STUDIES ON THE PATHOGENESIS OF STAPHYLOCOCCAL INFECTION*

II. THE EFFECT OF NON-SPECIFIC INFLAMMATION

BY KEIICHI GOSHI, M.D., LEIGHTON E. CLUFF, M.D.,
JOSEPH E. JOHNSON, 3RD, M.D., AND
C. RICHARD CONTI, M.D.

(From the Division of Allergy and Infectious Disease, Department of Medicine,
The Johns Hopkins University School of Medicine and Hospital, Baltimore)

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In the preceding paper it was shown that large numbers of pathogenic staphylococci must be injected intradermally into normal rabbits to induce a local skin infection (1). The apparent low infectivity of staphylococci in normal rabbit skin is similar to that observed by Elek (2) in human beings. Elek, however, demonstrated an increase in infectivity in the skin of volunteers when the microorganisms were inoculated with a foreign body such as a silk suture. In naturally occurring staphylococcus disease of man it is usual to observe infection in areas of tissue injury. For example, the most frequent site of infection is in skin wounds or abrasions and in obstructed hair follicles. This suggests that inflammation may play a role in altering the susceptibility of skin and other tissues to infection by the staphylococcus. That non-specific inflammation may significantly influence the evolution of an infection has been demonstrated many times (3–13), although the age of the inflammation and the nature of the bacterium probably play important roles in determining whether or not the inflammation increases or decreases the microorganisms' infectivity. Established inflammation, for example, has been shown to increase local resistance to the streptococcus, Bacillus pyocyaneus, and anthrax bacillus (6, 9, 12, 13). On the other hand established inflammation has been shown to enhance the infectivity of Treponema pallidum and fowl cholera bacillus (8, 9).

As indicated in the preceding paper, the presence in rabbits of delayed hypersensitivity to the staphylococcus is associated with a significant increase in infectivity of staphylococci injected into the sensitized animal (1). As the occurrence of a hypersensitivity reaction might be influencing the infectivity of the staphylococcus because of its associated inflammation, studies were done to evaluate the effect of a variety of types of non-specific inflammation upon

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local infectivity by the organism. It was shown that non-necrotizing inflammation did increase the infectivity of the staphylococcus although not to the same degree observed in animals with specific delayed hypersensitivity.

Methods

Bacteria. — *Staphylococcus aureus*, bacteriophage type 80/81, as described previously (1), was used in these experiments. The bacteria were cultured, as described in the preceding paper (1), and were washed twice with 0.85 per cent NaCl after 18 hours' growth. The number of bacteria injected was quantified by colony count of agar pour plates prepared just prior to each injection.

Rabbits. — White male rabbits weighing between 2500 and 3000 gm. were used in all experiments. Their dorsal and flank skin was exposed for inoculation with electric hair clippers. The animals were given water *ad libitum* and were fed with Purina pellets. They were usually kept in separate cages.

Thermal Injury. — Circular burns measuring approximately 2.5 cm. in diameter were produced in the rabbit skin by application of the flat surface of a metal tube containing water and a heating coil encased in glass and connected to a rheostat to regulate the electrical current. The temperature of the water was kept constant and with regularity burns of varying severity could be induced. With a water temperature of 75°-80°C., applied for 10 to 30 seconds, an erythematous, non-necrotizing burn could be produced. A more intense erythematous burn, associated with edema, could be induced with a temperature of 100°C. applied to the skin for 3 seconds.

Chemical Injury. — Undiluted croton oil was applied topically to rabbit skin producing marked erythema and edema without necrosis.

Bacterial Injury. — Group A streptococci, cultured for 6 hours at 37°C. in trypticase soy broth, were inoculated into rabbit skin in a volume of 0.2 ml. of diluted or undiluted culture, producing erythema, induration, and necrosis, depending upon the dose.

Arthus Hypersensitivity Reaction. — Rabbits were sensitized to human serum albumin by weekly intramuscular injections of 50 mg. (1.0 ml.) of Cohn fraction V emulsified in 1.0 ml. of “complete” adjuvant (Difco-Freund’s). After 8 to 10 weeks, erythematous, indurated and occasionally hemorrhagic lesions could be produced by intracutaneous inoculation of 1.0 mg. (0.2 ml.) of the albumin.

Tuberculin Hypersensitivity Reactions. — Rabbits were sensitized by weekly intramuscular injections of 1.0 mg. (1.0 ml.) dry, heat-killed *Mycobacterium tuberculosis* emulsified in 1.0 ml. of adjuvant, as described above. Within 3 weeks erythematous and indurated lesions could be produced in these rabbits by intracutaneous inoculation of 0.1 ml. Old Tuberculin diluted 1:10 with 0.85 per cent NaCl.

