INFECTION AND IMMUNITY, Dec. 1970, p. 757-761
Vol. 2, No. 6

Printed in U.S.A.

Immunologically Induced and Elicited Local Resistance to *Staphylococcus aureus*

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Received for publication 6 July 1970

Allergy characterized by delayed hypersensitivity was induced in populations of mice by injection, respectively, of *Staphylococcus aureus* and of tubercle bacilli. Eliciting doses of staphylococcal antigen or of old tuberculin antigen were injected subcutaneously into the nape of the neck of samples of these mice. A challenge dose of *S. aureus* was injected into the site of the eliciting injection, and the subsequent survival curves of the infecting staphylococci were determined by plate counts. Survival of the challenge staphylococci was significantly reduced only when the eliciting antigen was homologous with the inducing antigen. Thus induction and elicitation were specific, but the local resistance was nonspecifically effective against staphylococci.

Cell-mediated resistance has received greatly increased attention during the last decade. Excellent reviews are available (1, 3, 5–7, 9). Nevertheless, the role of cell-mediated resistance in infection with *Staphylococcus aureus* has remained ambiguous (8, 10). The present communication is one of a series in which experimental models in the mouse are examined in the hope of clarifying this role.

In an earlier study (12), it was shown that delayed-type hypersensitivity could be induced in mice by repeated infection with *S. aureus* and was demonstrated by injection of staphylococcal antigen into the footpad. This hypersensitivity was transferred to normal recipient mice by viable spleen cells from hypersensitive donors, but not by nonviable cells, cell-free extracts, or plasma of hypersensitive donors. Histopathological study of the footpads of normal and hypersensitive mice injected with staphylococcal extract were carried out by J. Součková-Štěpánová (11). Injection into normal mice produced acute inflammation with infiltration predominantly of polymorphonuclear leukocytes. Injection into hypersensitive mice produced inflammation and infiltration which became a predominantly mononuclear granuloma.

Populations of mice in which allergy characterized by delayed hypersensitivity, respectively, to staphylococcal extract and to old tuberculin (OT) were studied by Taubler and Mudd (13). Splenic explants from such populations were examined for cell migration in vitro. Elicitation of the migration-inhibition phenomenon was accomplished, respectively, with staphylococcal extract and with OT. Inhibition of cell migration in vitro was found only when specific antigen was used as the eliciting agent, i.e., migration of cells from spleens of mice hypersensitive to staphylococcus was inhibited by staphylococcal extract but not by OT; migration of cells from tuberculin-hypersensitive mice was inhibited by OT but not by staphylococcal extract. In the concentrations used, cells from normal animals were not significantly inhibited. Thus induction in mice of allergy characterized by delayed hypersensitivity and elicitation of the migration-inhibition phenomenon in vitro have both been shown to be immunologically specific.

The present study demonstrates local resistance against staphylococcal challenge, i.e., the effector mechanism, of such induction and elicitation. The effector mechanism is found to be nonspecific; i.e., mice prepared by infection with tubercle bacilli and local elicitation by OT are as well protected against local challenge with viable staphylococci as are mice prepared by staphylococcal infection and local elicitation by staphylococcal antigen. This result is in agreement with other systems studied, in particular with ones by Mackaness (6, 7).

MATERIALS AND METHODS

Strains used. *S. aureus* strain 18Z, previously described (4), and *Mycobacterium tuberculosis* strain H37Ra were used in the present study.

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Sensitization of mice. Female albino Swiss mice, weighing 24 to 26 g, were used throughout. Altered reactivity characterized by delayed hypersensitivity was induced by infection, respectively, with *S. aureus* 18Z or with H37Ra as previously described (12). Mice were injected subcutaneously with 10^8 viable staphylococci, in alternate flanks, once a week for 8 weeks.

Mice were made hypersensitive to tubercle bacilli by giving an intraperitoneal injection, once every 2 weeks for 4 weeks, with approximately 3×10^7 organisms (cell concentrations estimated by the McFarland nephelometric method). Tuberculin hypersensitivity was determined by footpad testing with 200 μg of OT in a 0.02-ml volume 1 to 2 weeks after the second infection. Measurements and determination of the net footpad swelling have been described (12). These measurements were made at 30 min and at 1 and 2 days.

