INFECTION AND IMMUNITY, July 1978, p. 114-123
0019-9567/78/0021-0114$02.00/0

Vol. 21, No. 1
Printed in U.S.A.

Pulmonary Infection of Mice with *Staphylococcus aureus*

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Received for publication 8 February 1978

The survival of *Staphylococcus aureus* in the lungs of mice was studied under various conditions. Doses of 10^7 to 10^9 washed staphylococci were quantitatively introduced into the lungs after intratracheal inoculation in mice under either ether or sodium pentobarbital anesthesia. Mice were sacrificed at intervals, the lungs were excised and homogenized, and the cocci were enumerated by plate count. The 50% lethal dose was 6×10^8 cocci per mouse, and mice died within 24 h but without proliferation of the inoculum. Mice given 10^8 cocci intratracheally under pentobarbital anesthesia regularly survived and eliminated the organisms over a 48-h period. The use of ether anesthesia resulted in persistence of the inoculum for up to 48 h, but the organisms were then eliminated. Inability to proliferate did not appear to result from a lack of iron because pretreatment of the mice with ferric ammonium citrate or Imferon did not alter inoculum survival. Staphylococci inoculated intratracheally in mice infected with influenza virus 3 to 21 days previously showed no enhanced persistence or multiplication. Cocci preclumped with fibrinogen, inocula mixed with 10 times the number of Formalin-killed staphylococci, or inocula of the encapsulated Smith strain did not survive any better than conventional inocula, suggesting that phagocytosis might not be the sole mechanism for elimination. However, a sedimentable fraction from normal or infected lung homogenates proved either inhibitory or cidal for staphylococci in vitro.

Much of the available information regarding the survival of *Staphylococcus aureus* in the lungs of mice is based on studies with challenge doses of less than 10^5 organisms administered either by the aerosol route or by direct intranasal instillation under ether anesthesia (10, 11, 23, 29). Doses of 10^5 cocci introduced into the respiratory tract by inhalation are eliminated from the lungs at an exponential rate and fail to initiate an inflammatory response or other pathological changes in lung tissues (5, 7, 20). Comparable results have been observed after intranasal inoculation with 10^7 cocci (29).

Because it appeared that experimental models utilized in the investigation of *S. aureus* host-parasite interactions frequently required large inocula to establish a focus of infection (19), it was deemed worthwhile to determine whether larger doses could survive better in the lungs of mice than could smaller doses.

It was found that staphylococci, even at doses capable of eliciting pneumonitis and death, did not multiply in the lungs of mice but were rapidly eliminated, and an antecedent influenza virus infection did not diminish this elimination. Contrary to previous reports (6), our data suggest that phagocytosis may not be the primary mechanism for the clearance of *S. aureus* from the lungs of mice.

MATERIALS AND METHODS

Staphylococcal strains. *S. aureus* TG, a representative toxigenic strain, produced exfoliatin, the alpha-, beta-, and delta-toxins (hemolysins), and was of phage type 55/71 (19, 24). *S. aureus* TG Sm^r was derived from strain TG as a streptomycin-resistant mutant.

The encapsulated *S. aureus* Smith mucoid (diffuse) strain has been previously described (9). *S. aureus* Smith Sm^r was derived from the Smith strain as a streptomycin-resistant mutant. To enhance capsule production, the culture was passed intraperitoneally successively through three mice. Encapsulation was confirmed by failure of organisms to clump in plasma and by negative staining with India ink.

S. aureus 18Z-G was an alpha-toxin-negative variant derived from *S. aureus* 18Z (18) after UV irradiation. *S. aureus* 18Z-G Sm^r was derived from strain 18Z-G as a streptomycin-resistant mutant.

Influenza virus. Influenza virus A_2/Aichi/2/68, a mouse-adapted strain of influenza virus, was originally obtained from the Bureau of Biologics, U.S. Food and Drug Administration. Antiserum prepared in guinea pigs, with neutralization titers of 1:1,280, and research reference influenza A_2/Aichi/2/68 for viral identification were obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases.

Animals. Female white Swiss mice, weighing 25 to 30 g and allowed food and water ad libitum, were used throughout.

Bacterial cultures. Staphylococci, grown overnight in Trypticase soy broth with constant aeration, were recovered by centrifugation. The sedimented cocci were washed and suspended in the same medium, then diluted with an equal volume of saline and stored at −70°C until needed. Before inoculation, organisms were routinely washed three times and diluted as required with saline. To confirm the dose, plate counts were also done on the suspensions inoculated.