Blood Cultures. — Blood was collected from ear vein or by cardiac puncture at intervals, was cultured in trypticase soy broth, and was subcultured on blood agar for identification of bacteria.

RESULTS

As described before (1), inoculation of $10^4$, $10^5$, $10^6$, and $10^8$ staphylococci into normal rabbit skin consistently resulted in production of small abscesses only with $10^6$ and $10^7$ bacteria. Fewer numbers of organisms produced either faint erythema or no reaction at all. To evaluate the effect of non-specific inflammation upon local infectivity by staphylococci the following series of experiments were performed, in which inflammation was induced in a number of different ways.
Inflammation Produced by Thermal Injury.—An erythematous, non-indurated, non-necrotizing burn was produced in 5 areas on the side of 3 rabbits as described above. Four of these burns were injected immediately with $10^8$, $10^7$, $10^6$, and $10^5$ staphylococci, respectively. In addition, the opposite side of the rabbit, where no burn had been induced, was similarly injected with the same concentrations of staphylococci. The lesions produced were photographed at 24 and 48 hours. Additional rabbits were treated in the same way but the staphylococci were inoculated at intervals of time varying from 3 hours to 4 days after production of the burn. Identical experiments were performed in which a more severe but non-necrotizing burn was induced as described in Methods.

Infection of the burned skin was induced with one-tenth the dose of staphylococci required to produce infection in normal rabbit skin. The abscesses produced with $10^8$ and $10^7$ organisms were large, associated with erythema, and were maximal 2 to 3 days after inoculation. This increased infectivity by the staphylococcus in an area of thermal injury, however, was demonstrable only when the organisms were inoculated within 3 days after inducing the burn. After the 3rd day, no increased infectivity by the staphylococcus was observed in an area of non-necrotizing thermal injury, and, in fact, lesions produced in such established areas of inflammation seemed to be somewhat less severe than lesions produced in normal skin. Blood culture and autopsy examination of these animals did not show bacteremia or metastatic infection.

Inflammation Produced by Chemical Injury.—Croton oil was applied topically to the skin of groups of rabbits in the same manner as the thermal injury was induced in the preceding experiment. Similar 10-fold dilutions of staphylococci were inoculated into the skin treated with croton oil and into normal skin at varying intervals of time after production of the chemical injury. The skin of rabbits treated with croton oil but not inoculated with staphylococci showed erythema at 6 hours, erythema and induration at 24 hours, and occasionally slight hemorrhage was seen, but necrosis was not evident. After 2 days, the area of chemical injury showed a decrease in erythema and by the 4th day all signs of active inflammation had subsided, and crusting over the area of inflammation appeared.

Some increase in infectivity of staphylococci injected into the area of chemical injury was observed when the bacteria were injected immediately after induction of the inflammation. This was characterized by the occasional appearance of a small abscess at the site of inoculation of $10^8$ staphylococci, and the lesions produced with $10^8$ bacteria tended to be somewhat larger than those observed with the same inoculum in normal skin. Injection of staphylococci into the areas of chemical injury 1 or 4 days after induction of the inflammation did not show an increased infectivity of the bacteria when compared with infection produced in normal skin at the same time. Blood cultures of these animals were negative and autopsy examination did not show metastatic abscesses.
Additional studies in which subcutaneous injection of 0.1 ml. 10 per cent croton oil, and 2 per cent tragacanth were done to produce inflammation, and staphylococci were injected as described above. These chemical irritants produced intense inflammation with necrosis and sterile abscesses within 24 to 48 hours. Injection of staphylococci into the prepared skin immediately and 4 days after inoculation of the chemical did not result in clearly definable infection, but no particular differences were observed between the infected and uninfected chemically inflamed skin lesions.

**Inflammation Produced with Group A Streptococci.**—Six hour cultures of streptococci were injected into 5 skin sites in groups of rabbits, in the same way as described in the previous experiments. Inoculation of 0.2 ml. of the culture diluted 1:50 resulted in lesions characterized by erythema and induration, a localized cellulitis, without abscess formation. These lesions were maximal at 6 hours, at 24 hours began to subside, and by 48 hours were sterile and the inflammation had almost completely disappeared. None of the animals expired from the streptococcal infection over several days observation. Three groups of rabbits prepared with such cutaneous streptococcal infections were injected into the sites of cellulitis with 10⁸, 10⁷, 10⁶, and 10⁵ staphylococci, respectively, as described before, immediately or 6 hours after injection of the streptococci. The third group of animals was injected in the same way with staphylococci 6 hours after inoculation of the streptococci and at the same time were given 1,000 units penicillin G intramuscularly, to interrupt the streptococcus infection.