Preparation and challenge infection of mice. The posterior neck region of each mouse was depilated with Nair one day prior to challenge infection. A 0.1-ml amount of a staphylococcal suspension (10^6 colony-forming units/ml), the concentration of which was predetermined by plate count and subsequently checked at time of inoculation, was injected subcutaneously in the nape of the neck of mice anesthetized with nembutal. The cocci were suspended in 1:100 dilution of Trypticase Soy Broth in saline.

Determination of staphylococcal population at challenge injection site. At the sampling time, the entire tissue around the site (skin and subcutaneous tissue) was excised and ground with sterile sand. The ground tissue was suspended in 5.0 ml of 1% Trypticase Soy Broth. The number of viable organisms at the challenge infection site at the various sampling times was estimated by determining by plate counts the median value for at least 15 mice at each sampling time.

Antigens. The staphylococcal antigen was prepared as previously described (12). The mycobacterial antigen, OT, was old tuberculin (Wyeth).

Antigen injection. A 0.1-ml amount of staphylococcal antigen (300 μg/dose in saline) was injected 24 hr prior to and 24 and 72 hr after the challenge injection of *S. aureus*, the antigen injection site being the same as for the challenge injection.

A 0.1-ml amount of OT (400 μg/dose in saline) was injected 24 hr prior to and 24 hr after the challenge injection of *S. aureus*, the antigen injection site being the same as for the challenge injection.

RESULTS

Survival of staphylococci in normal and staphylococcal-hypersensitive mice. The survival of cocci in subcutaneous tissue of staphylococcal-sensitive mice, without antigen, and normal mice, with and without antigen, was approximately the same throughout the duration of the experiment (Fig. 1). There is no significant difference as determined by the *t* test (Table 1). Complete elimination of cocci is seen only in staphylococcal-hypersensitive mice injected with staphylococcal antigen 24 hr before and 24 and 72 hr after challenge

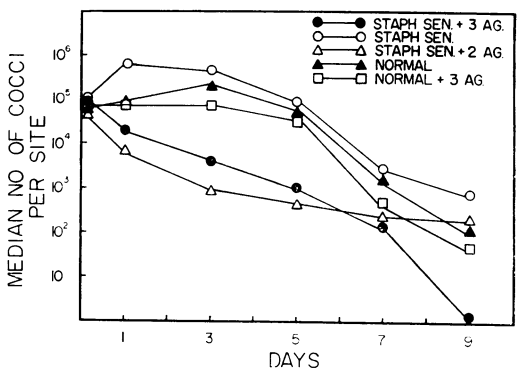

FIG. 1. *Survival of staphylococci in subcutaneous tissue of mice challenged with 10^5 viable staphylococci under various experimental conditions. The designation 2 AG indicates antigen injection 24 hr prior to and 24 hr after challenge injection; 3 AG indicates antigen injection 24 hr prior to and 24 and 72 hr after challenge injection. Local resistance to Staphylococcus was increased only when staphylococcal antigen was used locally as eliciting agent in mice in which hypersensitivity had been induced by infection with S. aureus.*

TABLE 1. *Probabilities derived statistically when the survival of staphylococci in normal mice (with or without antigen) and staphylococcal-hypersensitive mice (with or without antigen) are compared*

Conditions[a]	Probabilities				
	1 day	3 day	5 day	7 day	9 day
SS + 3 Ag vs. normal	<0.10	<0.001	<0.001	<0.01	<0.05
SS + 3 Ag vs. SS	<0.001	<0.001	<0.001	<0.001	<0.001
SS + 3 Ag vs. normal + 3 Ag	<0.02	<0.001	<0.001	<0.02	<0.02
SS vs. normal	<0.005	<0.20	<0.20	<0.20	<0.01
SS vs. normal + 3 Ag	<0.001	<0.001	<0.10	<0.10	<0.05
Normal vs. normal + 3 Ag	<0.80	<0.10	<0.70	<0.70	<0.80
SS + 2 Ag vs.; SS + 3 Ag	<0.02	<0.01	<0.05	<0.40	<0.05

[a] SS, staphylococcal-sensitive; Ag, staphylococcal antigen. Designation 2 Ag indicates antigen injection 24 hr prior to and 24 hr after challenge; 3 Ag, 24 hr prior to and 24 and 72 hr after.

injection. This elimination was continuous throughout the experimental period and by 9 days postchallenge no organisms could be recovered. There is a significant difference between hypersensitive mice with antigen and normal mice with and without antigen, as determined by the *t* test. When two injections of antigen (24 hr before and 24 hr after challenge) were given to mice hypersensitive to staphylococci, the survival curve was similar to the curve obtained with three injections of antigen as late as day 7. Thereafter, no additional decrease in the number of cocci at the challenge site was noted.

