associate the enhancement of resistance with either the soluble carbohydrate or the mucopeptide fraction of the cell walls. The soluble carbohydrate and the insoluble residue were prepared from cell walls by extraction with formamide at 180°C.

The resistance of mice to streptococcal challenge following pretreatment with cell walls, carbohydrate, or mucopeptide was detected as follows. A set consisting of three groups of ten 22 gm Swiss male mice were injected intraperitoneally with each of the three preparative materials 24 hours prior to a challenge with Group A, Type 6, streptococci, Strain S43/100. Another set of three groups of control mice received a preparative dose of saline prior to

TABLE II

Increased Survival of Mice which Received an Intraperitoneal Preparative Injection of Cell Wall Mucopeptide Prior to Intraperitoneal Challenge with Group A Streptococci

Preparative injection* intraperitoneal	Challenge dose of S43/100 streptococci 0.2 ml intraperitoneal	No. of mice injected	Survivors		
			Days after challenge		
			2	4	7
Saline	10^{-2} dilution	10	1	1	0
	10^{-3} "	10	1	1	0
	10^{-4} "	10	9	7	5
Cell walls	10^{-2} "	10	6	5	4
	10^{-3} "	10	10	10	10
	10^{-4} "	10	10	8	8
Carbohydrate	10^{-2} "	10	1	0	0
	10^{-3} "	10	2	1	1
	10^{-4} "	10	8	6	5
Mucopeptide	10^{-2} "	10	5	2	2
	10^{-3} "	10	8	8	8
	10^{-4} "	10	10	9	8

* Preparative injections were administered 24 hours prior to the challenge dose of streptococci: saline, 0.25 ml sterile saline; cell walls, 250 μg of T6 cell walls; carbohydrate, 250 μg of carbohydrate from T6 cell walls; mucopeptide, 250 μg of mucopeptide from T6 cell walls.

challenge. The challenge doses of streptococci for the three groups of mice for each set are as follows: 0.2 ml of a 1×10^{-2}, 1×10^{-3}, and 1×10^{-4} dilutions of the standard culture. The surviving mice 2, 4, and 7 days after challenge are recorded in Table II. It is clear that the cell walls and the mucopeptide enhanced survival of the mice, whereas the carbohydrate was ineffective in this respect.

A possible criticism of this experiment is that contamination with endotoxin of Gram-negative bacteria may have inadvertently occurred during the preparation of the cell walls. If such were the case, the enhanced resistance exhibited

by the mice following injection of cell walls might be dependent upon the contaminating endotoxin and not upon a cell wall constituent. It is unlikely, however, that the activity of the mucopeptide is dependent upon contaminating endotoxin, because this cell wall fraction has undergone treatment with formamide at 180°C, conditions which should inactivate the protective effect of endotoxin. To clarify this point Difco endotoxin was heated with formamide in the same manner employed to extract carbohydrate from cell walls. It is clear from the data in Table III that endotoxin following such treatment did not enhance the resistance of mice to subsequent challenge.

TABLE III

Survival of Mice which Received an Intraperitoneal Preparative Injection of Active and Inactivated Endotoxin Prior to Intraperitoneal Challenge with Group A Streptococci

Preparative injection* intraperitoneal	No. of mice injected	Survivors		
		Days after challenge		
		2	4	7
Saline	10	0	0	0
Cell walls	10	10	10	10
Endotoxin	10	10	8	8
Endotoxin, inactivated (hot formamide)	10	1	1	0

Challenge dose: S43/100; 0.2 ml of 10^{-4} dilution of standard culture.

* Preparative materials were injected intraperitoneally in a 0.25 ml volume 24 hours prior to challenge: cell walls, 250 μg; endotoxin, 5 μg; endotoxin, inactivated; Difco endotoxin was treated with formamide at 180°C for 30 minutes. The precipitate which formed with the addition of 2 volumes of acid alcohol was discarded and 5 volumes of acetone added. The precipitate was collected from the alcoholic solution by centrifugation, taken up in a minimal volume of water, dialyzed, and lyophilized. 5 μg was injected.

