Immunity in Staphylococcal Infection: Effect of Gamma Globulin on Burn Infections.* (31197)

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Previous studies on the pathogenesis of staphylococcal skin infection in rabbits indicated that a necrotic burn showed a significant increase in susceptibility to infection with *Staphylococcus aureus*(1). Not only was the minimal infective dose of microorganisms markedly reduced, but resulting infections differed qualitatively from those induced in unburned normal skin. Characteristically, a spreading hemorrhagic lesion developed extending beyond the area of burn, in contrast to the typical pustular lesions which resulted from injections on the opposite side of the same rabbit. Prevention of the hemorrhagic lesion of burned skin was regularly conferred by antistaphylococcal alpha hemolysin antibody present in rabbits recovering from burn infections, or acquired either by active immunization with alpha hemolysin toxoid or by passive transfer of antiserum(1).

Staphylococcal infections (along with those due to *Pseudomonas*) are important in the morbidity and mortality of seriously burned patients, and the staphylococcal alpha hemolysin antibody may be of value in amelioration of human burn infections. Studies were undertaken, therefore, on the levels of antihemolysin in the serum of normal persons and in commercial pooled human gamma globulin. In addition, the effect of gamma globulin on the development of infection in burns of rabbit skin was investigated.

Materials and methods. Serum samples were obtained from 211 apparently healthy individuals donating blood to the hospital blood bank. Staphylococcal anti-alpha hemolysin titers were performed as described previously(2) using a standard staphylococcal toxin and antiserum (Burroughs Wellcome & Co., Inc., Staphylococcus α-Haemolysin (Dried), "Wellcome" brand and Staphylococcus anti-α-Haemolysin, "Wellcome brand") and normal rabbit erythrocytes. One unit of anti-hemolysin activity is defined as the amount of antibody which will neutralize one LH_{50} dose of standard alpha hemolysin. The LH_{50} dose is that amount of toxin which will produce 50% hemolysis of a 2% suspension of rabbit erythrocytes.

Pooled human gamma globulin samples from 21 separate production lots were furnished by Merck Sharp and Dohme Research Laboratories, West Point, Pa., and samples were obtained commercially from 5 additional lots produced by Lederle Laboratories, E. R. Squibb & Sons, and Pitman Moore Co. In addition, a de-aggregated gamma globulin (preparation MK-860) was furnished by Merck Sharp and Dohme.

Staphylococcal "hemagglutinin titers" were performed according to the method of Weld and Rogers(3), which detects antibodies capable of agglutinating erythrocytes coated with staphylococcal cellular antigen.

A series of standard necrotic burns was produced in the shaved skin of 30 male New Zealand white rabbits by means of a flat bottomed metal tube containing water at 100°C, as previously described(1,4). Eleven rabbits served as controls and 19 received gamma globulin in varying dose, route and time schedules. In each animal, one burn was injected with 10^7 staphylococci, one with 10^6 organisms, and one or more burn lesions left as controls. Injection of staphylococci was carried out within 30 minutes following induction of the burn. Four additional rabbits received intravenous injections of alpha hemolysin in normal skin.

The strain of *Staphylococcus aureus* used (Lafferty(1)) was coagulase positive, bacteriophage type 52/42B/80/81. It fermented

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TABLE I. Anti-Hemolysin Titers of 211 Normal Persons.

Anti-hemolysin titer (u/ml)	No.	%
0 *	99	47 %
1.0	11	5.2
1.5	64	30
2.0	4	1.9
3.0	23	10.9
3.5	3	1.4
4.0	1	.5
6.0	4	1.9
7.0	1	.5
12.0	1	.5
	211	100. %

* No detectable titer.

mannitol and produced alpha hemolysin. A frozen stock culture was thawed and used to inoculate trypticase soya broth which was then incubated at 37°C for 18 hours.

Results. Anti-alpha hemolysin in normal persons. The distribution of serum anti-alpha hemolysin titers in 211 apparently healthy individuals donating blood to the hospital blood bank is shown in Table I. Almost half (99 of 211) of these individuals had no detectable levels. By the method employed, titers less than 0.8 units were not detected. Thirty-seven persons had titers of 2 units or greater but only 6 had titers greater than 4 units.

