JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1984, p. 806–807
0095-1137/84/100806-02$02.00/0

Vol. 20, No. 4

NOTES

Patterns of Antibodies to Staphylococcal DNases in Dog Sera

ERIK NESS

Department of Microbiology and Immunology, The Norwegian College of Veterinary Medicine, Oslo 1, Norway

Received 22 March 1984/Accepted 18 June 1984

Sera from clinically healthy dogs and from dogs with a history of skin disease were examined for the presence of antibodies to the DNases of *Staphylococcus aureus*, *Staphylococcus hyicus* subsp. *hyicus*, and *Staphylococcus intermedius*. The antibodies found most frequently and in the largest amounts were those to the DNase of *S. intermedius*.

The routine differential classification of *Staphylococcus aureus*, *Staphylococcus intermedius*, and *Staphylococcus hyicus* subsp. *hyicus* is based on biochemical tests (1, 2, 3). All three species coagulate rabbit plasma and produce heat-stable DNases. According to Gudding (4), the DNases produced by these organisms are serologically different. The staphylococcal species most frequently isolated from canine specimens has been identified as *S. intermedius* (5, 7; R. Gudding and E. Ness, manuscript in preparation). The aim of this study was to investigate the presence, in dog sera, of antibodies against the DNases produced by *S. aureus*, *S. hyicus* subsp. *hyicus*, and *S. intermedius* and to determine whether there is any difference in the patterns of antibodies to DNase in sera from clinically healthy dogs and dogs suffering from skin disorders.

Bacterial strains. The three strains used for the production of DNases were *S. aureus* ATCC 29740 (bovine mastitis origin), *S. hyicus* subsp. *hyicus* ATCC 11249 (pig exudative epidermitis origin), and *S. intermedius* 3591 (Type Culture Collection, The Norwegian College of Veterinary Medicine, Oslo, Norway; dog skin origin).

DNase production. The supernatant used as the test material was obtained after centrifugation of an overnight static culture of the strains in heart infusion broth (Difco Laboratories) that had been incubated at 37°C. The same supernatants were used throughout the experiment.

Antisera. Undiluted sera from 205 dogs were examined. The dogs were of different breeds and their ages varied from 1 to 11 years. Of these dogs, 140 had a history of skin disease (including 29 with clinical furunculosis) at the time samples were collected. Approximately one-third of these dogs were hospitalized at the Department of Internal Medicine II, The Norwegian College of Veterinary Medicine, the rest of the samples were taken from dogs at a private polyclinic. Most of the dogs were in a chronic stage of skin disease. The remaining 65 samples were taken from dogs with no history of any clinical disorders. β-Toxic staphylococci were isolated from all of the 140 dogs with skin disorders, but further classification into subspecies was not performed.

Enzymoserological examination. A semiquantitative agar diffusion test with toluidine blue DNA agar (6) was used. A 50-μl amount of undiluted sera from dogs or the same amount of 0.9% NaCl was put into agar wells which had a diameter of 7.5 mm. The plates were then preincubated for 3 h. An equal amount of the heart infusion broth supernatant with the DNase was then added.

DNase activity was manifested by the development of clear and distinct pink zones in the otherwise blue agar. Antibody to DNase activity appeared as a reduction in the diameter of the pink zones as compared with that in the controls. The diameters of the zones were read by using a caliper with an accuracy of 0.1 mm. The diameter of the zones in the absence of serum varied slightly, being 25 mm on average. After subtraction of 7.5 mm for the diameter of the well and 1.5 mm for the natural DNases in the sera, a net activity zone of 16 mm was obtained. The sera were divided into four groups according to the intensity with which the sera inhibited the three different staphylococcal DNases, resulting in one weak, two intermediate, and one strong inhibition group. The weak inhibition group consisted of sera which produced a 0- to 4-mm decrease in diameter (inhibition), the weak intermediate inhibition group consisted of sera which gave a 4.1- mm to 8.0-mm reduction, the strong intermediate group comprised sera that produced an 8.1- to

TABLE 1. Percentage of sera inhibiting DNases from *S. aureus*, *S. hyicus* subsp. *hyicus*, and *S. intermedius* divided in four groups

Inhibition group	Sera from group:[a]	% Sera inhibiting DNase from:		
		S. aureus	*S. hyicus* subsp. *hyicus*	*S. intermedius*
Weak	A	86.1	100.0	35.4
	B	71.4	98.6	17.9
	C	72.4	100.0	10.3
Weak intermediate	A	6.2	0.0	12.3
	B	17.9	0.7	14.3
	C	13.8	0.0	10.3
Strong intermediate	A	7.7	0.0	18.5
	B	10.0	0.0	21.4
	C	13.8	0.0	20.7
Strong	A	0.0	0.0	33.8
	B	0.7	0.7	46.4
	C	0.0	0.0	58.6

[a] A, Sera from 65 dogs assumed to be healthy; B, sera from 140 dogs with a history of skin disorders; C, sera from 29 dogs with furunculosis (also included in B).