Because the protective effect of the mucopeptide may be primarily dependent upon the initiation of a peritoneal inflammatory reaction, an experiment was performed to compare the enhanced resistance achieved with mucopeptide to that obtained with other irritant substances. The results are presented in Table IV. It is to be noted that enhanced resistance is exhibited by the mice which received streptococcal cell walls and endotoxin but not by other materials including thorotrast, carbon, glycogen, and paraffin oil. Furthermore, mucopeptide solubilized by ultrasonic treatment was capable of inducing enhanced resistance. Thus, this property of the insoluble mucopeptide is not dependent solely on its particulate nature.

Pyrogenic Effect of Mucopeptide.—Rabbits which are injected intravenously with heat-killed streptococci develop elevations of body temperature (1). Although one or more streptococcal substances may be responsible for this pyro-

genic response, intravenous injection of solubilized mucopeptide elicited a characteristic endotoxin febrile reaction in rabbits. The temperature curves obtained in a typical experiment are depicted in Fig. 1. Rabbits which were injected with 2 mg of particulate mucopeptide did not exhibit a febrile response, whereas other rabbits which were injected with solubilized mucopeptide developed fever. In this experiment 2 mg of insoluble mucopeptide were treated for 20 minutes in the MSE ultrasonic disintegrator, 20 kc. The product, consisting of both solubilized and particulate material, was injected. It is conceivable that better means of solubilization will yield a mucopeptide preparation with greater pyrogenic properties. In other preliminary experiments designed to achieve a more active preparation, a febrile response was detected in rabbits after injection of 50 μg of solubilized mucopeptide.

TABLE IV

Survival of Mice Which Received an Intraperitoneal Preparative Injection of Particulate and Non-particulate Materials Prior to Intraperitoneal Challenge With Group A Streptococci

Preparative injection intraperitoneal*	No. of mice injected	Survivors		
		Days after challenge		
		2	4	7
Saline	10	0	0	0
T6 cell walls, 250 μg	10	8	8	7
Endotoxin, 5 μg	10	10	9	7
Thorotrast, 250 μg	10	0	0	0
Carbon, 250 μg	10	1	1	1
Glycogen, 250 μg	10	0	0	0
Paraffin oil, 0.1 ml	10	5	2	2
Mucopeptide, particulate	10	10	8	8
Mucopeptide, solubilized	10	10	8	7

Challenge dose: S43/100; 0.2 ml of 10^{-4} dilution of standard culture.

* Preparative materials were injected intraperitoneally in a volume of 0.25 ml, with the except of paraffin oil as noted above, 24 hours prior to challenge: particulate mucopeptide, 250 μg; solubilized mucopeptide, 500 μg of mucopeptide were exposed to ultrasonic treatment for 15 minutes and centrifuged at 10,000 RPM for 30 minutes. Under these conditions the supernatant contained 50 per cent of the total hexosamine in the initial insoluble material. The supernatant was employed as the preparative dose and in terms of hexosamine content was equal to the insoluble mucopeptide dose.

Enhanced Resistance Following Intravenous Injection of Cell Walls.—To circumvent the possibility that the enhanced resistance was dependent solely upon a peritoneal inflammatory reaction secondary to the preparative injection, the preparative dose of cell walls was injected intravenously and the

challenge dose of streptococci intraperitoneally. The cell walls were solubilized by ultrasonic treatment to minimize the microembolic effect due to intravenous injection of particulate material. A preliminary test indicated that solubilization of the cell walls did not destroy their capacity to stimulate enhanced resistance.

The results of an experiment with different time intervals between the intravenous preparative injection and subsequent intraperitoneal challenge are recorded in Table V. Ten mice were injected intravenously with the cell wall

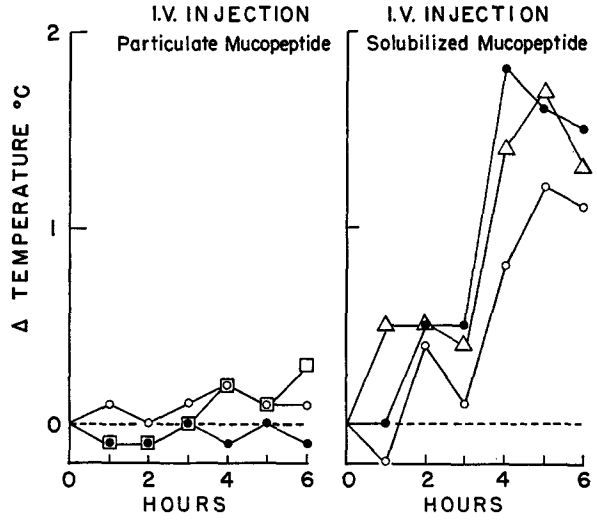

FIG. 1. Recorded on the right are the febrile responses for 3 rabbits after intravenous injection of 2 mg of solubilized streptococcal mucopeptide. Absence of fever after intravenous injection of 2 mg of insoluble mucopeptide is recorded on the left.

