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Effect of Type-Specific Active Immunization on the Development and Progression of Experimental *Pseudomonas aeruginosa* Endocarditis

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Rabbits with intracardiac catheters were immunized with heat-killed *Pseudomonas aeruginosa* or saline and challenged with either 10^9 (high inoculum) or 10^7 (low inoculum) pseudomonas. Immunization did not decrease the incidence of endocarditis when compared with controls, but it did significantly prolong survival. The longer survival of immunized rabbits after high-inoculum challenge was not due to prolongation of the course of endocarditis but to type-specific protection from early, overwhelming bacteremia. However, after low-inoculum challenge there were no early deaths and there was a significantly ($P < 0.01$) longer survival of immunized (17.4 days) than unimmunized (10.6 days) animals dying of endocarditis. Increased survival was associated with higher total and 2-mercaptoethanol-resistant hemagglutinating antibody titers 1 week after challenge in immunized as compared with unimmunized rabbits. Early (48 h after challenge) vegetation colonization was also significantly ($P < 0.05$) greater after type-specific as opposed to non-type-specific or saline immunization and low-inoculum challenge. However, whereas 67% of type-specifically immunized rabbits had colonized vegetations at 48 h, only 38.9% died with bacteremic endocarditis. Another 19.2% of immunized rabbits had vegetations colonized with $>10^5$ colony-forming units of pseudomonas at elective sacrifice 2 weeks after challenge but no bacteremia; no unimmunized rabbit exhibited similar late colonization. Preexisting antibody may be important in the pathogenesis of pseudomonas endocarditis in drug addicts, and its presence may explain the subacute and often protracted course of the disease.

Active or passive immunization of animals with pseudomonas vaccines has resulted in type-specific protection from lethal sepsis (6–8). However, the effect of preexisting antibody on the late consequences of pseudomonas bacteremia, such as endocarditis, is not clear. Antibody could have several influences on the development and progression of pseudomonas endocarditis. First, it may prevent endocarditis by inducing opsonizing antibodies that promote more rapid bacterial clearance (7, 14). This would be of benefit to patients undergoing insertion of prosthetic cardiac valves for prevention of early gram-negative prosthetic valve endocarditis. Second, preexisting antibody may modify endocarditis when it occurs, resulting in a subacute rather than an acute disease. A subacute presentation of pseudomonas endocarditis is often seen in intravenous-drug abusers (3). Finally, some investigators have suggested that preformed agglutinating antibody may actually be a predisposing factor in the pathogenesis of endocarditis, presumably because agglutinated masses of bacteria might be deposited more readily on damaged cardiac surfaces (13).

To study the influence of preexisting antibody on pseudomonas endocarditis, experimental *Pseudomonas aeruginosa* endocarditis was produced in rabbits after active immunization with whole-cell pseudomonas vaccines or saline. The effects of active immunization on survival, bacteremia, antibody titer, and vegetation colonization are summarized.

MATERIALS AND METHODS

Experimental endocarditis. Left-sided *P. aeruginosa* endocarditis was produced in male or female 3- to 5-lb (about 1.4- to 2.3-kg) New Zealand white rabbits by the introduction of a polyethylene catheter into the left ventricle and subsequent intravenous challenge with bacteria as prevously described (1). Endocarditis was felt to be present if animals exhibited continuously positive blood cultures, had progressive weight loss, and had $\geq 10^6$ colony-forming units (CFU) of the challenge organism per g of vegetation tissue cultured at autopsy (see below).

Immunization. Rabbits were immunized with

whole-cell vaccine made from either *P. aeruginosa* 231 or *P. aeruginosa* B-63; saline-immunized rabbits served as controls. *P. aeruginosa* 231, immunotype 3, has been previously characterized (2). *P. aeruginosa* B-63, immunotype 1 (kindly donated by Milagros Reyes), was recovered from the blood of a patient with endocarditis. Both organisms were serum resistant and differed in serotype (*P. aeruginosa* 231, Difco [Detroit, Mich.], serotypes 2, 5, and 16; *P. aeruginosa* B-63, serotypes 6 and 13) as well as immunotype.