12-mm reduction, and the strong inhibition group consisted of sera that reduced the diameter of the zones by more than 12 mm.

The degree of antibody to DNase activity in the sera varied considerably, in both healthy animals and those with various skin disorders. Inhibition of the DNase from *S. intermedius* was the type of inhibition most frequently encountered in the sera showing strong inhibition, in both dogs with a history of skin disorders as well as those assumed to be healthy (Table 1). Strong inhibition of the DNase from *S. hyicus* subsp. *hyicus* was found in only one serum sample. None of the sera showed strong inhibition of *S. aureus* DNase in combination with weak inhibition of *S. intermedius* DNase. Only six sera inhibited *S. aureus* DNase more strongly than *S. intermedius* DNase. These sera all belonged to the two weakest inhibition groups. Only eight sera, all from healthy dogs, did not inhibit any of the DNases. Among the 29 sera from dogs with a history of clinically manifest furunculosis, 17 (58.6%) showed strong inhibition of *S. intermedius* DNase (Table 1). Although none of these sera showed strong inhibition of the *S. aureus* DNase, 21 (72.4%) exhibited weak inhibition of this DNase. All of these sera reduced the zone of *S. hyicus* subsp. *hyicus* DNase by <4 mm.

The present work does not confirm the consistent results found in studies performed by Lachica (6) and Gudding and Ness (in preparation), in which it was reported, respectively, that 112 of 117 and 116 of 118 staphylococcal isolates from dogs were identified as *S. intermedius*. No serological cross-reaction existed between DNases produced by the staphylococcal species involved. Sandvik (8) has suggested that the immunological mechanism is very sensitive to staphylococcal DNases. This may partially explain the prevalence of weak antibody activity to the DNases from *S. aureus* and *S. hyicus* subsp. *hyicus*. The results strongly indicate that *S. intermedius* is the major pathogen in canine furunculosis, and this fact should thus be taken into account when preparing vaccines against the disease. One serum sample showed strong activity against *S. hyicus* subsp. *hyicus* DNase. In another study (Gudding and Ness, in preparation) strains of staphylococci were isolated in which the DNase was inhibited by antibodies to *S. hyicus* subsp. *hyicus* DNase. Based on other criteria, these strains were classified as *S. intermedius*. The fact that staphylococcal strains producing DNase were inhibited by antibodies to *S. hyicus* subsp. *hyicus* DNase explains the presence of such antibodies to DNase found in this study.

LITERATURE CITED

1. **Baird-Parker, A. C.** 1979. Methods for identifying staphylococci and micrococci, p. 201–210. *In* F. A. Skinner and D. W. Lovelock (ed.), Identification methods for microbiologists. Academic Press, Inc., Ltd., London.
2. **Devriese, L. A., and V. Hájek.** 1980. Identification of pathogenic staphylococci isolated from animals and foods derived from animals. J. Appl. Bacteriol. **49**:1–11.
3. **Devriese, L. A., V. Hájek, P. Oeding, S. A. Meyer, and K. H. Schleifer.** 1978. *Staphylococcus hyicus* (Sompolinsky 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogenes* subsp. nov. Int. J. Syst. Bacteriol. **28**:482–490.
4. **Gudding, R.** 1983. Differentiation of staphylococci on the basis of nuclease properties. J. Clin. Microbiol. **18**:1098–1101.
5. **Hájek, V.** 1976. *Staphylococcus intermedius*, a new species isolated from animals. Int. J. Syst. Bacteriol. **26**:401–408.
6. **Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich.** 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. **21**:585–587.
7. **Lachica, R. V. F., S. S. Jang, and P. D. Hoeprich.** 1979. Thermonuclease seroinhibition test for distinguishing *Staphylococcus aureus* from other coagulase-positive staphylococci. J. Clin. Microbiol. **9**:141–143.
8. **Sandvik, O.** 1974. The occurrence of antibodies against staphylococcal deoxyribonucleases in blood sera from different species. Acta Vet. Scand. **15**:631–635.