Intratracheal inoculation. Groups of mice were anesthetized with either ether delivered by inhalation or sodium pentobarbital given intraperitoneally, and secured on a surgery board angled to approximately 60°. After wetting the skin of the neck with ethanol, a 1-cm longitudinal incision was made over the trachea, the overlying muscle was retracted, and the trachea was exposed. A tuberculin syringe fitted with a short-bevel 26-gauge needle was used to introduce the 30-μl inoculum. The needle was inserted and threaded along the trachea for a distance of approximately 5 mm before inoculation. Application of pressure to the abdomen of the animal to deflate the lungs and its removal concurrent with the introduction of the inoculum into the trachea assured inspiration of the inoculum into the lungs. The incision was closed with two silk sutures, and the animals were left to recover from anesthesia.

Enumeration of staphylococci in lungs. Mice were sacrificed by cervical dislocation, the ventral skin was withdrawn laterally, and the thoracic cavity was exposed. Both lungs were aseptically removed, separated from the trachea, and homogenized with 5 ml of sterile saline in glass mortars with Teflon pestles fitted to a variable-speed motor. Serial 10-fold dilutions of the resultant homogenates were plated on Trypticase soy agar containing 60 μg of streptomycin per ml to enumerate the cocci.

In instances where mice were infected with plasma-clumped staphylococci, lung tissue, after initial homogenization, was treated with trypsin (Difco Laboratories, 1:250, 1.2 mg/ml of homogenate) and left to stand for 30 min. Specimens were then rehomogenized for plate counts.

Influenza virus pools and inoculation. The virus suspension originally obtained was passed successively through mice. Animals were infected intratracheally with 30 μl of the viral suspension under sodium pentobarbital anesthesia. A viral pool was prepared from the fourth passage by excising and homogenizing the consolidated areas of infected lung. The pool, consisting of a 10% lung suspension (by weight) in 1% peptone (Difco) with antibiotics, contained approximately 5,000 mouse 50% lethal doses per ml. The presence of virus was determined by the hemagglutination reaction with guinea pig erythrocytes (8).

Influenza virus isolation. Primary rhesus monkey kidney cell cultures (Flow Laboratories) were maintained for 24 h on a medium consisting of Eagle minimal essential medium plus Earle balanced salts (Flow Laboratories) with 100 U of penicillin G per ml, 100 μg of streptomycin per ml, 10 μg of polymyxin B per ml, and 2.5 ml of 7.5% sodium bicarbonate (International Scientific Ind., Inc.) per 100 ml and then inoculated with serial 10-fold dilutions of 10% lung suspensions from influenza virus-infected and sham-inoculated mice. Culture tubes were scored for evidence of cytopathic effect and hemabsorption of guinea pig erythrocytes after a 6-day period. The viral isolates thus obtained were identified serologically by neutralization with homologous monospecific antisera.

Preparation of Formalin-killed cocci. Formalin was added to an 18-h washed culture of *S. aureus* TG to give a final concentration of 0.4% (vol/vol), and the mixture was allowed to stand at room temperature until sterile. The killed organisms were washed three times with saline before use.

Aggregated *S. aureus* TG. Portions (0.1-ml) of citrated rabbit plasma (Difco) were added to 1-ml amounts of appropriately diluted suspensions of washed staphylococci in saline, and clumping was allowed to occur. The aggregated organisms were washed gently with saline and more finely dispersed by passage through a 26-gauge needle fitted to a tuberculin syringe.

Administration of exogenous iron. Groups of mice were inoculated intravenously with 0.1 ml of (5 mg of iron) Imferon (Lakeside Laboratories) 24 h before infection. The same animals were also given 3 mg of sterile ferric ammonium citrate (Pfaltz and Bauer) containing 18.5% iron intravenously 1 h before infection. The inoculum consisted of staphylococci suspended in a total volume of 1.0 ml containing 4.5 mg of ferric ammonium citrate and 0.15 ml of Imferon.

Lavage fluids. Mice were inoculated intratracheally under sodium pentobarbital anesthesia with either 0.03 ml of *S. aureus* TG suspension containing 10^8 cocci or with an equal volume of saline. Twenty-four hours later the mice were sacrificed with an overdose of sodium pentobarbital, their tracheas were cannulated in situ with an 18-gauge needle, and the lungs were lavaged with three 0.5-ml portions of sterile saline. Blood-tinged fluids were discarded. Lavage fluids from each group were pooled and stored at −70°C until needed.

Lung tissue homogenates. Mice were inoculated intratracheally with either 10^8 cocci or with saline. After 24 h the mice were sacrificed by cervical dislocation, and the involved portions of infected lungs were aseptically removed and pooled. Similar portions of lungs from saline-inoculated control animals were selected at random. The lung tissue was homogenized in glass mortars fitted with Teflon pestles and centrifuged at 40,000 × *g* at 4°C, and the sediment was washed three times with sterile saline. The washed sediment was made up as a 20% suspension in saline (wt/vol) and stored at −70°C until needed. Before use, the lung tissue suspensions were rehomogenized to insure even suspension.

