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Passive Immunity Against Pseudomonas Sepsis During Granulocytopenia

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Specific passive immunity against *Pseudomonas aeruginosa* sepsis was assessed in granulocytopenic dogs. Dogs were infused with either normal or anti-pseudomonas immune plasma 24 h before pseudomonas challenge. They were challenged intravenously with 10^7 serotype 6 *P. aeruginosa* during granulocytopenia. Treatment was evaluated by observation of survival periods, febrile responses, type 6 pseudomonas antibody titers, and quantitative cultures of blood and tissues. The results demonstrated that passively immunized dogs did not survive infection. Both normal-plasma and immune-plasma recipients had bacteremia at death, with median values of 980 and 470 pseudomonas per ml of blood, respectively. All dogs had marked febrile responses 24 h after pseudomonas challenge and had high concentrations of pseudomonas in their lung tissue at death, with median values of 10^8 pseudomonas per g of wet tissue weight. After plasma infusion, immune-plasma recipients had high concentrations of anti-pseudomonas antibody, with total antibody titers ranging from 256 to 1,024 and a median value of 1,024. These titers were comparable to titers attained in a previous study from our laboratory using active immunization with pseudomonas lipopolysaccharide vaccine, where the median total anti-pseudomonas antibody titer was 2,048. Actively immunized animals, however, were significantly protected against pseudomonas sepsis and had prolonged survival periods and prevention of bacteremia. The present study demonstrates that circulating type-specific antibody is not solely responsible for the protection afforded to granulocytopenic dogs actively immunized against pseudomonas.

The combined activity of serum opsonins and granulocytes is a primary defense in normal hosts against *Pseudomonas aeruginosa* infections (17). Removal of one or both of these factors in compromised hosts often results in pseudomonas infections which may terminate in septicemia and death. Our laboratory has previously reported an experimental model of *Pseudomonas aeruginosa* infection in myelosuppressed dogs (6). We have used the leukopenic dog model to evaluate the efficacy of (i) combined active preimmunization and granulocyte transfusions (12) and (ii) serotype-specific as well as non-type-specific active immunity alone (11) on the course of pseudomonas sepsis. Our previous investigations demonstrated that serotype-specific active preimmunization with pseudomonas lipopolysaccharide vaccine significantly prolonged survival and prevented bacteremia in leukopenic, pseudomonas-infected dogs. The protectiveness achieved with active immunization alone could not be attributed to granulocytes, since there were few granulocytes present and these cells had decreased phagocytic activity (1). The protective effect of active immunity was slightly augmented when granulocyte transfusions were also given. In addition, in vitro serum bactericidal assays demonstrated that specific anti-pseudomonas antibody and complement did not directly kill the organisms. These findings suggested that macrophages in actively immunized animals were providing significant defense against pseudomonas infections during leukopenia.

The present study used the same leukopenic dog model with pseudomonas infection, and was undertaken to evaluate the protection afforded by serotype-specific passive immunity against sepsis. Since serotype-specific active immunization with pseudomonas vaccine was significantly protective against pseudomonas sepsis in granulocytopenic animals (11), this study used comparable levels of passively administered anti-pseudomonas antibody in identically infected dogs in an attempt to clarify the protective role of type-specific anti-pseudomonas antibody.

MATERIALS AND METHODS

Dogs. Healthy mongrel dogs ranging in weight from 8 to 18 kg, immunized against distemper and

hepatitis and dewormed, were observed 2 weeks before use.

Plasma collection and infusion. Normal dogs having serotype 6 *P. aeruginosa* total antibody titers≤32 were used as donors of normal plasma. Other healthy dogs were repeatedly immunized with serotype 6 *P. aeruginosa* lipopolysaccharide vaccine (lot X41722, supplied by M. Fisher and H. Devlin of Parke-Davis Co., Detroit, Mich.) and used as immune-plasma donors. Dogs were initially immunized intramuscularly with a series of six vaccine injections (50 μg/kg of body weight) at 3- to 4-day intervals and then boosted weekly with a single injection of vaccine during the period of plasma collection. Donor animals were repeatedly plasmapheresed from femoral veins under sodium thiamylal anesthesia. Blood was collected in 500-ml Blood-Pack units (Fenwall Laboratories, Morton Grove, Ill.), containing 67.5 ml of anticoagulant citrate dextrose. Extracted plasma was stored in sterile blood bags at −60°C until infusion, and the erythrocytes were returned to the donor animal.