Survival of staphylococci in normal and tuberculin-hypersensitive mice. The survival of staphylococci in subcutaneous tissue of normal mice, with and without antigen (OT), was similar (Fig. 2). There is no significant difference as determined by the *t* test (Table 2). The survival curve of staphylococci in tuberculin-sensitive mice without OT was also similar as late as day 7 postchallenge; however, at 9 days no cocci could be found.

Tuberculin-sensitive mice injected twice with OT (24 hr prior and 24 hr after staphylococcal challenge) eliminated the staphylococci at a faster rate than tuberculin-sensitive mice without OT. There is a significant difference as determined by the *t* test. No cocci could be recovered from these antigen-injected mice by the 7th postchallenge day.

The elimination of staphylococci in mice with three OT injections showed decreased survival in comparison with normal mice but quantitatively less decreased survival than with two OT injections.

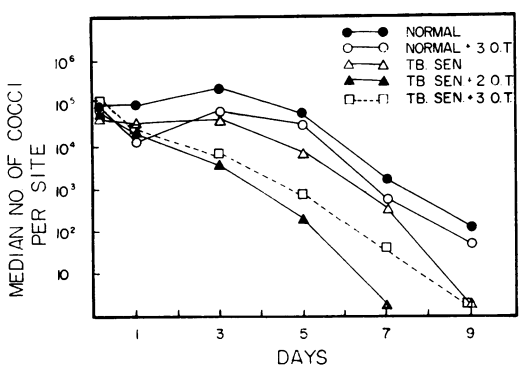

FIG. 2. *Survival of staphylococci in subcutaneous tissue of mice challenged with 10^5 viable staphylococci under various experimental conditions. The designation 2 OT indicates OT injection 24 hr prior to and 24 hr after challenge injection; 3 OT indicates OT injection 24 hr prior to and 24 and 72 hr after challenge injection. Local resistance to Staphylococcus was increased significantly only when tuberculin was used locally as eliciting agent in mice infected with tubercle bacilli.*

TABLE 2. *Probabilities derived statistically when the survival of staphylococci in normal mice (with or without OT) and tuberculin-hypersensitive mice (with or without OT) are compared*

Conditions[a]	Probabilities			
	1 day	3 day	5 day	7 day
TBS vs. TBS + 2 OT	<0.20	<0.001	<0.005	<0.01
TBS vs. normal	<0.30	<0.005	<0.50	<0.20
TBS vs. normal + 3 OT	<0.40	<0.40	<0.90	
TBS + 2 OT vs. normal	<0.005	<0.001	<0.001	<0.001
TBS + 2 OT vs. normal + 3 OT	<0.70	<0.001	<0.01	<0.01
Normal vs. normal + 3 OT	<0.10	<0.001	<0.10	<0.20
TBS + 2 OT vs. TBS + 3 OT	<0.40	<0.01	<0.40	<0.50

[a] TBS, tuberculin-sensitive; OT, old tuberculin mycobacterial antigen. Designation 2 OT indicates antigen injection 24 hr prior to and 24 hr after challenge; 3 OT, 24 hr prior to and 24 and 72 hr after.

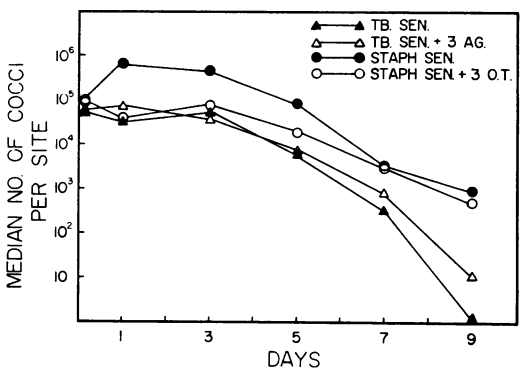

FIG. 3. *Survival of staphylococci in subcutaneous tissue in mice challenged with 10^5 viable cocci under various experimental conditions. The designation 3 AG indicates injection of staphylococcal antigen 24 hr prior to and 24 and 72 hr after challenge injection; 3 OT indicates injection of OT 24 hr prior to and 24 and 72 hr after challenge injection. Local resistance to Staphylococcus was not increased when elicitation was attempted with heterologous antigens.*