Histological sections. Mouse lungs were removed and placed in neutral buffered Formalin. Sections 6 μm thick were stained with hematoxylin-eosin.

RESULTS

Fifty percent lethal dose of *S. aureus* inoculated intratracheally. The 50% lethal dose of *S. aureus* TG Sm^r inoculated intratracheally in mice was 6×10^8 cocci per mouse regardless of the anesthesia employed. With doses of 10^8 or

greater, labored breathing became evident in the test animals after 8 to 10 h, and, in animals destined to die, death was usually preceded by expiration of copious amounts of blood-tinged fluid. These latter animals died within 24 h after infection but without proliferation of the inoculum. Postmortem examination revealed accumulation of fluid in the pleural space and grossly hemorrhagic lungs. In those animals surviving, organisms were essentially eliminated from the lungs by 48 h. Ability to recover some staphylococci from pleural fluids or from liver and spleen tissues indicated that some degree of dissemination had occurred, probably via the circulation.

Survival of *S. aureus* in the lungs. Groups of infected mice were sacrificed, the lungs were excised and homogenized with sterile saline, and the cocci were enumerated by plate counts on medium containing streptomycin. Streptomycin-resistant strains were used throughout to reduce the possibility of including contaminants which were sometimes present in the lower respiratory tract.

Mice anesthetized with sodium pentobarbital regularly survived doses of 10^7 to 10^8 cocci and eliminated the organisms over a 48-h period (Fig. 1). The use of ether anesthesia resulted in a persistence of the inoculum for up to 24 h, but the organisms were then eliminated (Fig. 1). Test animals inoculated under ether anesthesia manifested greater variability in the number of cocci demonstrable in the lungs after 24 h than did those infected under pentobarbital anesthesia. Generally, in mice given ether anesthesia the bacterial counts were distributed over a broader range, and an occasional animal showed a persistence of the inoculum for up to 7 days.

Histological sections prepared from lungs obtained immediately after infection readily permitted visualization of the inoculated cocci. The organisms were scattered throughout regions of both lungs and did not appear clumped. Sections prepared from lungs removed 4 to 8 h after infection with 10^8 cocci revealed an intense neutrophil influx, consolidation of alveolar spaces, and tissue necrosis, but organisms could no longer be recognized. At 4 to 8 h after infection with doses of greater than 10^9 organisms, numerous foci of cocci, circumscribed by areas of degenerating leukocytes, were scattered throughout the lungs. Control sections from lungs of noninfected etherized animals removed at zero time revealed moderate fluid accumulation and a slight increase in cellularity of alveolar septa.

Effect of supplemental iron. The parenteral administration of iron compounds to experimental animals has been shown to enhance

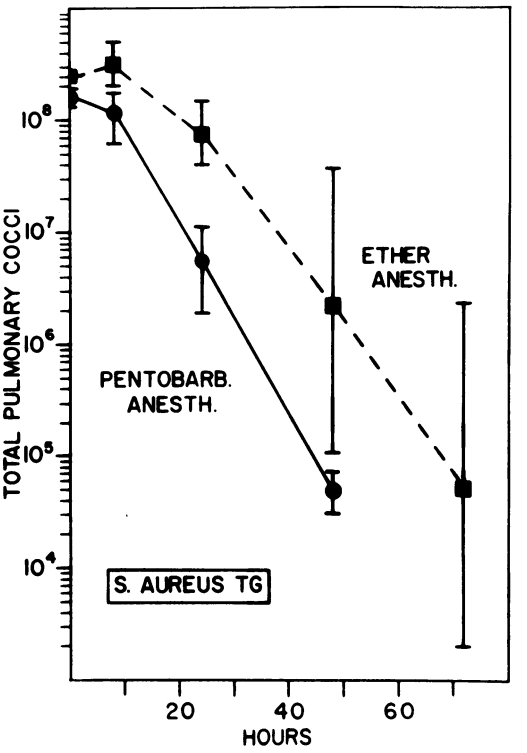

FIG. 1. *Elimination of S. aureus TG from the lungs of mice after intratracheal inoculation under ether or sodium pentobarbital anesthesia. Each point represents the geometric mean from three animals. Vertical bars indicate the standard error of the mean.*

multiplication of other microorganisms in certain experimental infections (2, 27). To determine whether staphylococci did not multiply because of an iron deficiency, groups of mice were inoculated with Imferon and ferric ammonium citrate. Animals receiving exogenous iron regularly survived doses of 10^8 cocci, and the inoculum was eliminated as effectively as in control animals not given additional iron.

Survival of *S. aureus* in influenza-infected mice. To explore the role of an influenza virus infection on the survival of staphylococci, groups of mice were inoculated intratracheally with sublethal doses of influenza virus A_2/Aichi/2/68 under sodium pentobarbital anesthesia. After 3 to 21 days, randomly selected subpopulations were infected intratracheally with 5×10^7 to 5×10^8 *S. aureus* under either ether or sodium pentobarbital anesthesia.