Preliminary plasma infusions in volumes of 60 ml per kg of body weight at a rate of 2 to 4 ml/min were tested in two normal, noninfected dogs. One of the animals was given cyclophosphamide and the other was not. Plasma infusions of this volume and rate were well tolerated by the dogs and did not produce adverse side effects.

Experimental model. All dogs were injected intravenously with a single dose of cyclophosphamide on day 0 to induce myelosuppression. Dogs were infused on day 3 with either normal or serotype 6 *P. aeruginosa* immune dog plasma in a volume of 60 ml of plasma per kg of body weight at a rate of 2 to 4 ml/min. On day 4, the animals were leukopenic (≤600 granulocytes/mm^3 of blood) and were challenged with an intravenous inoculum of 10^7 viable, serotype 6 *P. aeruginosa*. The bacterial suspension was prepared as described by Epstein et al. (6). The dogs were leukopenic on days 4 through 6 in this model.

Antibody titration. Serotype 6 *P. aeruginosa* antibodies were titered by the passive hemagglutination method of Neter et al. (16), modified by sensitizing sheep erythrocytes with type 6 lipopolysaccharide vaccine. Hemagglutination titers due to immunoglobulins G and M (IgG + IgM) were estimated from twofold dilutions of untreated samples. IgG antibody was titered after treating serum samples with equal volumes of 0.2 M 2-mercaptoethanol for 18 h at room temperature. All animals had type 6 *P. aeruginosa* IgG + IgM antibody titers of 16 or less and IgG titers of 2 or less before plasma infusion.

Leukocyte counts, temperatures, and quantitative cultures. Daily leukocyte counts and rectal temperatures were measured. Quantitative blood cultures were performed just before pseudomonas challenge, 5 min and 1 h after challenge, and daily thereafter. Animals were autopsied within 2 h of death, and blood from the right ventricle and sections of lung, liver, kidney, and spleen were obtained for quantitative culture. Quantitative cultures were performed by the agar overlay technique.

Statistical analyses. Survival period, temperature, and quantitative blood culture data were analyzed by using the Wilcoxon rank sum test (15). Analyses were performed by using a two-tailed test.

RESULTS

Survival periods and blood cultures. The survival periods of all dogs are shown in Table 1. All animals in this study died of pseudomonas infection within 72 h of challenge. Immune-plasma recipients had a median survival period 22 h longer than that of normal-plasma recipients.

Results of quantitative blood cultures obtained at autopsy are also shown in Table 1. Normal-plasma recipients had positive cultures ranging from 100 to 3,500 pseudomonas per ml of blood, with a median value of 980 pseudomonas. Four of five immune-plasma recipients also had positive blood cultures, ranging from 190 to 680 pseudomonas with a median value of 470 pseudomonas per ml of blood. The quantitative blood culture results of immune- and normal-plasma recipients were not significantly different.

Temperatures and leukocyte counts. Mean granulocyte counts and temperatures for both groups of dogs during the 5-day period after cyclophosphamide injection are plotted in Fig. 1. There were no significant differences between the granulocyte levels or temperatures during the 5-day period. All animals were leukopenic and had ≤600 granulocytes per mm^3 of blood on the day of challenge. Their granulocyte counts were even lower during infection (on day 5) and at death, with mean values of less than 100 granulocytes per mm^3 of blood. Before pseudomonas challenge all dogs had temperatures within the normal range (indicated by the shaded area); however, 24 h after challenge they demonstrated marked increases in temperatures, with means of 105.4°F (40.8°C)

TABLE 1. *Survival period and quantitative blood cultures at autopsy*

Dog no.	Normal-plasma recipients		Immune-plasma recipients	
	Survival[a]	Culture[b]	Survival[a]	Culture[b]
1	40	980	52	190
2	49	2,000	51	0
3	37	3,500	71	470
4	41	100	63	650
5	44	400	72	680
Medians	41	980	63	470

[a] Survival period in hours after pseudomonas challenge.

[b] Right-ventricular blood culture within 2 h of death; represents numbers of type 6 *Pseudomonas aeruginosa* per milliliter of blood.