There was no particular difference between the lesions induced with inoculations of staphylococci and streptococci together and the lesions produced by the streptococcus and staphylococcus inoculated separately into rabbit skin, and there were no differences between the observations made in the three groups of animals. None of the blood cultures taken during the course of the experimental infections were positive.

**Inflammation Produced by Arthus Hypersensitivity Reaction.**—Rabbits were sensitized to human serum albumin as described in Methods and Arthus reactions induced by intracutaneous inoculation of 1.0 mg. albumin in 0.2 ml. 0.85 per cent NaCl. Sensitized animals uniformly developed erythematous and edematous lesions within 2 to 3 hours after skin testing, and by 24 hours these became large, more intense and were occasionally associated with hemorrhage. These lesions had largely subsided by 48 hours leaving a faintly pigmented area. When the hemorrhagic reaction was severe, necrosis developed and a small ulceration appeared after a few days. Groups of sensitized rabbits were injected with the skin test dose in 5 separate sites, as described in the other experiments, one area serving as control, while the remaining 4 sites were inoculated with 10⁶, 10⁷, 10⁶, and 10⁵ staphylococci, respectively, either immediately or 24 hours after injection of the albumin. Similarly, injections of staphy-
lococci were made into normal skin on the opposite side of the animals in the same way as in all other experiments, as control.

Some increase in severity of the Arthus reactions injected with $10^8$ and $10^7$ staphylococci was seen, in comparison with the control lesions. However, infection was not produced by inoculation with fewer than $10^6$ organisms. Injection of staphylococci into necrotic Arthus reactions resulted in infection with $10^6$ bacteria, but these were not striking. It was of some interest, that injection of $10^8$, $10^7$, and $10^6$ staphylococci into an area inoculated immediately before with albumin served to prevent full development of the Arthus reaction, when compared with the control Arthus reactions in the same and other animals. Blood cultures of all rabbits were negative and autopsy did not reveal metastatic infection.

**Inflammation Produced by Tuberculin Hypersensitivity (Delayed) Reactions.**—Rabbits sensitized to *Mycobacterium tuberculosis* were skin-tested with Old Tuberculin as described in Methods and these inflammatory reactions were injected with $10^8$, $10^7$, $10^6$, and $10^5$ staphylococci in the same way as before, immediately and 24 hours after intracutaneous injection of the tuberculin.

No change in the character of the staphylococcus infection was observed when the organisms were injected into areas prepared by prior injection of tuberculin in sensitized animals compared with control tuberculin reactions and staphylococcus infections.

**DISCUSSION**

That "delayed" hypersensitivity unassociated with serum antibody to the staphylococcus may result in increased infectivity by the microorganism in the sensitized animal was shown in the previous paper (1) and confirms the observations reported by Johanovsky (14). The influence of delayed hypersensitivity upon the pathogenesis of infection has been examined most closely in tuberculosis (10), and it is of interest that in tuberculosis, as in staphylococcus infection, specific bacterial hypersensitivity is associated with an intensification of tissue injury produced by the bacteria but no obvious increase in host resistance. In addition, the evidence suggests that hypersensitivity to the staphylococcus may be important in increasing host susceptibility and the infectivity of the bacteria. Because the inflammatory reaction attributed to bacterial hypersensitivity might be responsible for the increase in host susceptibility, the studies reported here were done to evaluate the effect of non-specific inflammation upon staphylococcus infection.

To find that early inflammation produced in different ways could increase the infectivity of staphylococci is certainly not surprising, and the observation of a decrease in infectivity in established areas of inflammation is a direct confirmation of the findings in other experiments with other bacteria (3-13). Indeed, it has been repeatedly shown that during the early stages of inflam-
tion many bacteria tend to extend more rapidly, leading to a more severe infection (6, 9, 12, 13), and this has been associated with the presence of inflammatory edema and increased flow of lymph (11). In the studies reported here, no evidence of dissemination of infection was found after injection of staphylococci into areas of early inflammation, but the local lesion was intensified. There was some variation in the effect of non-specific inflammation upon the infectivity of staphylococci dependent upon the way in which inflammation was produced. However, the general similarity of the effect suggests a common mechanism was operative. The effect of non-specific inflammation upon staphylococcus infection, however, was never as impressive as that observed with specific delayed hypersensitivity (1); and although it is not possible to account for this difference, it would seem most reasonable to assume that each enhances infection in the same way. The effect of inflammation upon infection is determined in many instances by the age of the inflammatory reaction, and the possible differences of this factor in specific delayed hypersensitivity and non-specific inflammation may account for the observed variation in severity of infection and host susceptibility.