Infection with influenza virus in control animals not secondarily infected with *S. aureus* was confirmed by viral isolation with rhesus monkey kidney cell cultures. Lungs from animals infected with influenza virus showed typical plum-colored areas of consolidation varying in size

from areas approximately 5 mm in diameter at 3 days to areas involving one or more lobes by 10 to 12 days.

In mice challenged with staphylococci under sodium pentobarbital anesthesia, the total pulmonary staphylococcal population decreased in influenza-infected mice at the same rate as it did in control animals not previously infected with virus (Fig. 2).

When virus-infected mice were challenged with 10^8 staphylococci under ether anesthesia, the ether-induced lag before elimination began was still evident in mice infected with influenza 3 days previously, but was no longer evident in animals infected with virus 5 to 21 days earlier (Fig. 3). The total pulmonary staphylococcal population was significantly less ($P < 0.001$) 24 h after challenge with staphylococci in animals infected with virus 5 to 21 days previously as compared with animals infected 3 days after viral infection.

In neither instance was bacterial multiplication observed, and the combined infections did not result in death or other obvious manifestations of synergy.

Attempts to interfere with phagocytosis. Because elimination of bacteria from the lungs is usually considered due to phagocytosis by alveolar macrophages or neutrophils, attempts were made to assess the role of phagocytes in eliminating the staphylococci by altering of the inoculum in ways which could be expected to interfere with phagocytic mechanisms.

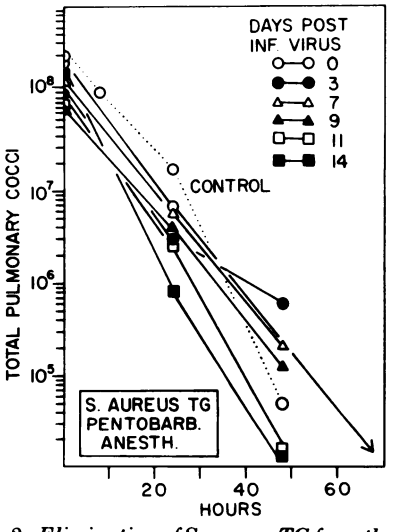

FIG. 2. *Elimination of S. aureus TG from the lungs of mice infected with influenza virus A_2/Aichi/2/68 0 to 14 days previously. Influenza virus and staphylococci were given intratracheally under sodium pentobarbital anesthesia. Each point represents the geometric mean from three animals. There are no significant differences in the elimination rates among the various groups.*

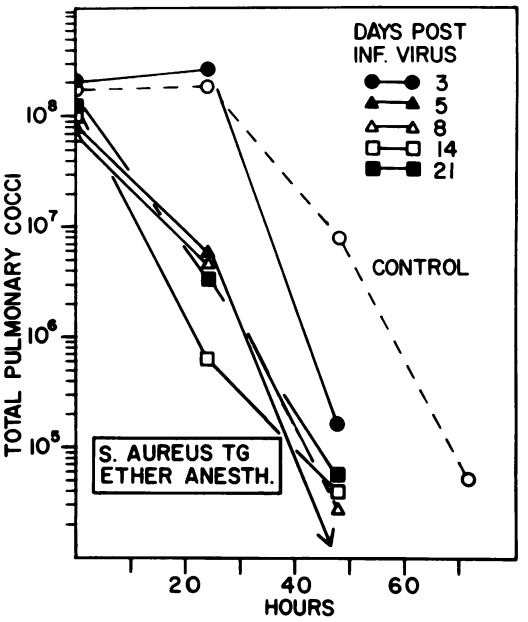

FIG. 3. *Elimination of S. aureus TG from the lungs of mice infected 3 to 21 days previously with influenza virus A_2/Aichi/2/68. Influenza virus was given intratracheally under sodium pentobarbital anesthesia and staphylococci were inoculated intratracheally under ether anesthesia. Twenty-four hours after inoculation with S. aureus, the pulmonary bacterial population in mice given influenza virus 3 days previously is significantly greater than that in animals infected with virus 5 to 21 days previously ($P < 0.001$).*

If phagocytosis played a prominent role in the removal, it appeared reasonable that the presentation of an excess number of killed organisms in combination with the viable cocci might reduce the elimination of the latter. Accordingly, mice were infected with a dose of 10^8 live organisms mixed with 10^9 washed Formalin-killed staphylococci. However, even the presence of a 10-fold excess in killed staphylococci failed to diminish the rapid and continuous elimination of the live organisms (Fig. 4).

