Serological Examination of Some Strains That Are in the Mycobacterium avium-intracellulare-scrofulaceum Complex But Do Not Belong to Schaefer’s Serotypes

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One hundred strains belonging to the Mycobacterium avium-intracellulare-scrofulaceum (MAIS) complex but not agglutinating with antisera type-specific for Schaefer’s 23 MAIS serotypes were examined using antisera against seven other such strains. Four of the 100 strains were found to be of the same serotype as one of the 7 against which antisera were prepared; 4 other strains were of the same serotype as another of those against which antisera were prepared. Although the strains against which antisera were prepared were serologically distinct from each other, no strains serologically identical to 5 of them were found. This suggests that numerous serotypes might have to be defined if strains such as those examined are to be assigned to their respective serotypes.

Antisera type-specific for the 23 serotypes recognized by Schaefer (3, 4; Mycobacterial Culture Collection, 1972, U.S. Department of Health, Education, and Welfare, publication no. NIH, 72-289) among organisms of the Mycobacterium avium-intracellulare-scrofulaceum (MAIS) complex do not agglutinate almost a third of the strains belonging to the MAIS complex and isolated at the Tuberculosis Section of the Laboratory of Microbiology and Pathology (TSLMP), Queensland Department of Health, Brisbane. The possibility that most of these nontypable strains belonged to only few yet undefined serotypes prompted us to prepare antisera against 7 such strains and then screen 100 other such strains with the antisera prepared.

MATERIALS AND METHODS

The 107 strains were from a collection of mycobacteria kept at the TSLMP. Four hundred and five were isolated at the TSLMP between 1969 and 1973; one (strain Harrison) was received in 1972 from B. O’Connor, Institute of Clinical Pathology and Medical Research, Lidcombe, New South Wales; and one (strain Ranchod) was received in 1971 from G. Woods, Cornwall Hospital, Auckland, New Zealand. Those isolated at the TSLMP were from clinical material, usually sputum, from persons in south-eastern Queensland who were under investigation for mycobacterial disease; the other two were from lymph nodes of children with lymphadenitis. All strains selected were from material from different persons. All strains were members of the MAIS complex, i.e., they grew slowly (in 10–20 days) on Lowenstein-Jensen medium at 22 to 25°C, were negative in Dawson’s version (1) of the niacin test, were negative in the nitrate reduction test of Virtanen (5), and were negative in the Tween 80 hydrolysis test of Wayne and Doubek (6). All were non-autoagglutinable, i.e., they formed stable suspensions in phenolized phosphate-buffered saline. All had been screened by Schaefer’s serumagglutination test as modified by Reznikov and Leggo (2) and were found to be non-agglutinable by antisera type-specific for Schaefer’s MAIS serotypes, namely, 1, 2, 3, IIIa, IIIb, IV, V, VI, VII, Altmann, Arnold, Boone, Chance, Darden, Davis, Dent, Gause, Howell, Lunning, Scrofulaceum, Watson, Wilson, and Yandle.

The seven strains against which antisera were prepared were Harrison, Ranchod, Brockett 2350, Corey 2540, Cox 1994, Hammelswang 1984, and Wendt 2188. The first two listed were chosen intentionally; the others were chosen at random. Antisera were prepared by hyperimmunization of rabbits as described by Schaefer (3), but the bacterial suspensions used were prepared as described by Yoder and Schaefer (8).

To determine the agglutinin titers, 0.1-ml volumes of progressive dilutions of the antisera in phenolized phosphate-buffered saline (NaH2PO4, 0.1%; KH2PO4, 0.04%; NaCl, 0.8%; phenol, 0.5%) and equal volumes
of the respective bacterial suspensions prepared in the same way as those used for immunization of rabbits were mixed in scratch-free tubes (7 by 50 mm) and incubated at 35 C for 20 h. Agglutination was read in oblique fluorescent light against a black background in a darkened room. Agglutination that was complete or almost complete and is scored as 4+ or 3+ on the scale used by Schaefer (3) was recorded as +; agglutination that is 2+ on Schaefer's scale was recorded as (+); and the absence or virtual absence of agglutination scored as 0 or 1+ by Schaefer was recorded as −.

To demonstrate that the seven strains against which antisera were prepared were indeed serologically distinct from those in Schaefer's 23 MAIS serotypes, the seven antisera at concentrations corresponding to four times their titer were tested with bacterial suspensions of reference strains for Schaefer's MAIS serotypes. The agglutination test was performed, read, and scored as described above.

To determine whether the strains against which antisera were prepared were serologically distinct from each other, the seven antisera were tested (as outlined above) with bacterial suspensions of the seven strains.

To determine how many of 100 nontypable test strains were serologically similar to any of the seven against which antisera were prepared, the bacterial suspensions of the 100 strains were tested (as outlined above) with the seven antisera.

Since Schaefer (personal communication) considers the capacity of a test strain to reduce the agglutinin titer of an antiserum by 16-fold as proof of homology, test strains agglutinated by any of the seven antisera were examined by agglutinin absorption.

This was carried out essentially as described by Yoder and Schaefer (8).

RESULTS AND DISCUSSION

Agglutination seen when reference strains for Schaefer's MAIS serotypes were tested with the seven antisera is shown in Table 1.

Agglutination seen on testing the seven strains against which antisera were prepared by the antisera prepared is shown in Table 2.

Agglutination of 100 test strains by the seven antisera is shown in Table 3.

A summary of the agglutinin-absorption tests is presented in Table 4.