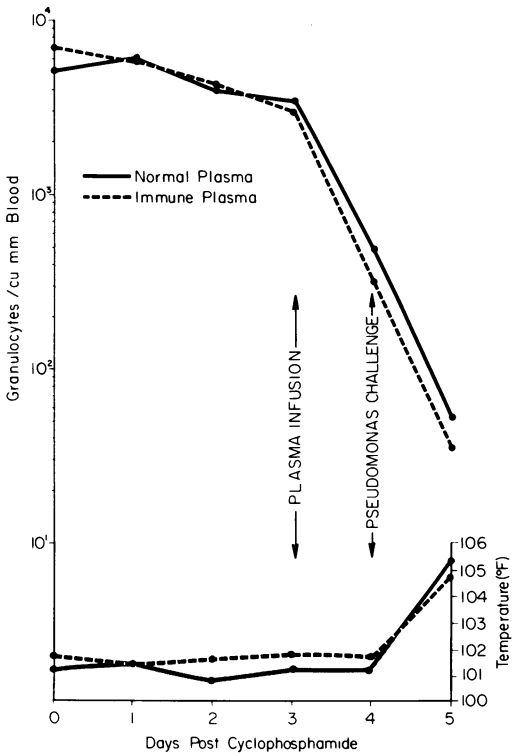

FIG. 1. *Average peripheral granulocyte counts and temperatures after cyclophosphamide administration. Shaded area represents the normal temperature range of dogs.*

for normal plasma recipients and 104.9°F (40.5°C) for immune-plasma recipients.

Antibody levels. Type 6 *P. aeruginosa* hemagglutinating antibody titers are plotted in Fig. 2 for all animals in this study. Before plasma infusion, dogs had IgG + IgM antibody titers of 16 or less (Fig. 2A) and IgG titers of 2 or less (Fig. 2B). Immune-plasma recipients attained IgG + IgM antibody titers of 256 to 1,024, with a median value of 1,024 (Fig. 2A), and IgG titers of 32 to 128, with a median value of 64 (Fig. 2B). The antibody levels remained the same throughout infection and were not depressed on the day of death. These titers attained by passive immunization were comparable to those of actively immunized animals whose total antibody titers ranged from 1,024 to 2,048 and whose IgG titers ranged from 16 to 256 (11).

Quantitation of pseudomonas from tissues. High concentrations of pseudomonas were cultured from lung, spleen, kidney, and liver tissues of dogs autopsied within 2 h of death. The numbers of pseudomonas in tissues of immune-plasma recipients were not significantly different from those in normal-plasma recipients. The lungs most often harbored the highest concentration of organisms. Table 2 contains the results of quantitative cultures from lung tissue homogenates. The median values of the two groups are nearly identical. This same relationship was found when quantitative cultures of spleen, kidney, and liver tissues from both groups were compared.

DISCUSSION

Other investigators have evaluated the protection afforded by passive immunity against pseudomonas in animals with normal granulocyte levels (2, 9, 13) and in patients with a variety of pseudomonas infections (7, 8, 10, 14). Feller and co-workers have demonstrated substantial protection against lethal pseudomonas infection in rabbits passively immunized with anti-pseudomonas immune plasma 24 h before challenge or within 2 h after challenge (9). Clinical trials using hyperimmune globulin or plasma as adjunctive therapy in patients with serious pseudomonas infections have also provided encouraging results (7, 8, 10, 14). The reports of passive immunization in humans are difficult to interpret, however, because of varied types of pseudomonas infection as well as treatment with antibiotics, leukocyte transfusion, and other supportive measures. The only controlled studies demonstrating significant protection with specific passive immunity have been in animals with normal numbers of phagocytic leukocytes (2, 9, 13).

Our present study demonstrates that specific passive immunity alone provides only minimal protection against pseudomonas sepsis in granulocytopenic hosts. In our earlier study (11), when identically infected granulocytopenic animals were actively immunized with type-specific lipopolysaccharide vaccine, they failed to develop bacteremia, had reduced tissue infection, and survived significantly longer than passively immunized dogs. Type-specific active immunity provided complete recovery in two of five dogs tested and a median survival period of 77.5 h in the dogs that died of infection. The survival period was significantly greater than in the group passively immunized dogs that had no survivors and had a median survival period of 63 h. Actively immunized dogs also had significantly lower concentrations of pseudomonas in their blood and tissues. They had negative blood cultures and a median of 2.5×10^6 pseudomonas per g of lung tissue compared with passively immunized animals, which had medians of 470 pseudomonas per ml of blood at death and 6.3×10^8 pseudomonas per g of lung tissue.