Survival of plasma-clumped cocci. Because aggregation of *S. aureus* with plasma has been shown to impede phagocytosis in the peritoneal cavity of mice (15), the survival of plasma-clumped staphylococci in the lungs was examined. Suspensions were clumped with rabbit plasma, washed, and partially resuspended before being inoculated intratracheally. Trypsin was added to lung homogenates to aid in the redispersal of cocci before enumeration. However, aggregated cocci administered under pen-

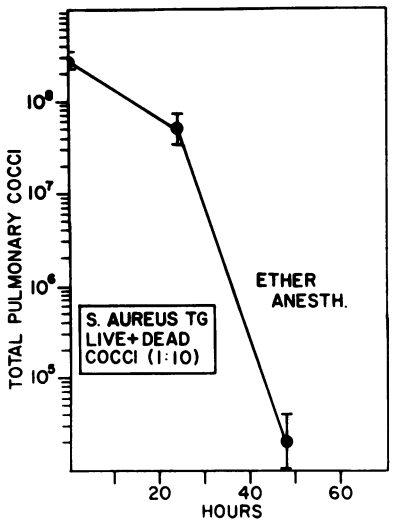

FIG. 4. *Survival of S. aureus TG in the lungs of mice inoculated intratracheally with 2 × 10^8 live organisms mixed with 2 × 10^8 Formalin-killed cocci. Staphylococci were inoculated under ether anesthesia. Each point represents the geometric mean of three animals. Data shown represent the elimination of viable cocci from the lungs.*

tobarbital anesthesia were eliminated even more rapidly than were nonclumped organisms (Fig. 5). When ether anesthesia was employed, the pulmonary population also decreased, although at a slower rate, but organisms were no longer recoverable by 96 h. Histological sections prepared from lungs removed shortly after inoculation revealed staphylococcal aggregates of various size distributed throughout the lungs.

Effect of encapsulation on survival. The *S. aureus* Smith (diffuse) strain is known to possess a permanent capsule which is effective in hindering phagocytosis to a significant degree (9, 15). Doses as high as 2 × 10^9 encapsulated cocci failed to kill test animals after intratracheal instillation, and there was no evidence that encapsulation reduced elimination of the pulmonary population over a 48-h period (Fig. 6). Histologically, lungs from mice infected with the Smith diffuse strain 24 h previously showed consolidation and thickening of alveolar septa comparable to that caused by the TG strain, but individual cocci were difficult to discern due to the intensity of the inflammatory response.

In vitro incubation of staphylococci in lavage fluids. Because these data suggested that phagocytosis may not be the exclusive mechanism responsible for the elimination of the inocula, the survival of staphylococci in lung lavage fluid was examined. Lavage fluid was obtained from either normal mice or animals infected intratracheally with 10^8 *S. aureus* TG (streptomycin sensitive) 24 h previously. Lavage fluid was seeded with *S. aureus* TG Sm^r, and the mixtures were incubated at 37°C in a water bath with shaking. Organisms were numerated by

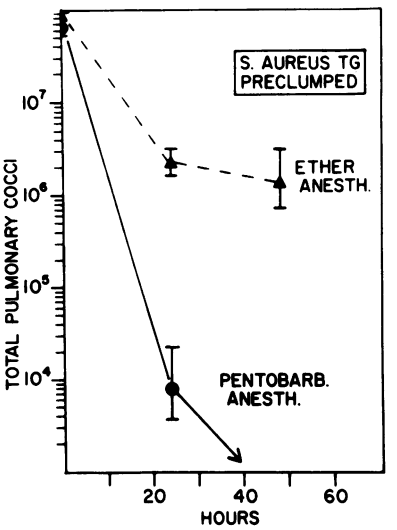

FIG. 5. *Survival of aggregated S. aureus TG in the lungs of mice. Staphylococci were aggregated with rabbit plasma and washed, and suspensions containing 10^8 cocci were inoculated intratracheally under either ether or sodium pentobarbital anesthesia. Each point represents the geometric mean derived from three animals. Arrow indicates a decline to less than 10 organisms per lung by 48 h.*

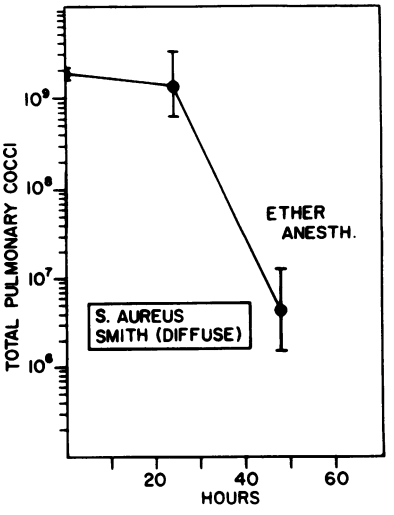

FIG. 6. *Survival of encapsulated S. aureus Smith (diffuse) in the lungs of mice after intratracheal inoculation under ether anesthesia. Each point represents the geometric mean derived from three animals.*

plate counts on medium containing streptomycin.

