SEROLOGICAL RELATIONSHIP OF
MIMEAE, MORAXELLA, DIPLOCOCCUS
MUCOSUS AND NEISSERIA WINOGRADSKYI

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SUMMARY: For the proper classification of the Mimeae, Moraxella, Diplococcus mucosus and Neisseria winogradskyi, the serological relationship should be considered. In a serological comparison using strains of Moraxella, N. winogradskyi and D. mucosus against polyvalent Mima polymorpha antiserum, adsorbed polyvalent Herellea antiserum and Bacterium anitratum serotypes 1-10, it was found that one of the five D. mucosus strains was serologically the same as B. anitratum, type 7. The other four strains of D. mucosus and N. winogradskyi were agglutinated by adsorbed polyvalent Herellea antiserum and share partial antigens with certain serotypes of B. anitratum. Henriksen (1952) suggested further study was necessary before Mima polymorpha var. oxidans could be included in the genus Moraxella. In the present study, 57% of the Moraxella strains studied were agglutinated by polyvalent M. polymorpha antiserum and M. polymorpha var. oxidans (pool of 3 sera). The strain of Bacterium lwoffii var. brevis included was agglutinated only by Moraxella lacunata antiserum.

In a previous publication a method for the differentiation of the tribe Mimeae De Bord (1942) from the Neisseriae was reported (Cary et al., 1958). At that time, no strains of Moraxella, Diplococcus mucosus von Lingelsheim (Seelinger 1953) or Neisseria winogradskyi Lemoigne et al. (1952) were included. Consequently, this laboratory has

obtained many of these strains for a study of the serological relationship of these cultures to the Mimeae using polyvalent Mima polymorpha antiserum, polyvalent adsorbed Herellea antiserum, and the 10 serotypes of Bacterium anitratum described by Ferguson and Roberts (1950). It was felt that such a study was necessary for the proper classification of these organisms.

MATERIALS AND METHODS

The strains used in this study included the 10 distinct serotypes of Bacterium anitratum designated by Ferguson and Roberts (1950). The Moraxella strains were furnished by Dr. S.D. Henriksen of the State Institute for Public Health, Oslo, Norway and by Dr. W.A. Clark of the American Type Culture Collection, Washington, D.C. One strain of Neisseria winogradskyi was obtained from Dr. M. Lemoigne of the Pasteur Institute. Five cultures of Diplococcus mucosus, isolated by Dr. H. Seeliger, and one of Bacterium lwoffii var. brevis were secured from Miss E.O. King of the Communicable Disease Center, Atlanta, Ga.

Antigens for the precipitin tests using Maxted's filtrate were prepared according to the method previously described (Cary et al., 1956) except for the following modification to insure a sufficient quantity to perform all the tests desired. The cultures of Moraxella were grown on freshly prepared Casman's blood agar at 37°C for 18 hr under 10% CO₂ to prevent drying. All the other cultures were grown on trypticase soy agar containing 5% glycerin. After incubation, growth was placed in 2 ml of Maxted's filtrate in a 48°C water bath until the fluid cleared to a point indicating that most of the cells were lysed.

Immune sera were prepared in the following manner. All cultures of Moraxella were grown on Casman's blood agar and the other strains on trypticase soy agar with 5% glycerin. Formalized vaccines, approximating 1,500,000 cells per ml, were made for the inoculation of rabbits in a series of 4 intravenous injections a week for a 2-3 wk interval. Four hours before the first injection of 1 ml, 10 ml of 10% dextrose solution were given intravenously (Holstein, 1948). At the end of 2 wk, all were bled and checked. If the titers were 1:1280, the animals were exsanguinated. Most required a 3-wk series of injections.

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Eighteen-hour living cultures were used as adsorbing antigens. Two-tenths of a milliliter of cells centrifuged at 2500 rpm for 30 min were added to each ml of indicated antiserum, incubated at 50° C for 1 hr, and centrifuged. The adsorbed sera were tested at a dilution of 1:2 for the precipitin tests and diluted 1:20 through 1:1280 for tube agglutinations. The latter were incubated at 50°C for 18 hr.

For slide agglutinations the antigens were prepared as previously described (Cary et al., 1958). One drop of polyvalent antiserum and one drop of alkali-treated cells resuspended in saline were placed on a spot plate. The plate was shaken for 3 min and read. A live antigen was also prepared for comparison with the alkali-treated cells.

The observation made in the original study (Cary et al., 1958) on the loss of group- and type-specific antigens when the cultures were maintained in the laboratory over long periods applied to the present work. Mice (14-16 gm) were injected intraperitoneally with heavy suspensions of microorganisms grown on blood agar. After 18 hr, they were exsanguinated. From two to twenty mouse passages were required to obtain the results reported here.

