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ORIGINALS

Virulence of Enterococci in Experimental Pyelonephritis

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Summary. Enterococci are well established in the aetiology of urinary tract infection and pyelonephritis. Little is known, however, of the relative virulence of the species of bacteria in this group. The virulence of group D streptococci was examined by determining the bacterial counts per gram of infected kidney after the intravenous injection of 1×10^9 bacteria. The descending order of virulence for the strains was S. faecalis var. zymogenes and S. faecalis var. liquefaciens, S. faecium, and S. durans.

Key words: Pyelonephritis - Enterococci.

Group D streptococci have been variously reported to account for 2-31% of pyelonephritis depending on the character of patient material and the methods of isolation (10). There have been few studies of the relative virulence of the species of bacteria in group D streptococci or even the frequency of isolation of the various species from urine.

Pyelonephritis has been readily produced in rats without manipulation of the urinary tract by injecting group D streptococci intravenously (7). The present study has compared species within the group D streptococci by determining the ability of a number of strains within each species to produce haematogenous pyelonephritis in rats. In this study we have examined the group D streptococci usually considered to be enterococci, S. faecalis var. zymogenes, S. faecalis var. liquefaciens, S. faecium and S. durans.

MATERIALS AND METHODS

Seventeen enterococcal strains were tested. The species and number of strains studied were S. faecium 5, S. durans 4, S. faecalis var. liquefaciens 3 and S. faecalis var. zymogenes 5. Strain F 24 was obtained from Dr. H. Gooder, and one strain of S. durans was isolated from the urine of a patient. Three strains of S. faecalis var. zymogenes were obtained from Dr. R. W. Jackson and 2 strains were clinical isolates. The other strains were obtained from the American Type culture collection. To maintain virulence the strains were passaged 4 times in rats by injecting overnight cultures into the tail vein and recovering the organism from the kidneys 48-72 hours later.

The techniques used to produce haematogenous pyelonephritis in rats and the methods of bacteriological evaluation have been described previously (7). Eighteen hour cultures in brain heart infusion (Difco) were adjusted to approximately 1×10^9 bacteria per ml and 1 ml injected into a tail vein of outbred Wistar rats. The number of bacteria in the inoculum was adjusted to approximately the same concentration to produce a consistent challenge for the rats. Groups of eight to ten animals were sacrificed at two, four, and six weeks, and microbial population was determined for each kidney separately. Several colonies from each plate were confirmed as enterococci by growth

Table 1. Comparison of renal infection induced by different species of group D streptococci

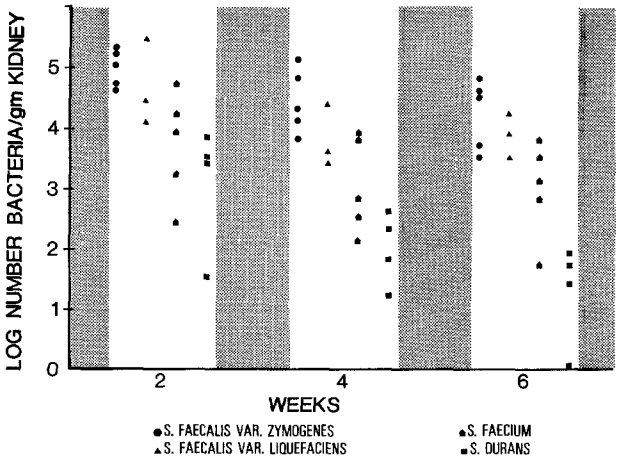
	Log bacteria/gm kidney			
Duration of infection (weeks)	S. faecalis var. zymogenes (5 strains)	S. faecalis var. liquefaciens (3 strains)	S. faecium (5 strains)	S. durans (4 strains)
2	4.95 ± 0.13[a]	4.71 ± 0.41	3.73 ± 0.42	3.16 ± 0.52
4	4.45 ± 0.23	3.86 ± 0.29	3.06 ± 0.34	2.00 ± 0.31
6	4.26 ± 0.26	3.92 ± 0.22	3.03 ± 0.36	1.30 ± 0.45
Total	4.55 ± 0.14	4.16 ± 0.21	3.27 ± 0.22	2.15 ± 0.32