preparation and 10 control mice were injected intravenously with saline, and 4 hours later both groups received the challenge dose of streptococci intraperitoneally. Similar groups of prepared and control mice were challenged intraperitoneally at 12 and 24 hours. The number of surviving mice during the 1st week are recorded in the table. It is to be noted that when the interval between the preparative dose and the challenge dose was 12 hours, the majority of the mice survived challenge. This protective effect was of short duration, however, because no appreciable protective effect was detectable when the interval was lengthened to 24 hours.

Long Term Survival of Mice Receiving a Preparative Injection of Cell Walls.— In all of these experiments resistance has been enhanced by an intraperitoneal preparative injection so that the majority of the mice inoculated with a lethal dose of streptococci survived at least 7 days. Although mice without a pre-

parative injection usually die within the 1st week after a streptococcal injection, it was possible to demonstrate enhanced survival beyond the 1st week for the mice which received a preparative injection of cell walls. The results of a typical experiment are depicted in Table VI. Eighty control mice received a preparative intraperitoneal injection of saline and 60 mice received 250 μg of cell walls. 24 hours later both groups of mice were injected with 0.5 ml of a 10^{-6} dilution of a standard culture of S43/100. The surviving mice were recorded at 1, 2, and 6 weeks. From an inspection of the data in Table VI it

TABLE V

Increased Survival of Mice Which Receive an Intravenous Injection of Solubilized Group A Cell Walls Prior to Intraperitoneal Challenge with Group A Streptococci

Intravenous preparative injection*	Interval between preparative injection and intraperitoneal challenge‡	No. of mice injected	Survivors		
			Days after challenge		
			2	4	7
	hours				
Cell walls	4	10	5	0	0
Saline	4	10	2	0	0
Cell walls	12	10	10	8	7
Saline	12	10	5	2	2
Cell walls	24	10	5	2	1
Saline	24	10	4	2	1

* Preparative injection: Volume 0.25 ml; 250 μg of T6 cell walls solubilized by ultrasonic disintegration.

‡ Challenge dose: 0.2 ml of a 10^{-4} dilution of a standard culture of S43/100.

is obvious that the preparative injection of cell walls enhances survival at least to the 6th week after challenge. Bacteriologic study at the time of autopsy of the surviving mice revealed streptococci in some mice as late as 6 weeks after challenge. Recovery of streptococci from blood and organs is enhanced, however, if the mice are given cortisone for 4 days prior to killing. Such persistence is associated in some instances with visible abscesses, although autopsy search of many mice does not reveal an obvious infectious process. While the anatomical focus for the persistence of the streptococcus may be obscure, persistence is minimized if the mice are injected with cell walls 24 hours prior to challenge.

The experiment recorded in Table VII demonstrates the influence of a preparative injection of cell walls on the subsequent recovery of streptococci. One half of the prepared and the control mice which survived 2 weeks after

challenge were given cortisone, and the other half saline injections. A similar scheme was employed for the mice which survived through the 7th week. All mice were autopsied and cultures taken from hearts' blood, liver, spleen, kidney, and peritoneum. For the 7-week mice, at the time of death or killing, Group A streptococci were cultured from the majority of the mice which had not received a preparative injection of cell walls prior to challenge. In contrast, Group A streptococci were detected infrequently in those mice which had had a preparative injection.

Bacteriologic Studies on the Persistent Infection.—The foregoing studies indicate that Group A streptococci will persist for many weeks in mice which

TABLE VI

Long Term Survival of Mice Which Received an Intraperitoneal Preparative Injection of Cell Walls Prior to Intraperitoneal Challenge with Group A Streptococci

Preparative injection intraperitoneal*	No. of mice injected	Survivors		
		Weeks after Challenge		
		1	2	6
Saline	80	55 (69%)	43 (54%)	35 (46%)
Cell walls	60	54 (90%)	53 (89%)	50 (84%)

Challenge dose: 0.5 ml of 10^{-6} dilution of a standard culture of S43/100.