Vaccine was prepared as follows. An 18- to 24-h culture of the organisms grown confluently on Mueller-Hinton agar (BBL, Cockeysville, Md.) was harvested from the surface of the agar by scraping with a glass rod. The organisms were suspended in physiological saline, washed three times, and resuspended in saline to the turbidity of a MacFarland standard no. 4 (approximately 1.2×10^9 CFU/ml). Phenol (Mallinckrodt, St. Louis, Mo.) was added to the bacterial suspension in a sterile vaccine bottle so that the final phenol concentration was 0.5%. The vial then was heated for 1 h at 56°C in a water bath. A 0.1-ml sample of the vaccine was subcultured to confirm loss of viability of the organisms.

Rabbits were assigned to receive either one of the pseudomonas vaccines or normal saline and were distributed so that there were no sex or weight differences among the immunization groups. On day 1, each of the three vaccine preparations was mixed with Freund complete adjuvant in a 1:1 ratio, and 0.5 ml was injected into both rear footpads of each rabbit. On day 3, each rabbit received 0.5 ml of vaccine intravenously into the marginal ear vein, and on day 6 and every 3rd day thereafter, 0.5 ml was given subcutaneously for a total of five doses. After the final immunization, catheters were inserted into the left ventricles of all rabbits and left in place for the duration of the experiment. Rabbits were challenged on the 3rd day after catheter placement. This was 6 days after the last immunization and 3 weeks after the first immunization. Groups of 20 rabbits, containing an equal number of pseudomonas- and saline-immunized animals, were studied at a time.

Challenge. All rabbits were challenged with *P. aeruginosa* 231 by rapid injection into the marginal ear vein. Rabbits were given either one of two challenge inocula. Some animals received 1 ml of a saline-washed overnight culture of organisms, adjusted to the turbidity of a MacFarland standard no. 4 (1.2×10^9 CFU/ml). Other rabbits were given 1 ml of a 10^{-3} dilution of an overnight culture that had been allowed to grow for 3 h at 37°C in Mueller-Hinton broth (BBL); this resulted in an inoculum of a mean of 0.9×10^7 CFU of log-phase organisms per ml (range, 0.7×10^7 to 1.4×10^7 CFU/ml).

Culture. Blood was obtained for culture from all animals in the study. Samples were drawn from the marginal ear vein in a 1-ml syringe containing 50 U of heparin and divided so that 0.2 ml was incorporated into each of two pour plates of Mueller-Hinton agar and 0.6 ml was injected into a tube containing 10 ml of Mueller-Hinton broth; blood was cultured within 5 min of being drawn into the syringe. Colony counts were determined after the pour plates had incubated for 48 h at 37°C (quantitative culture), and growth in broth was determined by subculture of broth to solid agar after 48 h of incubation (qualitative culture). Blood cultures were drawn at the following times after challenge: 1 h, 4 h, 8 h, 24 h, and daily until death or sacrifice.

Infected material adhering to the catheter, aortic valve, aortic wall, or ventricular endocardium was removed at autopsy, combined, washed gently in normal saline to remove blood, weighed wet, homogenized in normal saline with a mortar and pestle, and serially diluted in saline. A 0.1-ml sample of each dilution was plated on Mueller-Hinton agar and incubated for 24 to 48 h at 37°C. Colonies were counted, and the number of CFU per gram of vegetation tissue was determined. All *P. aeruginosa* colonies were identified by their characteristic appearance on agar and by the production of green pigment.

Sacrifice. In one set of experiments, animals were followed until they died, were moribund, or had negative blood cultures and no evidence of weight loss for 14 days. Animals dying were autopsied within 8 h of death, whereas moribund rabbits and those with negative blood cultures were sacrificed and autopsied immediately; infected tissue was removed and cultured as described above. In another set of experiments, animals were sacrificed and tissue cultured 48 h after challenge. Sacrifice was performed by the rapid intravenous injection of 150 mg of sodium pentobarbital in all cases.

