Antagonistic Action of *Streptococcus salivarius* and *Streptococcus faecalis* to *Mycobacterium tuberculosis*

CHARLES L. DARLING* AND GAROLD D. HART

Department of Pathology, U.S. Air Force Pulmonary Disease Center Laboratory, U.S. Air Force Medical Center Scott, Scott Air Force Base, Illinois 62225

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Streptococcus salivarius and *Streptococcus faecalis* were found to inhibit the growth of *Mycobacterium tuberculosis* on Löwenstein-Jensen and Middlebrook 7H11 agars, but not on the latter medium when antibacterial drugs were added. *S. faecalis* was found to be more inhibitory than *S. salivarius* to 15 strains of *M. tuberculosis*. *S. salivarius* produced little or no inhibition of growth of Runyon group III organisms but was very antagonistic to Runyon group I mycobacteria.

Although the antagonistic action of viridans streptococci on many different bacteria is well known, documentation of inhibition of acid-fast bacilli is limited. In 1974, Gelbart et al. (1) described the inhibitory effect of an alpha-hemolytic streptococcus on Runyon group III mycobacteria. The following report was prompted by the observation that strains of *Streptococcus salivarius* and *Streptococcus faecalis* inhibited the growth of *Mycobacterium tuberculosis* on media designed for the isolation of mycobacteria.

MATERIALS AND METHODS

Cultural procedures for mycobacteria. The *N*-acetyl-L-cysteine-sodium hydroxide procedure was used for sputum digestion and decontamination (2). The sediment from each specimen was inoculated on two slants of Löwenstein-Jensen medium (BBL) and a biplate that contained Middlebrook 7H11 agar (Difco) on one side and selective 7H11 medium (3) on the other side. The antibacterial agents used in the selective 7H11 medium were those recommended by McClatchy et al. (3), except that amphotericin B was not used. Biplates were enclosed in polyethylene bags and incubated in an atmosphere containing approximately 5% CO₂. All media were incubated at 35°C. Once each week, all media were observed for the presence of growth of acid-fast bacilli.

Test to detect streptococcal antagonism to mycobacteria. Streptococci were grown overnight in Todd-Hewitt broth (Difco) at 35°C. Todd-Hewitt broth was added until the turbidity matched that of a McFarland no. 1 standard. The culture was then diluted 1:10 with the same broth. Mycobacterial strains were inoculated in Middlebrook 7H9 broth (Difco), which contained 0.1% Trypticase (BBL). These cultures were incubated at 35°C until the turbidity matched that of a McFarland no. 1 standard. The culture was then diluted 1:10 with the same broth. A swab was used to streak the mycobacterial inoculum on both sides of a biplate that con-

tained Middlebrook 7H11 agar. A drop of the diluted streptococcal inoculum was allowed to fall and run across one side of each biplate. Todd-Hewitt broth was dropped on the other side of the biplate in the same manner. *S. salivarius* and *S. faecalis* were each tested against 32 strains of mycobacteria, which were isolated from clinical specimens in our laboratory. Plates were enclosed in polyethylene bags and incubated at 35°C in an atmosphere that contained approximately 5% CO₂. After 17 days of incubation, all cultures were observed for zones of inhibition of growth of the mycobacteria.

RESULTS

During routine culturing for acid-fast bacilli from clinical material, two examples of streptococcal antagonism to *M. tuberculosis* were observed. One example of this antagonism may be seen in Fig. 1. *M. tuberculosis* was observed to grow on the side of the plate that contained Middlebrook 7H11 agar and antibacterial drugs. Inhibition of *M. tuberculosis* may be seen on the other side of the plate that did not contain antibacterial drugs. On Löwenstein-Jensen medium there was growth that looked like that of a yellow scotochromogen. Subcultures from the side of the plate that did not contain antibacterial drugs and the two tubes of Löwenstein-Jensen medium revealed no growth of *M. tuberculosis*. However, an alpha-hemolytic streptococcus, which subsequently was identified as *S. salivarius*, was isolated from these three cultures on peptone agar (Difco) that contained 5% sheep blood. In the second example (Fig. 2) similar results were noted, except that the antagonistic bacterium was *S. faecalis* and there was "blueing" of the Löwenstein-Jensen medium. It may be seen that only 13 colonies of *S. faecalis* were required to completely inhibit the growth of *M. tuberculosis*.

* Send reprint requests to: Clearwater Clinical Laboratory, 714 S. Ft. Harrison Ave., Clearwater, FL 33516.