* Preparative injection of 250 μg of Type 6 cell walls was administered 24 hours prior to challenge.

survive the initial challenge of streptococci. These bacteria of the same serologic type as those in the initial injection were recovered from hearts' blood and various organs including kidney, liver, and spleen. In general, the bacteria were recovered from all the organs of those mice which received cortisone, but were much less likely to be disseminated when cortisone was withheld.

Wilson (7) described the occurrence of Group A-variant streptococci (8, 9) during serial mouse passage of Group A streptococci, and Lancefield (10) identified these variants in mice suffering from a cervical adenitis due to Group A streptococci Type 50. A similar antigenic alteration was noted in the persistent streptococci of the experiments reported here. For instance, Type 6, Group A-variant streptococci were cultured 5 weeks after injection from 8 of 10 mice which were positive for Type 6 streptococci. In the case of Type 14, 4 of 8 positive mice harbored Group A-variant streptococci and 1 mouse harbored Group A intermediate streptococci.

Throughout these studies numerous attempts were made at the time of autopsy to isolate L forms from peritoneal fluid, the organs, and the hearts' blood of mice which died or were killed within 48 hours after infection, and

from similar material of mice which survived for longer periods. Although L forms were readily recovered, particularly from the peritoneal exudate, in no instance were the L forms recovered from material which did not reveal Group A streptococci. Thus, it is conceivable that the L forms were not recovered from the autopsy material but were, in fact, produced *in vitro* from the streptococci in the inoculum by the penicillin in the L form media.

To answer this question the peritoneum of infected mice was washed with 2 per cent NaCl broth and an aliquot passed through a Millipore filter with a

TABLE VII

Recovery of Streptococci from Mice Which Survive 2 and 7 Weeks after Challenge: Effect of a Preparative Injection of Cell Walls on Streptococcal Persistence

After Challenge	Preparative injection intraperitoneal*	Cortisone treatment	No. of mice	No. of mice at death or killing with Group A streptococci
weeks				
2	Cell walls	+	10	6
	Cell walls	0	10	0
2	Saline	+	10	10
	Saline	0	10	7
7	Cell walls	+	10	2
	Cell walls	0	10	0
7	Saline	+	9	6
	Saline	0	8	6

Challenge dose: 0.2 ml of 10^{-3} dilution of standard culture of S43/100.

Cortisone treatment: 1.1 mg of cortisone intramuscularly daily for 4 days at the end of the 2nd and 7th week after challenge.

* Preparative injection of 250 μg of cell walls was administered 24 hours prior to challenge.

porosity of 0.65 μ. The filtered and non-filtered peritoneal fluid was cultured on regular blood agar and L form agar. For 1 mouse the peritoneal fluid, prior to filtration, contained 1×10^5 colony-producing L forms, and after filtration, 1×10^3. This diminution in numbers after filtration has been noted with other experiments and probably represents not only retention but also destruction of the filtered forms by the Millipore membrane. Representative colonies of the filterable L forms were identified serologically by previously described immunofluorescent techniques (6). In all cases the M protein was identical to that of the initial streptococci injected into the mouse. The peritoneal washing, prior to filtration, contained 10^8 colony-producing streptococci as detected on blood agar plates, whereas the cultures of the filtered material did not exhibit streptococcal colonies after 18 hours of incubation. During additional incuba-

tion over a 48 hours period, a few small atypical colonies with a faint hemolytic zone appeared on the blood plates. These streptococci, after subculture, developed into typical beta hemolytic colonies and were identified as Group A Type 6. It should be emphasized that repeated cultures of the Millipore filtrates of regular streptococcal cultures were sterile on both blood agar and L form agar. These data suggest that a predominant number of the viable filterable forms grew as L forms, but a few had the capacity to revert to streptococci. In addition, it is conceivable that a limited number of the filterable forms are intermediate structures which have the potential, depending upon the cultural conditions, to develop either as L forms or as bacteria. The identification in the peritoneal washing of viable filterable forms which develop as L form colonies supports the view that the streptococcal L forms were the result of alterations of streptococci within the mouse and do not represent *in vitro* conversion of the bacteria to L forms.