There was no evidence that the lavage fluid contained inhibitory substances because the cocci multiplied and growth was comparable in lung lavage fluids obtained from either infected or normal animals. In both cases growth in lavage fluids surpassed by 2 logs the growth observed in control tubes containing suspensions diluted with saline.

In vitro incubation of staphylococci in lung tissue homogenates. Failure to observe inhibitory substances in lavage fluids prompted an investigation of lung tissues from infected animals as a possible source of antibacterial activity. Lung homogenates were prepared from mice either infected 24 h previously with 10^8 *S. aureus* TG (streptomycin sensitive) or given sterile saline. The washed tissue suspensions were inoculated with an equal volume of *S. aureus* TG Sm^r and 18Z-G Sm^r suspensions (10^4 to 10^5 cocci per ml of 8% Trypticase soy broth in saline). The mixture was incubated at 37°C in a shaking water bath, and portions were removed periodically for plate counts on medium containing streptomycin.

S. aureus TG Sm^r, when added to washed homogenized normal lung tissue and maintained in vitro, exhibited more than a 10,000-fold increase in the population over a 10-h period (Fig. 7).

The same strain incubated in homogenized lung tissue from infected mice revealed less than a 100-fold increase during the same interval. Although the rate of growth in homogenates from infected animals paralleled that seen in normal lung homogenates during the first 4 h, the final concentration attained was only 10-fold greater than that obtained with bacterial suspensions in diluent (Trypticase soy broth-saline).

To ascertain whether the observations were specific for strain TG, the investigation was repeated with *S. aureus* 18Z-G.

S. aureus 18Z-G Sm^r showed results comparable to those obtained with strain TG when incubated in homogenized lung preparations from normal animals (Fig. 7). However, in homogenates from infected mice, there was only slight multiplication followed by a persistence of the population for 40 h.

The occurrence of an 8- to 12-h lag before the cessation of multiplication suggested a possible activation process in the infected lung homogenates. Therefore, the study was repeated with lung homogenates which had been incubated at 37°C for 24 h before the addition of the test organisms. This prior incubation was designated

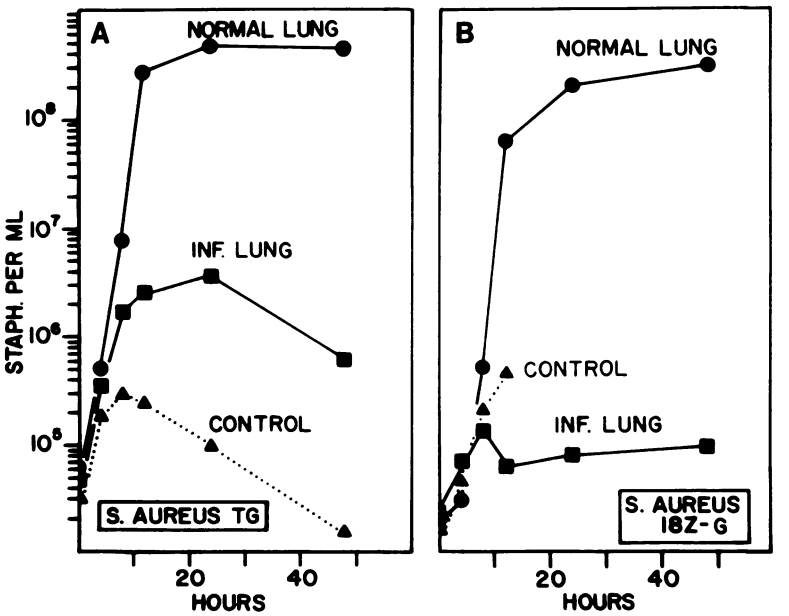

FIG. 7. *Survival of S. aureus TG Sm^r (A) or 18Z-G Sm^r (B) in homogenates from infected mouse lungs or normal lungs maintained at 37°C. Lungs were removed from mice 24 h after intratracheal inoculation of either 10^8 S. aureus TG Sm^s or saline. Cocci added to the homogenates were enumerated by plating portions on media containing 60 μg of streptomycin per ml. Cocci added to the diluent used in preparing the homogenates served as controls.*