Antibody determination. Antibody was determined by both passive hemagglutination in microtiter trays (Cooke Engineering Co., Alexandria, Va.) and bacterial agglutination. Antigen for sensitization of erythrocytes was obtained from the two immunizing organisms as follows. An overnight growth of *P. aeruginosa* was harvested from the surface of two Mueller-Hinton agar plates, washed three times, and suspended in 20 ml of physiological saline. The saline suspension was autoclaved for 1 h at 121°C and then sonically disrupted for 1 min, using a 3/8-inch (about 0.96-cm) titanium probe at 13 kc/s with an Artek Sonic 300 dismembrator (Artek Systems Corp., Farmingdale, N.Y.). The cells were confirmed as disrupted by phase-contrast microscopy. The sonically treated cells were centrifuged at $5,000 \times g$ for 1 h, and the clear supernatant, containing the antigen, was saved. Human type O erythrocytes were sensitized with a 1:10 dilution of the crude antigen in phosphate-buffered saline (PBS; pH 7.4) at 37°C for 30 min with tumbling. Sensitized erythrocytes were washed two times in PBS and resuspended to a concentration of 2% by volume. This erythrocyte suspension was added to unknown sera diluted serially in PBS and human serum albumin (5% by volume). After 2 h of incubation, macroscopic hemagglutination was observed in microtiter wells. The hemagglutinating antibody titer was recorded as the reciprocal of the highest dilution giving agglutination. Controls run concomitantly were sensitized erythrocytes with diluent only, serum with a suspension of washed but unsensitized erythrocytes, and serum plus erythrocytes sensitized with 100 μg of *P. aeruginosa* lipopolysaccharide per ml of the same immunotype as that of the crude pseudomonas antigen (kindly donated by C. Heifetz, Parke-Davis Co., Detroit, Mich.). Erythrocytes sensitized with the crude antigen generally gave an antibody titer within one

dilution of erythrocytes sensitized with known pseudomonas lipopolysaccharide of the same immunotype.

The relative amounts of immunoglobulin G (IgG) and IgM in antibody-containing sera were estimated by the addition of 0.2 M 2-mercaptoethanol (2-ME) to sera, followed by overnight incubation. Sera with and without 2-ME were serially diluted in plastic tubes 15 by 150 mm (Falcon Plastics, Oxnard, Calif.), sensitized erythrocytes were added, and hemagglutination was determined. The titer remaining after 2-ME treatment was called IgG (2-ME resistant), whereas the difference in titers before and after 2-ME treatment was called IgM (2-ME sensitive). Hemagglutinating titers before 2-ME treatment performed in microtiter or in plastic tubes agreed within one dilution.

Passive bacterial agglutination was performed on glass slides with a thick suspension of organisms; macroscopic agglutination of bacteria in the presence of twofold dilutions of sera was recorded and expressed as the reciprocal of the highest dilution showing agglutination.

Statistics. Differences in days of survival and numbers of bacteria in the blood among groups of animals were examined for significance by the Wilcoxon rank sum test. Numbers of rabbits in each group with vegetations colonized or not colonized were compared by the chi-square test. P values of ≤ 0.05 were considered significant.

RESULTS

Antibody titers. Antibody titers after immunization and challenge are summarized in Table 1. High titers of antibody were found before bacterial challenge in all immunized rabbits, whereas normal rabbits and saline-immunized rabbits had no or very low antibody titers. Hereafter, animals immunized with *P. aeruginosa* 231 will be designated 231 Im, animals immunized with *P. aeruginosa* B-63 will be designated B-63 Im, and those given saline will be designated NIm. There were no cross-reactions between sera from 231 Im and B-63 Im rabbits (Table 2). Hemagglutinating antibody titers performed on sera from 231 Im and B-63 Im rabbits showed the 231 isolate to be a better immunogen, resulting in fourfold-higher titers than were produced by the B-63 isolate. Titers of hemagglutinating and bacterial agglutinating antibody paralleled one another, but hemagglutinating titers were generally four- to eightfold higher.