FIG. 1. (A) Colonies of *M. tuberculosis* that grew on Middlebrook 7H11 agar plus antibacterial drugs. (B) Inhibition of growth of *M. tuberculosis* by *S. salivarius* on Middlebrook 7H11 agar.

FIG. 2. (A) Colonies of *M. tuberculosis* that grew on Middlebrook 7H11 agar plus antibacterial drugs. (B) Complete inhibition of growth of *M. tuberculosis* by 13 colonies of *S. faecalis* on Middlebrook 7H11 agar.

TABLE 1. *Streptococcal inhibition of mycobacteria*

<i>Mycobacterium</i> sp.	Inhibition (mm) produced by:	
	<i>S. salivarius</i>	<i>S. faecalis</i>
<i>M. tuberculosis</i> ^a	17	20
<i>M. tuberculosis</i> ^b	3(partial) ^c	Complete
<i>M. tuberculosis</i>	2(partial)	Complete
<i>M. tuberculosis</i>	5(partial)	13
<i>M. tuberculosis</i>	2	18
<i>M. tuberculosis</i>	0	17
<i>M. tuberculosis</i>	3(partial)	14
<i>M. tuberculosis</i>	5(partial)	12
<i>M. tuberculosis</i>	3(partial)	20
<i>M. tuberculosis</i>	0	14
<i>M. tuberculosis</i>	3(partial)	20
<i>M. tuberculosis</i>	10	15
<i>M. tuberculosis</i>	7	Complete
<i>M. tuberculosis</i>	12	27
<i>M. tuberculosis</i>	12	30
<i>M. kansasii</i>	20	Complete
<i>M. kansasii</i>	27	Complete
<i>M. kansasii</i>	30	Complete
<i>M. kansasii</i>	28	Complete
<i>M. kansasii</i>	15	Complete
<i>M. gordonae</i>	17	Complete
<i>M. gordonae</i>	5(partial)	15
<i>M. gordonae</i>	10	Complete
<i>M. scrofulaceum</i>	3(partial)	Complete
<i>M. intracellulare-avium</i> complex	0	5
<i>M. intracellulare-avium</i> complex	0	0
<i>M. intracellulare-avium</i> complex	0	7
<i>M. intracellulare-avium</i> complex	0	3
<i>M. intracellulare-avium</i> complex	3(partial)	3
<i>M. intracellulare-avium</i> complex	0	2
<i>M. fortuitum</i>	0	7(partial)
<i>M. fortuitum</i>	0	0

^a Culture originally inhibited by *S. salivarius*.^b Culture originally inhibited by *S. faecalis*.^c Colonies of mycobacteria smaller than on the control.^d Complete inhibition of growth on plate.

The results of the streptococcal antagonism study on the 32 clinical isolates may be seen in Table 1. *S. faecalis* exhibited more inhibition than *S. salivarius* to 30 of the 32 mycobacteria tested. Both species of streptococci markedly inhibited photochromogens. *S. salivarius* had little or no effect on Runyon group III organisms.

DISCUSSION

This is apparently the first report of streptococcal antagonism to *M. tuberculosis*. It is noteworthy that two species of streptococci were capable of completely inhibiting the growth of *M.*

tuberculosis on media specifically designed for the isolation of mycobacteria. If antibacterial drugs had not been added to one of the basic media used, *M. tuberculosis* would not have been isolated from the clinical specimens of two patients. It is recommended that antibacterial drugs be added to media designed for the isolation of acid-fast bacilli in order to prevent any streptococcal antagonism that might occur and to make possible more definitive diagnoses.

McClatchy et al. (3) and Mitchison et al. (4) reported that the antibacterial agents used in selective oleic acid albumin agar medium are not completely innocuous for all species of mycobacteria. Therefore, nonselective media such as Löwenstein-Jensen, 7H10, or 7H11 should also be used.

The *S. salivarius* and *S. faecalis* isolates studied were found to produce varying degrees of antagonism for 30 of the 32 strains of mycobacteria tested. The alpha-hemolytic streptococcus studied by Gelbart et al. (1) was antagonistic only to Runyon group III mycobacteria. These workers suggested the possible use of this selective antagonism for typing mycobacteria. In the present study it was found that a strain of *S. salivarius* had almost no effect against Runyon group III mycobacteria but was antagonistic to Runyon groups I and II. It had variable antagonism to *M. tuberculosis*. It appears that bacterial antagonism may be a useful tool for typing mycobacteria, but further studies are needed, using additional strains of streptococci and mycobacteria.

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