Immune Response to Chronic Osteomyelitis in the Rabbit (34891)

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Recent studies of the immune response to chronic active bacterial infection have revealed some interesting and unexplained differences between at least two such types of infection in humans, namely, subacute bacterial endocarditis and osteomyelitis (1, 2). A previous report (3) documented our attempts to simulate in rabbits an accurate model of these two infections which would permit convenient study of the immune response. These studies revealed that chronic intermittent staphylococcal bacteremia in rabbits induced an immune response similar to that seen in human subacute bacterial endocarditis including induction of extremely high levels of heat-stable opsonic serum factors. In experimental rabbit osteomyelitis opsonic factors were also found in high titer in contrast to human staphylococcal osteomyelitis in which heat-stable serum opsonic titers had been previously shown to be low or in the normal range (2). This difference provoked continuing studies of the rabbit osteomyelitis model. Longer periods of observation of rabbits with osteomyelitis (5 months) showed that chronic staphylococcal osteomyelitis in the rabbit closely parallels the situation which obtains in human osteomyelitis (2).

Protein A, a staphylococcal cell-wall component which has non-immunologic reactivity with the Fc portion of human and rabbit γG globulin (4, 5), was quantitatively measured in staphylococci isolated from chronically infected bones. The amount of protein A was assessed in bacteria in the original infecting culture, and bacteria freshly isolated from an osteomyelitic lesion.

Materials and Methods. The experimental model for osteomyelitis, the methods of production and confirmation of infection, the collection of serum samples, and culturing and preparation of bacteria were identical to those previously described (3). The strain of staphylococci used in chronic osteomyelitis studies was designated (Durkin) (3). It was phage type 86 at RTD and 42E/47/53/54/75/77/81 at 1000 $\times$ RTD. (Courtesy of the National Communicable Disease Center, Atlanta, Georgia.) In certain experiments the laboratory strain of *S. aureus* Wood 46 was used. This strain completely lacked protein A *in vitro* and *in vivo* (6).

Opsonic antibodies were determined by the quantitative *in vitro* phagocytosis test of Maaloe as modified by Hirsch and Strauss and by Laxdal *et al.* (7, 8). In brief, leukocytes were collected from peritoneal exudates of sensitized rabbits 4 hr after an injection of glycogen. These were tumbled with washed bacteria of the infecting strain and serum dilutions which had been inactivated for 30 min, at 56°, to remove heat-labile opsonic factors. The number of surviving bacteria in this mixture was determined by sampling after 0, 30, 60, or 120 min of incubation. The highest dilution giving 50% killing at 2 hr was reported as the opsonic titer.

Bacterial agglutination tests on whole serum were performed according to the method described by Laxdal *et al.* (8). In testing for inhibition of alpha-hemolysin the protocol of Quie and Wannamaker was followed (9).

Tests for complement-fixation activity employed the method of Casals and Palacios (10). Quantitative tests for protein A were

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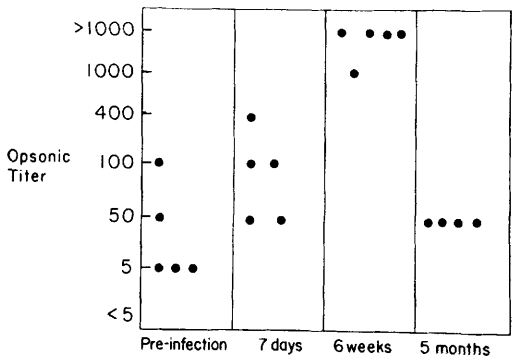

FIG. 1. Changes in heat-stable opsonic factors in rabbit osteomyelitis. Titer is expressed as reciprocal of the highest dilution of serum which permit PMN's to kill 50% or more of test organisms. Heat-stable opsonins increased markedly in the first weeks in response to experimental staphylococcal osteomyelitis. By the fifth month of infection levels of opsonic factors had returned to near normal levels.

performed using a recently developed radioactive method (11), which employed the combination of labeled γG myeloma protein with staphylococci.

Results. The opsonic titer of rabbits with osteomyelitis experimentally infected with *S. aureus* Durkin had previously been shown to increase markedly during the first 2 months of infection (3). After an additional 3 months, the measured levels of serum heat-stable opsonic capacity in these rabbits had fallen to levels no higher than preimmunization values (Fig. 1). This occurred although active infection persisted, as proved by X-ray and recovery of the infecting organism, *S. aureus* (Durkin) at necropsy, from chronically infected osteomyelitic lesions.