A large proportion of the antibody in immunized rabbits was IgG (2-ME resistant). After challenge with either 10^7 or 10^9 bacteria, total antibody and IgG titers in the immunized animals did not change over the course of 3 weeks of infection. In contrast, whereas NIm rabbits challenged with 10^9 bacteria developed total antibody titers 1 week after challenge similar to titers in 231 Im rabbits, NIm rabbits receiving 10^7 bacteria had titers at 1 week fourfold lower than those of either 231 Im animals or NIm animals given the high inoculum. Furthermore, the antibody in NIm animals 1 week after challenge was virtually all IgM (2-ME sensitive). IgG did not appear until 2 to 3 weeks after challenge in NIm rabbits developing endocarditis.

Titers of total antibody, IgG, or IgM were not helpful in distinguishing rabbits with endocarditis from those without in immunized animals. However, three NIm rabbits that did not develop endocarditis after challenge with a low inoculum (10^7 CFU) had four- to eightfold-lower total antibody and IgG titers in comparison with NIm animals that developed endocarditis 2 weeks after challenge.

Survival. The effects of antibody on survival are shown in Table 3. All animals challenged with 10^9 pseudomonas died with constant bacteremia; none was protected from bacteremic death by type-specific immunization. However, the course of infection was significantly altered

TABLE 1. *Antibody titer in rabbits with endocarditis after active immunization with P. aeruginosa or saline and homologous challenge*

Immune status	Challenge inoculum (CFU)	Reciprocal hemagglutinating antibody titer in relation to bacterial challenge					
		Prechallenge		1 wk		≥2 wk	
		Total	After 2-ME	Total	After 2-ME	Total	After 2-ME
Immune	$10^{9.0 \pm 0.1}$[a]	1,280[b] (1,280–5,120)[d]	320 (22)[c] (40–640)	2,560 (1,280–5,120)	320 (12) (80–320)	2,560 (1,280–5,120)	320 (7) (80–320)
Nonimmune	$10^{9.0 \pm 0.1}$	0 (0–8)	0 (22) (0)	2,560 (640–5,120)	<20 (9) (<20–40)	1,920 (640–5,120)	80 (4) (40–320)
Immune	$10^{7.0 \pm 0.1}$	1,280 (640–5,120)	320 (15) (160–640)	2,560 (640–2,560)	320 (8) (160–640)	1,280 (640–1,280)	320 (3) (40–320)
Nonimmune	$10^{7.0 \pm 0.1}$	2 (0–8)	0 (15) (0)	640 (160–1,280)	<20 (6) (<20)	2,560 (2,560)	80 (3) (80–160)

[a] Mean ± standard deviation.
[b] Median.
[c] Number of rabbits tested in each group.
[d] Range.

by the presence of type-specific antibody. There was a significantly longer survival in all animals given type-specific vaccine over those immunized with saline or the heterologous strain ($P < 0.05$ for each goup). Early (within 3 days after challenge) death due to overwhelming bacteremia accounted for the shorter survival of NIm animals. All rabbits surviving the first 3 days lived at least 8 days and died with a course characteristic of endocarditis (1). There was no difference in survival among animals dying late of endocarditis after challenge with 10^9 pseudomonas.

Challenge with the lower inoculum (10^7 CFU) produced a lower incidence of death from endocarditis, with no difference in mortality between immunized and nonimmunized animals. However, there were no early deaths as seen after challenge with the high inoculum, and there was a significantly longer survival ($P < 0.01$) in immunized versus nonimmunized rabbits with endocarditis.

Bacteremia. Early bacterial clearance did not distinguish between animals that would develop endocarditis and those that would not, but there were significant differences in clearance among groups.

Decreased bacterial clearance was associated with early death in rabbits challenged with 10^9 bacteria (Table 4). In those NIm rabbits dying during the first 3 days after challenge, there was a significant decrease in early bacterial clearance at each time period sampled (1, 4, 8, and 24 h after challenge) as compared with either 231 Im rabbits or NIm rabbits not dying early ($P < 0.01$ for each time period). However, there was no significant difference ($P > 0.05$) in bacterial clearance between 231 Im rabbits and NIm rabbits not dying early. Antibody that agglutinates bacteria can cause a falsely low plate count, but since the same association between clearance and early death was found within the NIm group as between the NIm and 231 Im group, the comparison should be valid.