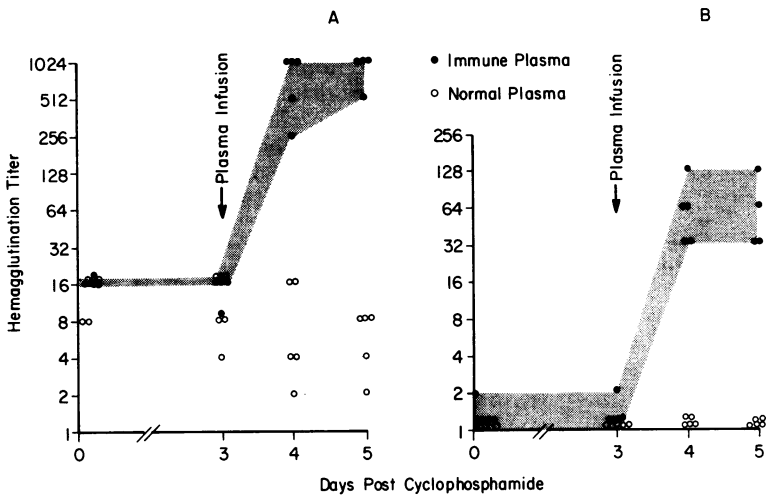

FIG. 2. *Hemagglutination titers of serum antibody against type 6 Pseudomonas aeruginosa after cyclophosphamide administration. Hemagglutination titer represents the reciprocal of the highest dilution giving hemagglutination.*

TABLE 2. *Quantitation of pseudomonas from the lung at autopsy*

Dog no.	Normal-plasma recipients	Immune-plasma recipients
1	1.3×10^{9}[a]	3.1×10^{10}[a]
2	4.0×10^{8}	1.0×10^{5}
3	1.3×10^{6}	6.3×10^{8}
4	4.4×10^{8}	1.1×10^{9}
5	1.0×10^{9}	1.4×10^{8}
Medians	4.4×10^{8}	6.3×10^{8}

[a] Numbers of serotype 6 *P. aeruginosa* per gram of wet tissue weight from animals autopsied within 2 h of death.

The dogs used in our investigations were markedly leukopenic on the day of bacterial challenge and had mean granulocyte counts of less than 100 cells per mm^3 of blood during infection. The small number of remaining granulocytes had decreased phagocytic ability (1) and were probably ineffective in killing pseudomonas. During leukopenia, fixed macrophages of the reticuloendothelial system served as the primary phagocytic cells. Investigations with mouse macrophages by Bjornson and Michael (4, 5) have shown that IgM anti-pseudomonas antibodies opsonize pseudomonas in the presence of complement for phagocytosis by macrophages. The results of our study indicate that opsonization by specific anti-pseudomonas antibody does not significantly protect granulocytopenic hosts. Since active immunization with type-specific vaccine is protective and passive immunization with comparable levels of specific antibody is only minimally protective, our studies indicate that another host defense mechanism besides circulating type-specific antibody functions against pseudomonas during leukopenia. Baughn and Bonventre have evidence for cell-mediated immune responses enhancing phagocytosis of *Staphylococcus aureus* (3). A similar mechanism may also function in actively immunized granulocytopenic hosts with pseudomonas infection, where cellular immunity could enhance macrophage phagocytic activity against the infecting pseudomonas strain. Unlike typical cellular immune mechanisms, however, the protective immunity that is operative in our actively immunized granulocytopenic dogs is specific.

The only protection afforded by passive immunization with type-specific plasma was an increase in the median survival period by 22 h. Since there were no significant differences in quantitative blood cultures or in quantitation of pseudomonas from tissues, it appears that prolonged survival was not attributable to bactericidal activity of immune plasma. Recent investigations using antibody against the core glycolipid of *Enterobacteriaceae* have suggested that nonspecific antibody may function in neutralizing pathophysiological effects of endotoxin (18). Ziegler and Braude (Clin. Res. 23:445A, 1975) reported significant protection against *P. aeruginosa* infections in granulocytopenic rabbits, using passively administered antibody to the core glycolipid of an *Escherichia coli* mutant. Both studies attributed major protection to anti-endotoxin activity of the antiserum. It is possible that plasma donor dogs receiving multiple injections of pseudomonas vaccine pro-

duced some nonspecific antibodies to core antigens of the pseudomonas. The production of anti-core antibodies with endotoxin-neutralizing activity could account for an increase in survival without significant differences in tissue infection as seen in the two groups of dogs in this study.

Optimal host defense against *P. aeruginosa* infections requires the presence of sufficient quantities of functional granulocytes and serum opsonins. We have previously demonstrated, however, that even in the absence of granulocytes, type-specific active immunization provides significant protection (11). The present study has clarified the protective role of anti-pseudomonas antibody and has demonstrated that type-specific anti-pseudomonas antibody is not solely responsible for enhanced host defense against pseudomonas infection in actively immunized granulocytopenic dogs. These studies suggest that macrophages of animals actively immunized with type-specific pseudomonas antigen play an important role in controlling pseudomonas infection during leukopenia.

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