Observations on 16 patients with recurrent staphylococcal furunculosis (Table II) revealed a similar distribution. Nine of the 16 had no detectable titers, and only one had a titer of 8 units. In contrast, 4 patients with staphylococcal osteomyelitis had titers of 8 to 24 units.

Pooled human gamma globulin. Anti-hemolysin titers on 26 lots of commercial pooled human gamma globulin ranged from 6 to 24 units per ml with a median titer of 12 units per ml. This approximated the expected titer since these preparations represented approximately 10-fold concentrations of human plasma gamma globulin, and the median titer of normal human serum was in the range of 1 to 1.5 units per ml. Anti-hemolysin titers of the pepsin digested "de-aggregated" gamma globulin preparation was 6 units per ml.

TABLE II. Anti-Hemolysin Titers.

	Anti-hemolysin titer (u/ml)	No.
Patients with recurrent furunculosis	0	9
	1.5	3
	2.0	1
	3.0	1
	8.0	1
		16
Patients with osteomyelitis	8	1
	16	1
	24	2
		4

Staphylococcal "hemagglutinating" antibodies, detected by their capacity to agglutinate erythrocytes coated with staphylococcal cellular antigen(3) were also present in the gamma globulin preparations in titers of 1:64 to 1:712 with a median titer of 1:356.

Effect of gamma globulin on rabbit burn infection. As shown in Table IV, characteristic spreading hemorrhagic infections developed in all 11 control animals receiving injections of 10^7 staphylococci in the areas of burned skin. Less severe lesions were produced by 10^6 organisms. The lesions reached maximum size and severity 24 to 48 hours following staphylococcal challenge.

TABLE III. Anti-Hemolysin Titers—Pooled Human Gamma Globulin (26 Lots).

Anti-hemolysin titer (u/ml)	No.
6	7
12	13
24	6
	26

Intravenous or intramuscular injection of gamma globulin in doses of 1 to 4 ml immediately following staphylococcal challenge consistently produced striking amelioration of the course of the burn infection (Fig. 1 and Table IV). "Protection" indicated that the burned area was indistinguishable from uninfected burned areas at 24 to 48 hours and "partial protection" referred to the presence of minimal erythema around the burned area. Doses of gamma globulin containing 12 to 24 units of anti-hemolysin activity produced protection in 60% of animals and partial protection in the remainder, while all 5 test animals receiving 48 units showed maximum protection. A delay of 4 hours between staphylococcal challenge and administration of gamma globulin resulted in partial protection in all animals.

Four additional animals received intradermal injections of 0.1 ml of staphylococcal alpha toxin. This dose consistently produced dermonecrosis in normal rabbit skin. Simultaneous intravenous administration of gamma globulin containing 12 units of anti-hemolysin activity in 2 animals significantly reduced the resulting area of dermonecrosis.

Discussion. Infection remains a serious problem in severely burned patients, and accounts for 40-60% of the mortality in patients with deep burns of greater than 30% of body surface(5,6). The use of antibiotics in such patients has been associated with an apparent decline in the importance of streptococcal infection and the emergence of the staphylococcus and *Pseudomonas* as organisms producing the most significant morbidity and mortality(7,8).

The factors which lead to increased susceptibility of burned skin to infection are incompletely understood. Undoubtedly interference with the integrity of the skin as a barrier to microorganisms is a factor. Alterations in cellular defense mechanisms may also play a role(9,10). While antibody synthesis does not appear to be impaired in most seriously burned patients(10), the increased

TABLE IV. Effect of Gamma Globulin on Burn-Infection in Rabbits.

Gamma globulin dose* (u)	Time of administration	Total animals	Protection†	Partial protection†	No protection†
12	Simultaneous with infection	6	4	2	
24	*Idem*	4	2	2	
48	"	5	5		
12–24	4 hr after infection	4		4	
0	——	11			11

* Expressed as units of staphylococcal anti-hemolysin (1 ml standard gamma globulin = 24 u; 1 ml intravenous gamma globulin = 6 u).