With few exceptions, any animal challenged with 10^7 bacteria had negative blood cultures at 1, 4, and 8 h after challenge. However, NIm rabbits that died with endocarditis became bacteremic significantly sooner (mean, 1.6 days; range, 1 to 4 days) than 231 Im rabbits with endocarditis (mean, 3.1 days; range, 2 to 8 days; $P < 0.05$); 64% of NIm rabbits (9 of 14) were bacteremic by 24 h after challenge, whereas only 7% (1 of 14) of 231 Im rabbits had positive blood cultures at this time.

Any animal with a positive blood culture 72 h after challenge with any inoculum became constantly bacteremic, with the number of bacteria in blood varying from <10 to several hundred CFU per ml from day to day. Bacteremia of $\geq 10^3$ CFU/ml usually heralded impending death.

TABLE 2. *Homologous and heterologous antibody titers in rabbits immunized with P. aeruginosa isolates of different immunotype*

Immunizing antigen	Reciprocal hemagglutinating antibody titer	
	Against *P. aeruginosa* 231	Against *P. aeruginosa* B-63
P. aeruginosa 231	640[a] (15)[b] (320–1,280)[c]	0 (15) (0)
P. aeruginosa B-63	0 (12) (0)	160 (12) (80–320)

[a] Median.
[b] Number of rabbits immunized.
[c] Range.

TABLE 3. *Influence of antibody on mortality in rabbits with intracardiac catheters challenged with P. aeruginosa 231*

Immune status	Challenge inoculum (CFU)	Cumulative mortality, animals dying/no. challenged (%)						Mean day of death (range)	Mean day of death in animals surviving first 3 days (range)
		3[a]	7	10	14	20	>20		
P. aeruginosa 231 immunized	10^9	0/22 (0)	0/22 (0)	4/22 (18.1)	13/22 (59.0)	18/22 (81.8)	22/22 (100)	14.8 (8–31)	14.8 (8–31)
P. aeruginosa B-63 immunized	10^9	5/12 (41.7)	5/12 (41.7)	7/12 (58.3)	10/12 (83.3)	11/12 (91.7)	12/12 (100)	8.8 (1–24)[b]	16.1 (8–24)
Saline immunized	10^9	8/21 (38.1)	8/21 (38.1)	10/21 (47.6)	15/21 (71.4)	19/21 (90.5)	21/21 (100)	10.4 (0.3–21)[b]	13.5 (9–21)
P. aeruginosa 231 immunized	10^7	0/36 (0)	0/36 (0)	0/36 (0)	7/36 (19.4)	9/36 (25.0)	14/36 (38.9)	17.4 (11–32)	17.4 (11–32)
Saline immunized	10^7	0/36 (0)	6/36 (16.2)	10/36 (27.0)	13/36 (35.1)	13/36 (35.1)	14/36 (38.9)	10.6 (4–24)[b]	10.6 (4–24)[b]

[a] Days after challenge.
[b] Survival significantly shorter ($P < 0.05$; Wilcoxon rank sum test) than for any group of animals receiving type-specific (*P. aeruginosa* 231) immunization.

Vegetation colonization. Vegetation colonization in the various groups is summarized in Table 5. All rabbits with endocarditis had similar numbers of organisms in vegetations ($10^{8.8 \pm 0.89}$ CFU/g; mean ± standard deviation) regardless of antibody status, challenge inoculum, or whether they had been sacrificed moribund or autopsied after spontaneous death. All rabbits with negative blood cultures for 14 days had sterile vegetations except seven rabbits in the 231 Im group challenged with 10^7 CFU. These rabbits with negative blood cultures 2 weeks after challenge had $>10^5$ CFU of *P. aeruginosa* 231 per g (mean, $10^{6.5}$ CFU/g; range, $10^{5.2}$ to $10^{8.8}$ CFU/g) cultured from cardiac vegetations.

To investigate the possibility that type-specific antibody increased early vegetation colonization, 231 Im, B-63 Im, and NIm rabbits were sacrificed 48 h after challenge with 10^7 *P. aeruginosa* 231. Vegetations were considered colonized if $\geq 10^5$ CFU/g was cultured from vegetations. Vegetation counts were either 0 or $>10^6$ CFU/g in all but 3 (2 231 Im, 1 NIm) out of 70 rabbits cultured. These three rabbits had from 10^3 to 10^4 CFU/g in small vegetations and were considered contaminated and not colonized. In those animals colonized, there was no difference among the groups in the number of organisms in vegetations. The difference in colonization between the 231 Im and both the NIm group and the B-63 group was significant (Table 5; 231 Im versus NIm, chi square = 4.697, $P < 0.05$; 231 Im versus B-63, chi square = 6.109, $P < 0.02$).

