INFECTION AND IMMUNITY, Apr. 1977, p. 152-154

Vol. 16, No. 1
Printed in U.S.A.

Differentiation of Several Isolates of *Peptococcus magnus* by Counterimmunoelectrophoresis

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Received for publication 15 November 1976

Uniform sonicated suspensions of three clinical isolates of *Peptococcus magnus* that were morphologically, chromatographically, and biochemically identical were used to produce hyperimmune antisera in rabbits. Similar hyperimmune serum was prepared to a single strain of *Peptostreptococcus anaerobius*. When these antisera and antigens were tested by counterimmunoelectrophoresis, the three isolates of *Peptococcus magnus* were immunologically distinct. None of the antisera to *Peptococcus magnus* formed precipitin lines with *Peptostreptococcus anaerobius*.

Peptococcus magnus occurs in normal throats and feces and has been isolated from suppuration in the lung and abdomen. However, firm data proving a pathogenic role for these anaerobic cocci have not been presented. Bacteria of similar morphological and biochemical characteristics may be serologically different. Differing antigenic properties have been clinically important with many bacteria as well as, for instance, aerobic streptococci (5). Here we differentiate several isolates of *Peptococcus magnus* by counter-immunoelectrophoresis (CIEP) (2, 4). We have undertaken this study in an attempt to differentiate strains of *Peptococcus magnus* and, ultimately, to relate specific strains (by their serological responses in man) to human disease.

MATERIALS AND METHODS

Peptococcus magnus isolates no. 1, no. 2, and no. 3 and a single strain of *Peptostreptococcus anaerobius* were identified by routine anaerobic methods using morphology, gas chromatography, and biochemical reactions (1). Identifications were confirmed in the clinical laboratory of Hutzel Hospital and by the Special Microbiology Laboratory of this University and by Virginia Polytechnic Institute Laboratory, Blacksburg. *Peptococcus magnus* were large gram-positive cocci in small grape-like clusters, whereas the single isolate of *Peptostreptococcus anaerobius* was similar tinctorially, but formed short linear chains. As determined by gas chromatography with each *Peptococcus magnus*, the major product was acetic acid and the minor one was lactic acid. Similar products of gas chromatography with *Peptostreptococcus anaerobius* were ethyl alcohol and acetic, isobutyric, butyric, isovaleric, and isocaproic acids. Biochemical tests indicated that the *Peptococcus magnus* isolates failed to ferment glucose, sucrose, maltose, mannose, or fructose and did not liquefy gelatin. (*Peptococcus magnus* does liquefy gelatin when Tween 80 is added to the medium.) The *Peptostreptococcus anaerobius* fermented only glucose and fructose.

Peptococcus magnus no. 1 was isolated in pure culture from a biopsy of the femur in a case of osteomyelitis, a 37-year-old man (J.M.). *Peptococcus magnus* no. 2 was isolated from the lingula at lobectomy in a refractory primary lung abscess of a 27-year-old alcoholic man (D.L.) with severe dental caries. *Peptococcus magnus* ATCC 14956, labeled *Peptococcus variabilis*, was obtained from the American Type Culture Collection, Rockville, Md. This is the type strain of *Peptococcus magnus*. *Peptostreptococcus anaerobius* was isolated from an abdominal wound in a 16-year-old woman after caesarean section.

Preparation of antigen. Cultures of *Peptococcus magnus* (no. 1, no. 2, and ATCC 14956) and *Peptostreptococcus anaerobius* were grown for 48 h in individual tubes of chopped-meat medium. Also, 2 ml of the growth in chopped-meat medium were used to inoculate 1,500 ml of Schaedler broth, and this was incubated for 48 to 72 h in an anaerobic glove box. Cells were harvested and centrifuged in a Sorvall RSV-2 at 10,800 × *g* for 30 min (4°C). Pellets were resuspended in sterile distilled water. After three similar centrifugations and washes, cell packs were resuspended in 4 to 6 ml of sterile water, lyophilized overnight (Virtis automatic freezer dryer), and stored (−20°C). When ready for use, pellets were weighed and suspended in distilled water (15 mg/2 ml). This material was then sonicated (Sonifier cell disrupter) in an ice bath for 3 min. Sonication or similar periods was repeated 4 times and was considered complete when, on Gram strain, at least 50% of the cells were disrupted. Finally, the 2-ml suspensions were brought to 10 ml with sterile distilled water and stored at −20°C until use as antigen, either for injection into rabbits or for CIEP.