† "Protection" indicates burned area indistinguishable from uninfected burned areas at 24 to 48 hr. "Partial protection" refers to presence of minimal erythema around burn. "No protection" indicates hemorrhagic lesion tracking down side of animal.

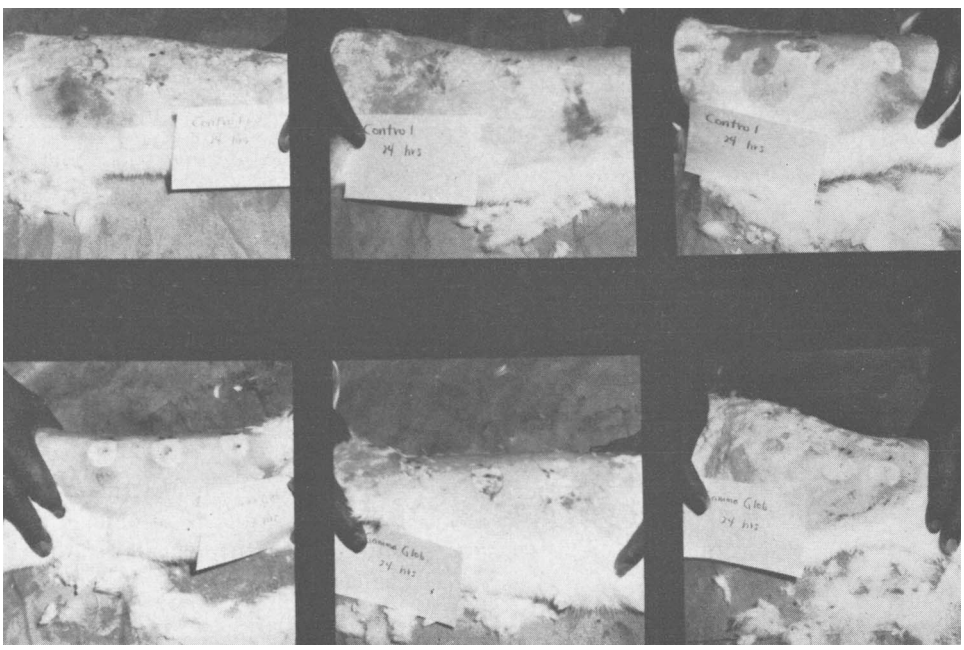

FIG. 1. Upper photographs: spreading hemorrhagic lesion resulting from staphylococcal injection into necrotic burn of rabbit skin. Lower photographs: amelioration produced by gamma globulin.

catabolism of gamma globulin and loss from the vascular compartment in the immediate post-burn period leads to depression of gamma globulin levels(5,7).

The importance of antibacterial antibodies in protection of the burned patient from infection undoubtedly varies with specific organisms. In the case of *Pseudomonas* infection immune serum with anti-pseudomonas agglutinins protected burned mice from septicemic death when challenged with *Pseudomonas*(11). Furthermore, the use of "hyperimmune" anti-pseudomonas human plasma has apparently produced a significant decrease in deaths due to *Pseudomonas* in burned patients(12).

With respect to staphylococcal infections, we have shown that staphylococcal anti-alpha hemolysin produced striking amelioration of the evolution of staphylococcal infection in the burned skin of rabbits(1). The significance of this antibody in human staphylococcal infections, however, remains uncertain. The present study indicates that staphylococcal anti-hemolysin titers are low or absent in a substantial part of the normal population. Furthermore, many patients with recurrent staphylococcal infections fail to develop significant increases in this antibody. Higher titers generally appear only in patients with deep-seated long standing infection such as osteomyelitis.

Anti-hemolysin titers in pooled human gamma globulin were found to show levels which might be anticipated from its approximate 10-fold concentration from normal plasma. The present study further indicates that human gamma globulin is effective in ameliorating the spreading hemorrhagic lesion produced by staphylococcal infection in the burned skin of rabbits. In addition to anti-hemolysin activity, pooled human gamma globulin also contains other staphylococcal antibodies including "hemagglutinating" antibodies. Previous experience with the rabbit-burn infection model indicates, however, that the specific protective effect observed in this instance is associated with anti-hemolysin. Furthermore, human gamma globulin was also found to exert a protective effect on the dermonecrotic action of alpha hemolysin injected into normal rabbit skin.