DISCUSSION

This study has shown that active immunization has significant effect on the development and progression of experimental *P. aeruginosa* endocarditis.

First, the presence of preexisting type-specific

TABLE 4. *Number of P. aeruginosa in the blood of immunized and nonimmunized rabbits in the first 24 h after challenge with 10^9 P. aeruginosa 231*

Immune status	Bacteria in blood (CFU/ml) at each time period (h)			
	1	4	8	24
Saline immunized, early death[a]	673[b] (8)[c] (80–2,000)[d]	245 (8) (75–618)	764 (5) (310–1,000)	3834 (4) (230–10,000)
Saline immunized, late death	202 (11) (28–675)	138 (11) (4–1,200)	27 (11) (0–208)	34 (11) (0–105)
P. aeruginosa 231 immunized	148 (22) (18–673)	16 (22) (0–73)	5 (19) (0–35)	92 (21) (0–1,325)

[a] Significantly greater number of bacteria ($P < 0.01$; Wilcoxon rank sum test) at each time period as compared with other two categories.
[b] Mean.
[c] Number of rabbits sampled at each time period.
[d] Range.

TABLE 5. *Relationship of vegetation colonization with P. aeruginosa 231 to the presence of preexisting antibody*

Immune status	Challenge inoculum (CFU)	No. of animals colonized[a] / no. of animals challenged (%)	
Saline immunized (late death or sacrifice)[b]	10^7	14/36 (38.9)	
			$0.1 > P > 0.05$
P. aeruginosa 231 immunized (late death or sacrifice)	10^7	21/36 (58.3)	
Saline immunized (early sacrifice)[c]	10^7	15/40 (37.5)	
			$P < 0.05$
P. aeruginosa 231 immunized (early sacrifice)	10^7	14/21 (66.7)	
			$P < 0.02$
P. aeruginosa B-63 immunized (early sacrifice)	10^7	6/21 (28.6)	

[a] Colonization = $>10^5$ CFU/g of vegetation.
[b] Sacrifice after 14 days of negative blood cultues.
[c] Sacrifice 48 h after challenge.

antibody did not reduce the mortality from endocarditis, but it did protect against early death and prolong the mean survival. Pseudomonas vaccines have been shown to afford type-specific protection of rabbits and granulocytopenic dogs from death occurring within the first 72 h after a massive bacterial challenge (6, 7). The protection may be due to both increased bacterial clearance, mediated by opsonizing antibody in the case of serum-resistant bacteria, and an abrogation of the pathophysiological consequences of endotoxemia. Hemagglutinating antibody, directed against bacterial lipopolysaccharide, correlates well with both opsonizing and protective antibody, and therefore hemagglutinating antibody titers can be used to predict these activities (4, 14). Our data confirmed the protection from early bacteremic death associated with high titers of hemagglutinating antibody. Early death occurred in 38 and 42% of animals given saline and heterologous immunization, respectively, followed by challenge with 10^9 pseudomonas; immunization with the challenge organism afforded 100% protection. Bacterial clearance was significantly less complete in animals dying early than in those not dying early.