[a]Mean ± standard deviation of mean

Table 2. Statistical analysis of differences between species[a]

	2 weeks	4 weeks	6 weeks	Total
S. faecalis var. zymogenes vs. var. liquefaciens	NS	NS	NS	NS
S. faecalis var. zymogenes vs. S. faecium	<0.025	<0.02	<0.05	<0.001
S. faecalis var. zymogenes vs. S. durans	<0.01	<0.001	<0.005	<0.001
S. faecalis var. liquefaciens vs. S. faecium	NS	NS	NS	<0.02
S. faecalis var. liquefaciens vs. S. durans	NS	<0.02	<0.01	<0.001
S. faecium vs. S. durans	NS	<0.01	<0.02	<0.01

[a]Differences in log numbers of bacteria/gm of kidney by student t test



ig. 1. The mean log number of bacteria per ram of kidney for each of the strains when amined at 2, 4, and 6 weeks

in S. F. medium (Difco) and 6.5% NaCl and failed to produce catalase.

RESULTS

The mean log number of bacteria per gram of kidney at 2, 4 and 6 weeks for each of the strains is shown in Figure 1. The different species were compared by determining the mean of the mean log number of bacteria in the kidney for each strain within the species (Tables 1 and 2). Overall, when the two, four, and six week harvests were added, S. faecalis var. zymogenes had greater numbers of bacteria in the kidneys than any of the other species. The difference between S. faecalis var. zymogenes and S. faecalis var. liquefaciens was not statistically significant but differences between all the other species were significantly different. The descending order of virulence was zymogenes and liquefaciens > faecium > durans. The results also indicated that the bacterial population in the kidneys remained relatively constant with the zymogenes, liquefaciens, and faecium but with a marked reduction with the durans.

DISCUSSION

Chronic pyelonephritis has frequently been produced in rats by injecting enterococci intravenously. Because progressive infection for the life of the rat results without manipulation of the rat kidney or urinary tract the model has permitted observations to be made of natural history, pathogenesis, renal function, and therapy of pyelonephritis (1, 5, 6, 7, 8, 9). We are not aware of any clinical or experimental studies examining the relative virulence of the species of bacteria in the group D streptococci. The present study indicates that virulence of different species within group D streptococci as measured by their ability to infect rat kidneys varies considerably. The descending order of virulence for the species studies was zymogenes and liquefaciens > faecium > durans. In a previous experimental study it was found that a single strain of S. durans failed to produce persistent renal infection in rats (6).

The factor or factors accounting for differences in virulence of enterococci for experimental pyelonephritis are not known. We have previously found differences in the virulence of bacteriocin producing and non-producing strains of S. faecalis var. liquefaciens (11). However, the differences with the S. faecalis var. liquefaciens strains that were described previously were small compared with the marked differences observed here between different species. S. faecalis var. liquefaciens and some strains of S. faecalis var. zymogenes are distinguished by the proteolytic activity. Heat denatured pepsin, casein gelatin protamine and wheat gluten are usually hydrolyzed by the actively proteolytic strains (2). The possible relationship of proteolytic enterococci to food poisoning has been considered in the past, but has been refuted (3).

There have been few studies of the incidence of the different species of group D streptococci in infections. Facklam (4) speciated 45 strains of urine isolates of group D streptococci received at the CDC, Atlanta, Georgia. He found that the number of isolates were S. faecalis 22, S. faecalis var. zymogenes 11, S. faecalis var. liquefaciens 5, S. durans 3, S. faecium 2, and S. bovis 1. The proportion of var. zymogenes isolates from the urine was somewhat greater than the proportion of var. zymogenes isolates from elsewhere. Whether the incidence of the various species isolated from human infections may relate to virulence has not been studied. The pathogenicity of group D streptococci deserves further study.

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