Determinations of agglutinating antibodies were simultaneously performed in serum samples from 0, 42 days, and 5 months after infection to insure internal consistency. These efforts were plagued by difficulties with false agglutination and lack of reproducibility, similar to those reported by Haukenes (12) and Lind (13). Because Haukenes reported these difficulties in agglutination tests were ameliorated by culturing the staphylococci used as antigen on mannitol salt agar, this procedure was used (Table I). Titers of agglutinating antibodies rose and remained elevated. Inhibition of staphylococcal alpha-hemolysin was increased by 6 weeks after onset of infection and was generally maintained at increased levels to the termination of the experiment.

Animals were sacrificed and their bones examined. All animals had lesions that appeared grossly and microscopically typical of chronic osteomyelitis. The cultures from the bone lesions as well as the original infecting strain were phage-typed at the National Communicable Disease Center. The recovered bacteria were identical to the infecting strain (phage type 86 at RTD and 42E/47/53/54/75/77/81 at 1000 × RTD.

The amount of protein A present in staphylococcal cultures was quantitated by capacity of a standard bacterial suspension to combine with radioactive myeloma globulin (11). When compared with a strain which produces large amounts of protein A (Cowan I), the infecting strain (Durkin) showed only 5% as much protein A. When freshly isolated from an osteomyelitis lesion however, the Durkin strain showed 50% of the radioactive uptake of Cowan I, a value 10 times higher

TABLE I. Antibody Titers[a] in Rabbit Staphylococcal Osteomyelitis.

	Agglutination			α-Hemolysin inhibition		
Rabbit	Preinfection	6 weeks	5 months	Preinfection	6 weeks	5 months
90	32	1024	2048	2	16	8
91	0	64	2048	4	8	8
93	0	128	1024	4	32	32
94	0	512	512	2	8	8
95	0	4096	4096	2	16	32

[a] Titers are reciprocals of the highest serum dilution giving agglutination or inhibition of α-hemolysin.

than that of the original subcultured infecting strain. It appeared that *S. aureus* Durkin had increased its content of cell-wall protein A 10-fold during the 5 months of infection.

Three rabbits with acute osteomyelitis produced by staphylococcal strain Wood 46 which lacked protein A showed no rise in opsonic, agglutinating, or complement-fixing antibodies. A rapid rise in these antibodies occurred in rabbits with osteomyelitis with *S. aureus* (Durkin) (3).

Discussion. The observed difference in the acute response to osteomyelitis in rabbits (3) and the chronic response in the human (2) previously reported could possibly be explained by the patient sample in the clinical survey. Of the 13 patients with staphylococcal osteomyelitis reported (2) only 2 had had the disease for less than 3 months. It was during this period that our experimentally infected rabbits showed unusually high titers of opsonic antibody. If a sequential study were undertaken in human osteomyelitic patients it seems possible that a similar early transient rise and subsequent fall in serum opsonins might be found.

The absence of elevated opsonic titers in rabbit osteomyelitis produced with the Wood 46 strain, lacking staphylococcal protein A, suggests some involvment of protein A in the animal's acute response to staphylococcal infection. An acute response may be dependent upon such factors as protein A which vary from strain to strain.

The explanation for normal or low titers of opsonins during chronic osteomyelitis remains elusive. In our chronic rabbit osteomyelitis model, it is certain that the infection was not eradicated, since the infecting organism could be recovered at the termination of the experiment. It seems likely that in the chronic state the infection becomes so completely localized that there is virtual isolation of the infecting agent antigens.

The presentation of antigens and early formation of antibody may also play a role in host opsonic response. It may be that central processing by the reticuloendothelial system in bacterial endocarditis gives a quantitatively different antibody response than peripheral processing in osteomyelitis. Polymorphonuclear leukocytes may process bacterial antigens differently than the phagocytic cells of the R-E system and this may be the basis for observed differences of antibody response in these two chronic infections.

The observation of apparent quantitative differences in staphylococcal protein A content between bacteria recently isolated from infected bone and bacteria repeatedly subcultured is of great interest. The bacteria obtained from bone contained 10 times more of protein A than did those subcultured in the laboratory. It seems possible that bacterial content of protein A may be increased by the particular local environment of the infection.

Summary. Experimental staphylococcal osteomyelitis with *S. aureus* (Durkin) containing protein A induces in the rabbit an acute rise in heat-stable opsonic factors. The high titers of heat-stable opsonins fall to preinfection levels by the fifth month even though active bone infection persists. The opsonic antibody response in chronic rabbit osteomyelitis, therefore, parallels that in human osteomyelitis. During chronic infection the content of protein A in the staphylococcal organisms increases at least 10-fold. If osteomyelitis is produced in rabbits with staphylococcal strain (Wood 46) lacking protein A, no increase in heat-stable opsonins occurs.

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