The shorter survival of unimmunized than immunized animals after challenge with 10^9 pseudomonas was due solely to early death in the unimmunized group; animals surviving the first 3 days had similar mean survival times regardless of the presence or absence of prechallenge antibody. Furthermore, the early death probably had little to do with endocarditis, since a similar early mortality has been reported in normal, unimmunized, uncatheterized rabbits challenged with 10^9 pseudomonas (6). However, after challenge with 10^7 bacteria, unimmunized rabbits died significantly sooner than immunized animals, and all of the deaths were due to endocarditis. There was no early death at this inoculum, and early bacterial clearance was rapid and similar in immune and nonimmune rabbits. Sixty-three percent of the unimmunized rabbits that died did so between the 4th and 10th days after challenge; no immunized rabbit that died did so before the 11th day after challenge. All dying rabbits had constant bacteremia, embolic lesions in kidneys, progressive weight loss, and high vegetation counts characteristic of animals with endocarditis. An explanation for the difference in survival may be the difference in antibody titers present 1 week after challenge. Although antibody was present in all unimmunized animals 1 week after challenge, titers were fourfold higher after challenge with the high (10^9 CFU) as compared with the low (10^7 CFU) inoculum. Furthermore, all of the antibody in unimmunized animals 1 week after challenge was IgM (2-ME sensitive), whereas immunized animals had high titers of IgG (2-ME resistant) antibody. Crowder et al. (5) have shown a significantly greater mouse protective effect against type-specific challenge of sera containing 2-ME-resistant as compared with sera containing 2-ME-sensitive antipseudomonas hemagglutinating antibody. Thus, the combination of comparatively low antibody titers and 2-ME-sensitive antibody may have afforded rabbits insufficient opsonophagocytic activity to prevent lethal bacteremia from endocarditis in the first 10 days after low-inoculum challenge.

High titers of type-specific 2-ME-resistant hemagglutinating antibody are also seen in patients with pseudomonas endocarditis. Reyes et al. (11) found these antibodies present in a reciprocal titer of 256 or greater in the sera of 13 of 14 patients with pseudomonas endocarditis at the time of initial presentation. These titers were achieved in our rabbits only after 3 weeks of immunization or 2 to 3 weeks of bacteremic endocarditis. They are in contrast to the absence of type-specific opsonic, hemagglutinating, and precipitating antipseudomonas antibody in acute-phase sera of hospitalized patients with pseudomonas bacteremia (14). Pseudomonas endocarditis in humans may thus be modified by antibody in a fashion similar to that of the experimental disease. Patients frequently present with a subacute disease and a history of weeks to months of symptoms before admission (3); high IgG titers at presentation confirm an ongoing period of hyperimmunization.

Second, the presence of type-specific antibody was associated with increased vegetation colonization in this study. Rabbits with homologous antibody had significantly increased rates of early (48 h) vegetation colonization after challenge with 10^7 pseudomonas in comparison with rabbits having no or heterologous antibody. However, there was not always an association between vegetation colonization and the eventual evelopment of bacteremic endocarditis. Although the early colonization rate of pseudomonas-immunized rabbits after homologous challenge with 10^7 organisms was 67%, only 38.9% of similarly immunized and challenged rabbits developed bacteremic endocarditis within 2 weeks of challenge. An additional seven rabbits that had negative blood cultures for 2 weeks, however, were found to have large numbers of organisms in vegetations at elective sacrifice; no unimmunized rabbit with negative blood cultures had bacteria recovered from vegetations. These seven rabbits may have developed bacteremic endocarditis after a longer period of observation. As evidence for the delayed appearance of endocarditis in immunized rab-

bits, 3 of 14 dying of endocarditis did not have the first blood culture positive until 6 to 8 days after challenge, whereas comparable unimmunized rabbits developing endocarditis all had positive blood cultures by 4 days after challenge and 64% were positive 1 day after challenge. Opsonizing antibody, by promoting more rapid clearance of blood-borne bacteria, may delay the clinical expression of pseudomonas endocarditis after challenge. Some vegetations, initially colonized, may also endothelialize and partially heal in the absence of reseeding through continuous bacteremia. Partial healing of vegetations in rabbits in the absence of bacteremia has been observed after challenge with *Streptococcus viridans* by McGeown (9) and by us after çhallenge with *P. aeruginosa* and treatment with gentamicin (2; G. Archer, unpublished observations).

Agglutinating antibody has been suggested by Weinstein and Schlesinger (13) as an important factor in the pathogenesis of endocarditis on the basis of early studies with pneumococci in horses and rabbits (10, 12). The proposed mechanism for increased colonization is a clumping of organisms which produces large bacterial masses more likely to be deposited by eddy currents on a platelet-fibrin thrombus and which results in a larger local inoculum. Other possible mechanisms may be increased "stickiness" of bacteria coated with antibody or immune adherence due to complement on the Fc portion of IgG attaching to complement receptors on leukocytes, platelets, or fibrin in the sterile vegetation. Type-specific bacterial agglutinating antibody was found in all rabbits with hemagglutinating antibody in our study and was absent in saline-immunized controls.