Preparation of antibody. Antigens in 0.5-ml portions of the three isolates of *Peptococcus magnus* and

the single strain of *Peptostreptococcus anaerobius* were given intravenously once weekly to young (3 to 4 lb [ca. 1,360 to 1,813 g]) male New Zealand white rabbits. (For *Peptostreptococcus anaerobius* only, weekly injections were given 5 times. Four weeks elapsed without injections, and, finally, weekly immunizations were given another 6 times. This entire series was necessary in order to produce high-titered antibody.) At least two rabbits were used for immunization with each bacterial isolate. Every 7 days samples of blood were taken from an ear vein, and the antibody titer was determined by CIEP. When titers to the injected antigen were stable on 2 successive weeks at dilutions of 1:8 or more, animals were anesthetized with an intravenous infusion of 75 mg of pentobarbital sodium and exsanguinated by cardiac puncture. Adequate titers were reached in 5 to 8 weeks with isolates for *Peptococcus magnus*, but, for reasons unclear to us, required 15 weeks with *Peptostreptococcus anaerobius*. Sera were stored in 5-ml portions at −20°C until use.

CIEP. A Gelman electrophoresis chamber using a Heathkit high-voltage (100 to 200 V) power source at 25 mA was used for 90 min per plate. Glass slides (3.25 by 4 inches [ca. 9.4 by 10.2 cm]) were coated with a 15-ml suspension of 1% agarose in barbital (0.737 g/100 ml)-sodium barbital (0.824 g/100 ml) buffer prepared in a 7:13 ratio, respectively. Parallel wells to contain 10 to 15 μl were cut 1 cm apart with a no. 1 corkborer. The Gelman chamber was filled with 900 ml of barbital-sodium barbital buffer.

RESULTS AND DISCUSSION

Homologous precipitin lines by CIEP at titers 1:16 to 1:64 were reached within 5 to 8 weeks with each of the three strains of *Peptococcus magnus* and in 15 weeks with the single isolate of *Peptostreptococcus anaerobius* (Table 1). When normal rabbit serum and the several hyperimmune sera were tested with the sonicated antigens, the three isolates of *Peptococcus magnus* were immunologically distinctive (Table 2). Homotypic titers to *Peptococcus magnus* no. 1, 2, and 3 were 1:16, 1:64, and 1:64, respectively. Heterotypic titers were 1:2 in each case. None of the strains of *Peptococcus magnus* cross-reacted with hyperimmune sera produced with the single isolate of *Peptostreptococcus anaerobius* that we used. Conversely, *Peptostreptococcus anaerobius* did not cross-react with any of the *Peptococcus magnus* antisera. None of the four sonicated antigens induced precipitins with normal rabbit serum.

Therefore, three morphologically, chromatographically, and biochemically similar isolates of *Peptococcus magnus* are separable immunologically. Other isolates of *Peptococcus magnus* from clinical specimens need to be tested by these methods. CIEP was used because of its sensitivity and availability. The significance of these preliminary experiments is uncertain, but these results appear to be the first immunological separations of strains of *Peptococcus magnus*.

Porschen and Spaulding have also recently studied antibodies produced in rabbits to a number of anaerobic cocci using fluorescence microscopy (3). No common genus antigen with *Peptococcus magnus*, *Peptococcus asaccharolyticus*, or *Peptococcus prevotii* was found. However, 10 strains of *Peptococcus magnus* reacted strongly with rabbit antisera to *Peptococcus magnus* 926-2, suggesting a common spe-

TABLE 1. *Homologous antibody titers by CIEP in rabbits to Peptococcus magnus and Peptostreptococcus anaerobius*

Antigen	No. of injections[a]	Reciprocal of antibody titer
Peptococcus magnus no. 1	8	16
Peptococcus magnus no. 2	5	64
Peptococcus magnus (variabilis) ATCC (14956)	8	64
Peptostreptococcus anaerobius	11	32

[a] One injection of antigen was given per week except in the case of *Peptostreptococcus anaerobius*, in which immunizations spanned 15 weeks.

TABLE 2. *Differentiation of three subspecies of Peptococcus magnus by CIEP*

Antigen	Hyperimmune rabbit antiserum[a]				Normal rabbit serum
	Peptococcus magnus			*Peptostreptococcus anaerobius*	
	No. 1	No. 2	ATCC 14956		
Peptococcus magnus no. 1	16	2	2	Neg[b]	Neg
Peptococcus magnus no. 2	2	64	2	Neg	Neg
Peptococcus magnus ATCC 14956	2	2	64	Neg	Neg
Peptostreptococcus anaerobius	Neg[b]	Neg	Neg	32	Neg

[a] Titers are expressed as reciprocal of dilution.

[b] No precipitin line occurred with undiluted serum.

cies antigen. These results differ from those reported here with CIEP.

ACKNOWLEDGMENTS

We thank Rex L. Hamilton for his helpful advice at the inception of this study.

We also wish to thank L. V. Holdeman of the Virginia Polytechnic Institute (VPI) for confirming the identification of these strains. These strains are now part of the VPI collection. The VPI designated numbers are *Peptococcus magnus* no. 1 (11031) and no. 2 (11032).

This work was supported by Public Health Service grant AI 00261-13 from the National Institute of Allergy and Infectious Diseases and by a grant from the Skillman Foundation.

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