Gamma globulin has previously been administered to burned patients in an effort to prevent infection, with a resultant apparent decrease in septicemia due to staphylococcus and *Pseudomonas*(5,7).

Controlled studies to evaluate further the protective effect of gamma globulin in burned patients are being established. Intensive bacteriologic investigation as well as analysis of the specific immunological basis of this protection are planned. Of particular interest is a preparation of de-aggregated gamma globulin suitable for intravenous administration. This material has been shown to retain anti-hemolysin activity and is effective in ameliorating staphylococcal infection in the rabbit-burn infection model.

Summary. 1. Determination of anti-staphylococcal alpha hemolysin titers in 211 blood donors revealed that almost half had no detectable titers. Thirty-seven had titers of 2 units or greater but only 6 had titers greater than 4 units. Patients with recurrent furunculosis revealed a similar distribution in contrast to titers of 8 to 24 units in patients with osteomyelitis. 2. Anti-hemolysin titers on 26 lots of commercial pooled human gamma globulin ranged from 6 to 24 units per ml with a median titer of 12 units. An investigational de-aggregated gamma globulin preparation had 6 units per ml. "Hemagglutinating" anti-staphylococcal antibodies were also present in high titer. 3. Administration of gamma globulin produced striking amelioration of the standard rabbit-burn infection, and also protected against dermonecrosis produced by alpha hemolysin in normal skin. 4. The role of gamma globulin in the prevention of infection in burned patients is considered.

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Effect of Hypophysectomy on Serum Lipids in Aminonucleoside Nephrosis.* (31198)

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Pituitary hormones are known to have an important influence upon lipid metabolism (1) and the concentration of lipids in the serum(2). It has been suggested that the pituitary plays a role in mediating the lipemia of nephrosis(3). Hypophysectomy reduced the lipemia and proteinuria(4) in rats made nephrotic by injections of anti-kidney serum (4).

Anatomic and physiologic disturbances similar to those of the nephrotic syndrome in man have been produced in rats by injection of the aminonucleoside of puromycin, 6 dimethylamino purine, 3-amino-d-ribose(5). In addition, this compound produces changes in oxidative phosphorylation(6) and influences the hormonal control of fatty acid release from adipose tissue(7).

This study was performed to evaluate the effect of hypophysectomy upon serum lipids in rats made nephrotic by aminonucleoside, and to correlate the lipemia with other findings in this type of experimental nephrosis.

Materials and methods. Normal and hypophysectomized Charles River rats, weighing 130-150 g, were used. All were housed in metabolic cages, and were maintained on Purina Lab Chow and 5% glucose water. The animals were weighed daily. A period of 7 days was allowed for stabilization before the aminonucleoside injections were started. Adequacy of hypophysectomy was confirmed by failure of weight gain, lack of testicular growth, and the absence of pituitary tissue when the pituitary fossa was examined after the rat was killed. The experimental animals were given daily subcutaneous injections of the aminonucleoside,† 1.5 mg/100 g body wt as 0.5% solution in water. Control animals received daily injections of equivalent amounts of distilled water. The nephrotic rats developed signs of edema and ascites 10 to 12 days after the start of the aminonucleoside injections. At this time they were anesthetized with ether, and were bled to death from the aorta. Control rats were bled and killed in the same manner.

Serum cholesterol was measured by the Zak method(8) and serum triglycerides were determined by the method of Van Handel and Zilversmit(9). Blood urea nitrogen determinations were made by the diacetyl method (10). Total serum proteins(11) and urinary protein(12) were measured by the biuret method.

Results. Aminonucleoside produced nephrosis in both normal and hypophysectomized rats. The hypophysectomized rats did not gain weight until ascites and edema appeared 7 to 8 days after the start of the aminonucleoside injections. Normal rats, made nephrotic, gained weight throughout the experimental period but had accelerated weight gain with the onset of fluid retention. Typical weight curves are shown in Fig. 1.

The results of the various biochemical determinations are given in Table I. Significant elevation of both cholesterol and tri-

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