If preformed antibody promotes the colonization of valves with *P. aeruginosa*, this may be one explanation for the surprisingly high incidence of pseudomonas endocarditis in addicts. These individuals may immunize themselves continually by injecting pseudomonas from contaminated intravenous drug paraphernalia. Another implication would be that the efficacy of pseudomonas vaccines in protecting patients from septic death would have to be weighed against the potential predisposition of individuals with abnormal valves to endocarditis.

This study has shown that preexisting type-specific antibody protected rabbits from early-septic death, increased the interval between challenge and the onset of bacteremic endocarditis, and prolonged the survival of animals that developed endocarditis in comparison with animals having no antibody. However, antibody did not protect animals from developing endocarditis and was associated with increased early vegetation colonization. Further studies to define the prevalence of antibody in groups at high risk for developing pseudomonas endocarditis, such as addicts, may help to define the role of antibody in the pathogenesis and modification of the disease. The differences in colonization between animals with and without antibody in this study were small and need confirmation by others. The use of larger numbers of animals, in vitro as well as in vivo models, passive immunization studies, and manipulation of models to increase the differences between groups may help to define the role of antibody in the pathogenesis of pseudomonas endocarditis.

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LITERATURE CITED

1. **Archer, G., and F. R. Fekety.** 1976. Experimental endocarditis due to *Pseudomonas aeruginosa*. I. Description of a model. J. Infect. Dis. **134**:1-7.
2. **Archer, G., and F. R. Fekety, Jr.** 1977. Experimental endocarditis due to *Pseudomonas aeruginosa*. II. Therapy with carbenicillin and gentamicin. J. Infect. Dis. **139**:327-335.
3. **Archer, G., F. R. Fekety, and R. Supena.** 1974. *Pseudomonas aeruginosa* endocarditis in drug addicts. Am. Heart J. **88**:570-578.
4. **Crowder, J. G., H. B. Devlin, M. Fisher, and A. White.** 1974. Heat-stable opsonins in sera of patients with pseudomonas infections. J. Lab. Clin. Med. **83**:853-859.
5. **Crowder, J. G., M. W. Fisher, and A. White.** 1972. Type-specific immunity in pseudomonas diseases. J. Lab. Clin. Med. **79**:47-54.
6. **Feller, I., A. B. Vial, W. Callahan, and J. Waldyke.** 1964. Use of vaccine and hyperimmune serum for protection against pseudomonas septicemia. J. Trauma **4**: 451-456.
7. **Harvath, L., and B. R. Anderson.** 1975. Evaluation of type-specific and non-type-specific pseudomonas vaccine for treatment of pseudomonas sepsis during granulocytopenia. Infect. Immun. **13**:1139-1143.
8. **Harvath, L., B. R. Anderson, and H. L. Amirault.** 1976. Passive immunity against pseudomonas sepsis during granulocytopenia. Infect. Immun. **14**:1151-1155.
9. **McGeown, M. G.** 1954. Bacterial endocarditis: an experimental study of healing. J. Pathol. Bacteriol. **67**:179-186.
10. **Mair, W.** 1923. Pneumococcal endocarditis in rabbits. J. Pathol. Bacteriol. **26**:426-428.
11. **Reyes, M. P., M. W. Fisher, and A. M. Lerner.** 1974. Pseudomonas endocarditis: hemagglutinating antibodies to Fisher-Devlin-Gnabasik immunotypes in sera. J. Lab. Clin. Med. **83**:845-852.
12. **Wadsworth, A. B.** 1919. A study of the endocardial lesions developing during pneumococcus infection in horses. J. Med. Res. **39**:279-292.
13. **Weinstein, L., and J. J. Schlesinger.** 1974. Pathoanatomic, pathophysiologic and clinical correlations in endocarditis. N. Engl. J. Med. **291**:832-837.