DISCUSSION

Dubos and Schaedler have emphasized the reversible changes in the susceptibility of mice to bacterial infections brought about by injections of pertussis vaccines, fractions of mycobacteria, and bacterial entotoxins (3, 11). Since these reports, such alterations of resistance to infections following administration of various substances have been extensively reviewed (12–15), so that this subject need not be discussed in detail here. In most instances in which the subject has been investigated, these non-specific protective effects have been attributed to bacterial cell wall substances which have the chemical and biological properties of the endotoxins (14). The precise mechanism of the enhanced resistance initiated in mice by endotoxin has not been clarified. It has been shown that endotoxin markedly altered the clearance capacity of the reticuloendothelium system (15), and that the bacteriocidal power of the serum for *Escherichia coli* was elevated after injection (16). Serum from mice injected with endotoxin exhibited increased opsonic power as measured by blood clearance of P^{32}-labeled *E. coli* (15). Finally, Whitby *et al.*(14), and others, have suggested that preexisting antibody to the challenge organism is a requirement for the elucidation of the endotoxin-induced increased resistance. It is probable that none of these currently described mechanisms are solely responsible for this biological property of endotoxin.

The experiments reported here indicate that mucopeptide isolated from the streptococcal cell wall has at least two of the biological properties attributed to the endotoxins of Gram-negative bacteria. Administration of mucopeptide to mice is followed by increased resistance to subsequent streptococcal challenge, and injection into rabbits is followed by a febrile response. Stetson has studied in detail the endotoxic properties of the lysates of Group A streptococci (1). In this work, attention was directed to the cytoplasmic material

obtained after removal, by centrifugation, of the cell walls from streptococci which had been disrupted in a mechanical shaker. The endotoxic properties of the cytoplasm may have been dependent upon traces of cell wall mucopeptide solubilized during the mechanical disruption, or, alternatively, streptococci may contain a substance in addition to mucopeptide which possesses entotoxic properties. These alternatives will require further clarification. Nevertheless, the evidence suggests that streptococcal mucopeptide has certain of the biological properties of endotoxin isolated from Gram-negative bacteria. From a chemical point of view, however, there is no similarity between the chemical content of streptococcal mucopeptide and that of endotoxin. On a weight basis endotoxin is greater than 10 times as effective as mucopeptide in stimulating enhanced resistance, and is greater than 100 times as effective in eliciting a febrile response in rabbits.

A possible interpretation of these data is that the mucopeptide was contaminated with endotoxin at some point in preparation. This alternative is thought unlikely in view of the preparative procedures employed to isolate the mucopeptide. It is yet to be learned if the mucopeptide of other bacteria also possesses endotoxic properties.

The data reported here indicate that a preparative injection of endotoxin enhances the resistance of mice to subsequent challenge with Group A streptococci. Michael and Massell failed to demonstrate a protective effect, but this failure was probably dependent upon the large dose of virulent streptococci which was employed as the challenge in their experiments (17).

Other studies have drawn attention to the persistence in rabbits of Group A streptococci after injection (18), and the recovery of streptococci in mice after 6 to 10 weeks is consistent with these earlier studies. It was of interest that injection of mucopeptide prior to challenge with streptococci sufficiently stimulated resistance so that persistence of the streptococci was eliminated.

No attempt will be made here to discuss in detail the recovery of streptococcal L forms from mice infected with Group A streptococci. In agreement with the findings of Mortimer (19), the evidence suggests that these special forms of streptococci were developed in the host. Certainly the isolation of L forms from the animal material requires extensive confirmation before any biological significance can be attributed to these findings. There are an increasing number of reports, however, which suggest that L forms under certain circumstances can be isolated from experimental infections (20).

SUMMARY

The streptococcal cell wall mucopeptide when injected into mice either intraperitoneally or intravenously enhances the resitance to subsequent challenge with virulent Group A streptococci. Rabbits which are injected intravenously with solubilized mucopeptide develop a fever response which has a resemblance to that achieved with endotoxin.

Mice which survive 6 to 7 weeks after challenge with virulent Group A streptococci yield at autopsy search Group A streptococci serologically identical to the challenge organisms. A preparative dose of cell walls injected into mice prior to challenge diminished this late recovery of streptococci.

Group A-variant streptococci were recovered from mice which survived challenge and carried the organisms for several weeks. Filterable bacterial forms, which grew on L form media, were recovered from infected mice. The serologic type of the L forms was identical to that of the challenge organisms.

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