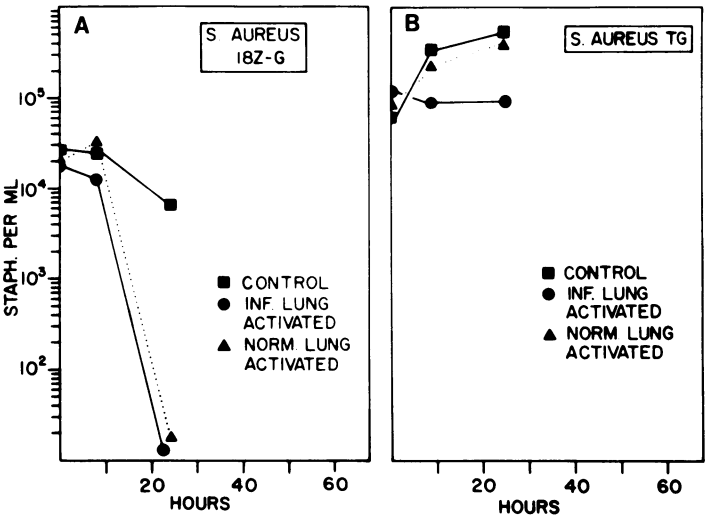

FIG. 8. *Survival of S. aureus 18Z-G Sm^r (A) or TG Sm^r (B) in activated homogenates from infected mouse lungs or normal lungs maintained at 37° C. Lungs were removed from mice 24 h after intratracheal inoculation of either 10^8 S. aureus TG Sm^r or saline. Cocci added to the homogenates were enumerated by plating portions on media containing 60 μg of streptomycin per ml. Cocci added to the diluent used in preparing the homogenates served as controls.*

"activation."

In the presence of activated normal lung homogenates, the growth of *S. aureus* TG Sm^r merely paralleled that seen in the diluent and consisted of a fivefold increase over a 24-h period (Fig. 8). Strain TG incubated in the presence of activated homogenates from infected lungs persisted for a 48-h period but failed to multiply.

With activated homogenates from either normal or infected lungs, strain 18Z-G Sm^r exhibited a 3-log decline in the population over the 24-h period of observation (Fig. 8).

DISCUSSION

Although other workers have introduced staphylococci into the lungs by aerosolization, we did not attempt to utilize this method because of inherent difficulties in delivering sufficient organisms to the lungs during a suitable period of time.

In preliminary studies we found the inoculation of mice by intranasal instillation to be an unreliable means of introducing known quantities of organisms into the lungs. Even under deep anesthesia, a variable but significant portion of the inoculum appeared to be expired and reflexively swallowed in at least half of the animals infected. Attempts to substitute direct inoculation into the trachea proved only slightly more consistent than intranasal inoculation because expiration of the inoculum still occurred in a portion of animals inoculated. This problem was eventually circumvented by modifying the inoculation procedure. By exerting pressure on the abdomen to deflate the lungs and then relaxing this pressure concurrent with expulsion of the inoculum from the needle, the animal was forced to inspire the entire inoculum. Immobilization of the mice at approximately 60° from the horizontal aided in this maneuver, presumably because of gravitational effects. With this method even large doses (10^7 to 10^9) were consistently introduced, as reflected in an average recovery from the lungs of 95 ± 8% (2 standard deviations) of the cocci inoculated into the trachea.

Our findings indicated that *S. aureus* TG, even in doses of 10^9, was unable to multiply in the lungs of mice and was, in fact, eliminated from the lungs at a rapid and continuous rate. Even though multiplication of the staphylococci did not occur, large doses were lethal for mice or capable of causing tissue destruction. These effects presumably resulted from the elaboration of one or more toxins by the organism in situ.

Factors responsible for the multiplication of staphylococci in mice are not well defined. The encapsulated Smith strain (mucoid) can multiply after intraperitoneal inoculation (9, 15), and certain exfoliatin-producing strains are able to proliferate in the subcutaneous tissues (17). Strain TG, a known exfoliatin producer, and the Smith mucoid strain, however, were unable to multiply in the lungs of mice. Therefore, capsule formation or exfoliatin production are not the sole requisites for proliferation in the mouse.

The reasons for the delay in staphylococcal

elimination observed in animals infected under ether anesthesia are unclear. Direct ether toxicity to phagocytic cells, damage to pulmonary surfaces, or secondary changes leading to fluid accumulation are possible explanations and are considered significant factors in the establishment of respiratory tract infections with other organisms after ether anesthesia (1, 4, 25, 28). If such factors were actually responsible for persistence, one could suspect that the exposure to ether was not sufficient to permit proliferation of the staphylococci, or else the effect was later negated by other factors so that the inoculum was eventually eliminated. The latter possibility appears plausible in light of other observations discussed below. Furthermore, experimental animals etherized 3 days before or 3 days after infection with 10^8 cocci revealed an elimination of the organisms comparable to that observed in animals exposed to ether at the time of inoculation. Attempts were not made to assess the effect of a greater exposure to ether.