RESULTS

In Table 1, the precipitin reactions using Maxted's filtrate are summarized. With pooled Mima polymorpha var. oxidans antiserum, 7 out of 10 strains of Moraxella reacted. In contrast only 2 Moraxellae reacted with polyvalent Mima polymorpha antiserum. The precipitin procedure used here was originally devised in 1956 for the detection of a group antigen in M. polymorpha. At that time only a type-specific antigen was demonstrated with this method. In the current study, the reaction of one Moraxella and a weak reaction of Moraxella non-liquefaciens indicated that these strains share an antigen with one or both of those included in the polyvalent antiserum. From the precipitin tests, a greater serological similarity between the Moraxellae and M. polymorpha var. oxidans was observed. Six strains of Moraxella reacted with Moraxella lacunata antiserum. Bacterium lwoffii var. brevis reacted only with the M. nonliquefaciens and M. lacunata antisera.

Table 1. Precipitin reactions.

	Polyvalent	Mima	Moraxella	
	Mima	polymorpha	Moraxella	non-
Mixed	polymorpha	var. oxidans	lacunata	liquefaciens
Extracts	Antiserum	(3 sera)	Antiserum	Antiserum
<u>Moraxella</u> sp. (5 strains)	1/5 +	5/5 +	3/5+	-
<u>Moraxella</u> <u>lacunata</u>	-	-	+	-
<u>Moraxella</u> <u>bovis</u>	-	+	-	-
<u>Moraxella</u> <u>anapestifer</u>	-	-	+	-
<u>Moraxella</u> <u>nonliquefaciens</u> ±		+	+	+
<u>Bacterium</u> <u>lwoffi</u> var. <u>brevis</u>	-	-	+	●
<u>Mima</u> <u>polymorpha</u> var. <u>oxidans</u> (3 strains)	3/3 +	3/3 +	3/3 +	1/3 +

The slide-agglutination technique with alkali-treated cells which previously has been found as the only means of detecting a group antigen in M. polymorpha (Cary et al., 1958) was employed with Moraxellae for the purpose of demonstrating whether they share a group antigen with the Mimae. Four out of seven Moraxellae were strongly agglutinated by the polyvalent M. polymorpha antiserum and M. polymorpha var. oxidans as indicated in Table 2. When these tests were run, two strains of Moraxella had become too rough to be used. B. lwoffi var. brevis was not agglutinated by either serum. From the results with living suspensions (Table 2), one finds out of 7 Moraxellae reacted with both antisera.

Using tube agglutinations, the homologous titers were between 1:320 and 1:1280. There were no cross reactions in the Moraxella group or with the M. polymorpha var.

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Table 2. Slide agglutinations.

Antigens	Polyvalent <u>Mima polymorpha</u> Antiserum	<u>Mima polymorpha</u> var. <u>oxidans</u> (3 sera pool)
Live Antigens		
<u>Moraxella</u> sp. (3 strains)	-	-
<u>Moraxella</u> <u>lacunata</u>	+	+
<u>Moraxella</u> <u>bovis</u>	-	-
<u>Moraxella</u> <u>anapestifer</u>	-	-
<u>Moraxella</u> <u>nonliquefaciens</u>	-	-
<u>Bacterium</u> <u>lwoffii</u> var. <u>brevis</u>	-	-
<u>Mima</u> <u>polymorpha</u> var. <u>oxidans</u>	+	+
Alkali-Treated Cells		
<u>Moraxella</u> sp. (3 strains)	+	+
<u>Moraxella</u> <u>lacunata</u>	±	±
<u>Moraxella</u> <u>bovis</u>	-	-
<u>Moraxella</u> <u>anapestifer</u>	±	±
<u>Moraxella</u> <u>nonliquefaciens</u>	+	+
<u>Bacterium</u> <u>lwoffii</u> var. <u>brevis</u>	-	-
<u>Mima</u> <u>polymorpha</u> var. <u>oxidans</u>	+	+

oxidans antisera. Cross reactions of 1:160 did occur within the M. polymorpha var. oxidans group.

Seeliger (1953) felt that Diplococcus mucosus and Bacterium anitratum were probably the same. Five of his strains were compared serologically to Ferguson's B. anitratum, types 1-10. In the original study (Cary et al., 1958), Ferguson's ten serotypes were agglutinated by polyvalent M. polymorpha antiserum. One of the five strains studied here with alkali-treated cells was strongly agglutinated by polyvalent M. polymorpha antiserum. A strong precipitin reaction was also observed with B. anitratum, type 7. Reciprocal adsorptions were performed with this strain of D. mucosus and B. anitratum, type 7 to ascertain whether they were homologous types. By using tube and slide agglutinations with adsorbed and unadsorbed sera, this strain proved to be the same serologically as B. anitratum, type 7. The remaining D. mucosus cultures were agglutinated by the polyvalent adsorbed Herellea antiserum. These strains also share partial antigens with B. anitratum, types 6 and 9. Alkali-treated cells of N. winogradskyi were agglutinated by polyvalent adsorbed Herellea antiserum, and this culture shares partial antigens with B. anitratum, types 3 and 5.

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