Early or acute inflammation, as a general rule, increases the infectivity of bacteria locally although a notable exception to this rule is Treponema pallidum. Chesney and Kemp (8) have shown that the infectivity of this microorganism may be less in early inflammation than in an established inflammatory reaction of some days duration. In addition, it has been shown by Halley et al. (9) that fowl cholera bacillus is lethal for rabbits when inoculated into established or early skin inflammation. The usual effect of established inflammation on bacterial infection resulting in a decrease in infectivity or increase in local resistance to infection, however, has been shown previously to occur with Streptococcus erysipelas, staphylococcus, anthrax bacillus, and Bacillus pyocyaneus in a number of animal species (6, 9, 12, 13).

The mechanisms determining the effect of inflammation upon bacterial infection are still poorly understood but appear to involve not only the properties of inflamed tissue but also the virulence of the bacterium. The influence of bacterial virulence is illustrated by fowl cholera bacillus in granulating wounds of rabbits (9). This microorganism is very invasive in the rabbit and leads to disseminated infection and death within a day or so, and the presence or absence of inflammation induced before inoculation of the bacillus does not influence its infectivity. It may be concluded with fowl cholera infection in rabbits, therefore, that mechanisms of non-specific resistance probably play little, if any, role in controlling the infection.

Inflammation could be expected to influence the infectivity of an organism only when factors of non-specific resistance play an important role in conditioning the organism's pathogenicity. It is probably for this reason that the effects of inflammation upon infection are shown best with organisms of
moderate pathogenicity, such as the streptococcus and pneumococcus. It should also be possible to show, however, that the infectivity of bacteria of low pathogenicity would be less influenced by inflammation; and this may, in fact, be the case with the staphylococcus. In a sense then it is possible to separate the effects of inflammation upon infectivity according to virulence of the microorganism: bacteria of high virulence produce lethal or severe infection, irrespective of the presence of inflammation; bacteria of moderate virulence produce infection preferentially in areas of acute or early inflammation; and the infectivity of bacteria of low virulence may be influenced very little by inflammation.

The factors of non-specific resistance to infection that influence the response to bacteria inoculated into an area of inflammation may vary depending upon the microorganism. For example, *Treponema pallidum*, although capable of producing infection in areas of acute or chronic inflammation of the skin, may not initiate infection when inoculted into normal skin (9). In this instance, therefore, inflammation, regardless of age, only increases susceptibility to infection. This is contrary to the observed effects of established inflammation found in this and other reports with the staphylococcus showing a lessened infectivity of the bacteria in subacute or chronic inflammation. The differences in these findings suggest that the factors operating in host resistance to syphilitic infection are separable from those operative in staphylococcus and many other bacterial infections.

It is not pertinent to discuss here the problem of non-specific resistance to infection, except as it relates to staphylococcus infection, and in this regard little can be said as so little is known. However, it seems likely that phagocytosis plays a most important role in host resistance to this organism (15); and the increase in numbers of phagocytes, particularly macrophages, in areas of established inflammation suggest that these may be of importance in determining the decreased infectivity of staphylococci in areas of inflammation of 3 days' duration or more. The role of macrophages in inflammation in increasing resistance to infection has been studied and shown previously (4, 12). Polymorphonuclear leukocytes also may phagocytose and destroy staphylococci (16), and these cells are predominant in inflammation in its early stages (15). The fact that early inflammation is associated with local increase rather than a decrease in infectivity of staphylococci might initially suggest that the polymorphonuclear leukocytes are of no importance in resistance; however, the many other dynamic processes in early inflammation such as vasodilatation, thrombosis, increasing capillary permeability, increased lymph flow and changes in tissue acidity may be responsible for the failure of these phagocytes to control the infection (17).

The possibility that non-specific necrosis of tissue may play a more important role than non-necrotic inflammation in increasing local susceptibility was found
when necrotic burns were infected with staphylococci, and this is the basis of
the following report.

SUMMARY

Studies have been described in which the effect of early and late or established
inflammation upon staphylococcus infection of rabbit skin has been evaluated.
Inflammation was produced in skin by thermal, chemical, bacterial and immu-
ological injury, and it was found that the area of inflammation was more
susceptible to staphylococcus infection than was normal skin if the bacteria
were injected within 2 to 3 days after the injury. When staphylococci were
injected into an area of inflammation of over 3 days' duration, there appeared
to be an increase in local resistance to infection. The way in which inflammation
was produced seemed to have a little influence upon the effects observed. This
influence of non-specific inflammation upon staphylococcus infection was
compared with the influence of specific bacterial hypersensitivity, which also is
associated with an increase in infectivity of the microorganism in sensitized
animals. It was concluded that specific bacterial hypersensitivity probably
increases susceptibility to infection with the staphylococcus in the same way
as non-specific inflammation. The general significance of non-specific inflam-
maction upon infection is also discussed.

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