Survival of staphylococci in staphylococcal- and tuberculin-hypersensitive mice. Survival of staphylococci in subcutaneous tissue of mice, hypersensitive to staphylococci, with and without heterologous antigen (OT), is similar; approximately 10^3 viable cocci remain after the 9th post-

TABLE 3. *Probabilities derived statistically when the survival of Staphylococci in tuberculin- and staphylococcal-hypersensitive mice (with heterologous antigen) are compared*

Conditions[a]	Probabilities			
	1 day	3 day	5 day	7 day
TBS vs. SS	<0.001	<0.001	<0.005	<0.005
TBS vs. TBS + 3 Ag	<0.50	<0.70	<0.80	<0.50
TBS vs. SS + 3 OT	<0.70		<0.25	<0.025
TBS + 3 Ag vs. SS	<0.005	<0.001	<0.005	<0.10
TBS + 3 Ag vs. SS + 3 OT	<0.60	<0.50	<0.10	<0.10
SS vs. SS + 3 OT	<0.001	<0.001	<0.10	<0.50

[a] TBS, tuberculin-sensitive; SS, staphylococcal-sensitive; Ag, staphylococcal antigen; OT, old tuberculin mycobacterial antigen. Designation 3 Ag or OT indicates antigen injection 24 hr prior to and 24 and 72 hr after.

challenge day (Fig. 3). There is no significant difference as determined by the *t* test (Table 3). With regard to the survival of staphylococci in mice hypersensitive to tuberculin, with and without heterologous antigen (staphylococcal antigen), the survival of staphylococci is similar throughout the experimental period. There is no significant difference as determined by the *t* test. At the 9th postchallenge day, no cocci could be recovered from tuberculin-hypersensitive mice; similarly, only a few cocci could be recovered from tuberculin-hypersensitive mice with heterologous antigen (staphylococcal antigen).

DISCUSSION

This study shows that survival of staphylococci in the subcutaneous tissue of mice can be altered by the injection of antigens to which the mice are hypersensitive.

In the staphylococcal system (Fig. 1), complete elimination of cocci was seen only in mice hypersensitive to staphylococci receiving three staphylococcal antigen injections. In staphylococcal-hypersensitive mice receiving two staphylococcal antigen injections, the staphylococcal survival curve paralleled that of hypersensitive mice receiving three antigen injections as late as the 7th postchallenge day, after which time no further decrease was noted.

Regarding the survival of staphylococci in mice hypersensitive to tuberculin (Fig. 2), elimination of staphylococci occurred by the 9th postchallenge day without the injection of antigen (OT). Some degree of local cellular immunity thus may have existed in these animals prior to any injection of extrinsic antigen.

The fact that the challenge staphylococci survived longer in mice hypersensitive to tuberculin with three eliciting injections of OT than with two eliciting injections of OT deserves comment. Probably living tubercle bacilli were still present in the mice hypersensitive to tuberculin. The third injection of tuberculin very likely was excessive. This interpretation is in agreement with other observations, which suggest that the injection of eliciting antigens needs to be correct in amount and timing (E. G. Allen and S. Mudd, *unpublished data*).

The site of antigen injection needs some comment. Dannenberg (1, 2) has shown that the greatest level of cellular immunity develops in the local lesion. Thus the antigen injection site and the challenge injection site in the present study were purposely placed in close proximity. The antigen was injected directly into the challenge injection site.

In earlier studies, we have shown that contact of staphylococcal antigen with a source of macrophages (splenic fragments) of staphylococcal hypersensitive mice specifically inhibits migration of the macrophages. It seems altogether probable that such migration inhibition in the present experimental model serves to trap macrophages at the site of challenge. In fact, the injection of antigen in hypersensitive mice produces conspicuously more local induration than in normal mice. The relative importance of numbers of macrophages at the local site and the activity of the individual macrophages remain to be ascertained. Experiments to this end are in progress.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI 05473 from the National Institute of Allergy and Infectious Diseases and by the U.S. Veterans Administration Central Office Research Service, Stuart Mudd, responsible investigator.

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