It has been suggested that an antecedent viral infection of the respiratory tract predisposes the lungs to bacterial infection (3, 12, 13, 30). In our study an antecedent influenza virus infection did not permit staphylococci to multiply in the lungs, and survival of the bacterium was not enhanced. These findings are contrary to those reported by Sellers et al. (29), who observed an inhibition of clearance of staphylococci inoculated intranasally or by the aerosol route in mice previously infected with influenza virus A/Japan/305/57. In their study an inhibition of bacterial clearance, 1 to 3 days in duration, was observed in animals infected 7 days previously with virus. Aside from technical differences or disparities attributable to use of different strains of experimental animals, influenza virus, or challenge organisms, we have no explanation for these differences.

Jakab and Green (12) obtained evidence that staphylococcal multiplication was limited to consolidated regions of Sendai virus-infected lungs. To promote the deposition of cocci in close proximity to virus-infected regions, we infected mice with mixtures of virus and bacteria. However, our data showed no enhanced survival of cocci in mice challenged simultaneously with influenza and staphylococci.

It is usually assumed that phagocytosis by alveolar macrophages constitutes the major defense mechanism in the lungs (6, 26). Attempts to interfere with phagocytic mechanisms by prior clumping of the inocula, inoculation of killed cocci with the viable inoculum, or use of the encapsulated Smith strain, however, failed to reduce the rate of elimination.

Aggregation of *S. aureus* has been shown to impede phagocytosis of the cocci in the peritoneal cavity of mice (15). Intraperitoneal inoculation of more than 2×10^8 cocci possessing the bound coagulase (clumping factor) results in prompt clumping because of the interaction of the bound coagulase with fibrinogen in the cavity fluid. The clumping protects the inoculum from peritoneal leukocytes except for some minimal phagocytosis occurring on the surface of the clumps. In an attempt to create a similar situation in the lungs, the cocci were aggregated with citrated rabbit plasma before inoculation. Histological sections obtained shortly after infection confirmed the presence of aggregates of various sizes in the lungs. However, in spite of this aggregation the organisms inoculated under ether anesthesia were effectively eliminated and without evidence of a lag period. Because many clumps were estimated to contain thousands, or tens of thousands, of closely packed cocci, it seems unlikely that the destruction of these aggregated staphylococci was due to phagocytosis alone.

The capsule on the Smith mucoid strain has been shown to hinder phagocytosis to a significant degree when the organism is inoculated into the peritoneal cavity of mice (9, 15). Intraperitoneal doses of 10^8 encapsulated organisms, or greater, multiply and cause death of the experimental animals, presumably due to elaboration of alpha-toxin. Antibody to the capsular substance is known to promote phagocytosis and afford protection. Our experimental animals had no known prior exposure to this strain and readily succumbed to intraperitoneal infection, yet when introduced into the lungs this organism was eliminated just as rapidly as was the nonencapsulated TG strain.

Recent findings of Johnson and co-workers (14) indicate that *Neisseria gonorrhoeae* are rapidly eliminated from the lungs of mice after intranasal inoculation. Their data also suggest that phagocytosis may not be the major means of destroying gonococci in this site.

To explain the elimination of the cocci, lung lavage fluids were examined for possible bactericidal mechanisms, but no soluble inhibitory substances were detected in lavage fluids from either normal or infected animals. The multiplication that occurred in these lavage fluids was comparable to that noted by LaForce and coworkers (21, 22). Because they had observed multiplication of staphylococci in lavages from normal rats or rabbits, they also concluded that bronchopulmonary secretions from these animals do not have bactericidal activity against *S. aureus*.

Although it is conceivable that reduction in the pulmonary population might result from

dissemination of the organisms to other organs, this seems unlikely. Dissemination, to the extent required to satisfy the observations, would probably involve considerable seeding of the circulation, which in turn would lead to extensive renal involvement (16). No such evidence of massive dissemination was noted in infected animals.

The reduced growth or killing of *S. aureus* strains TG or 18Z-G in the presence of homogenized lung tissue suggests that substances may be generated during infection that can destroy staphylococci in vivo. The retention of activity after repeated freeze-thawing of these tissue homogenates tends to discount the role of viable leukocytes in this process, but not that of their lysosomal contents. No attempts were undertaken in this study to assess the presence of basic proteins, lactoferrin, or other known anti-staphylococcal leukocytic products in the washed tissue homogenates.

It is possible that the antibacterial activity found in lung tissue is analogous to a recently detected cidal system associated with abscesses produced by intraperitoneal infection of mice with *S. aureus* (E. S. Dye and F. A. Kapral, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, B70, p. 27). Homogenates of abscesses formed during this infection possess bactericidal activity which is also increased by prior incubation (activation). In this case the activity is due to a fatty acid capable of rapidly destroying staphylococci in vitro. Although the fatty acid responsible for the cidal activity has not as yet been identified, it exerts markedly different degrees of activity toward various *S. aureus* strains, with strain 18Z-G being significantly more sensitive than strain TG.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant AI-7826 from the National Institute of Allergy and Infectious Diseases.

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