In Table 1, strain P39 (the reference strain for Schaefer's serotype Boone) is shown as being partially agglutinated by antiserum Wendt 2188 at a concentration corresponding to four times its titer. But no agglutination was seen when strain J2970 (another reference strain for Schaefer's serotype Boone) was substituted. This partial agglutination is therefore disregarded.

That strains Harrison, Ranchod, Brockett 2350, Corey 2540, Cox 1994, Hammelswang 1984, and Wendt 2188 are serologically distinct from the reference strains for Schaefer's 23 MAIS serotypes, and from each other, is seen from Tables 1 and 2, respectively.

From agglutination results in Table 3 it can be seen that five, one, and four of the test

Table 1. Agglutination* of reference strains* for Schaefer's MAIS serotypes by the seven antisera

| Reference strains | Harri-son | Ran-cho-d | Brock-ett | Core-y | Cox | Ham-melswang | Wende-t | Control* |
|-------------------|-----------|-----------|-----------|-------|-----|--------------|---------|----------|
| 16909-2380 (1)*, 17752-372 (2), 6197 (3), Borne (Illa), 14186-1424 (Illb), Cheltenham 13528-1079 (IV), 5688-46 (V), 12315 (VI), Manten 157 (VII), Melnick (Alt-mann), Findley (Arnold), Chance (Chance), W552 (Darden), S. J. Bull No 2 (Davis), Simpson (Dent), Gause (Gause), P42 (Howell), Lunning (Lun-ning), Bridge (Scrofulaceum), 6450-204 (Watson), P54 (Wilson), Yandle (Yan-dle) | − | − | − | − | − | − | − | − |
| P39 (Boone) | − | − | − | − | − | − | (+) | − |

* Symbols: (+) and − indicate intermediate agglutination, and absence or virtual absence of agglutination, respectively.

* From the collection of W. B. Schaefer, National Jewish Hospital and Research Center, Denver, Colo.

* At concentrations corresponding to four times the titer.

* Consisting of a 0.1-ml volume of bacterial suspension and an equal volume of phenolized phosphate-buffered saline.

* Numbers and names in parentheses are Schaefer's designations for his Mycobacterium avium, M. intracellulare, and M. scrofulaceum serotypes.
Table 2. Agglutination* of strains against which antisera were prepared by the antisera prepared

| Strains             | Harrison | Ranchod | Brockett 2350 | Corey 2540 | Cox 1994 | Hammelswang 1984 | Wendt 2188 | Control* |
|---------------------|----------|---------|---------------|------------|-----------|------------------|------------|----------|
| Harrison            | +        |         |               |            |           |                  |            |          |
| Ranchod             |          | +       |               |            |           |                  |            |          |
| Brockett 2350       |          |         |               |            |           |                  |            |          |
| Corey 2540          |          |         |               |            |           |                  |            |          |
| Cox 1994            |          |         |               |            |           |                  |            |          |
| Hammelswang 1984    |          |         |               |            |           |                  |            |          |
| Wendt 2188          |          |         |               |            |           |                  |            |          |

* Symbols: + and – indicate complete or almost complete agglutination, and absence or virtual absence of agglutination, respectively.

Table 3. Agglutination* of 100 test strains by the seven antisera

| Strains                                      | Harrison | Ranchod | Brockett 2350 | Corey 2540 | Cox 1994 | Hammelswang 1984 | Wendt 2188 | Control* |
|----------------------------------------------|----------|---------|---------------|------------|-----------|------------------|------------|----------|
| Crothers 2162, Dossor 2430, Lane 3081,      |          |         |               |            |           |                  |            |          |
| McCarthy 2279, Waters 2778                   |          |         |               |            |           |                  |            |          |
| Clark 2502                                   |          |         |               |            |           |                  |            |          |
| Bond 1947, Mackenzie 2233, Schultz 2436,    |          |         |               |            |           |                  |            |          |
| Strong 2272                                  |          |         |               |            |           |                  |            |          |
| The remaining 90 strains                      |          |         |               |            |           |                  |            |          |

* See footnote a to Table 2.

Table 4. Summary of agglutinin absorption tests

| Antisera absorbed | Strains with which antisera were absorbed | Strains reducing agglutinin titer by 16-fold or more |
|-------------------|-------------------------------------------|--------------------------------------------------|
| Harrison          | Crothers 2162, Dossor 2430, Lane 3081,    | Crothers 2162, Dossor 2430, Lane 3081,            |
|                   | McCarthy 2279, Waters 2778                | McCarthy 2279,                                   |
| Corey 2540        | Clark 2502                                | Bond 1947,                                        |
| Cox 1994          | Bond 1947, Mackenzie 2233, Schultz 2436, | Mackenzie 2233,                                   |
|                   | Strong 2272                               | Schultz 2436,                                    |
|                   |                                           | Strong 2272                                       |

strains are serologically related to strains Harrison, Corey 2540, and Cox 1994, respectively; the other 90 are serologically distinct from the seven against which antisera were prepared.

From agglutinin absorption results in Table 4 it can be seen that (i) strains Crothers 2162, Dossor 2430, Lane 3081, and McCarthy 2279 are of the same serotype as strain Harrison, (ii) strains Bond 1947, Mackenzie 2233, Schultz 2436, and Strong 2272 are of the same serotype as strain Cox 1994, (iii) strain Waters 2778 is serologically related to strain Harrison, but the two are not of the same serotype, and (iv) strain Clark 2502 is serologically related to strain Corey 2540, but the two are not of the same serotype.

Our findings therefore suggest that nontypable strains such as those examined are not confined to few serotypes but, instead, show much serological diversity.

It may be noteworthy that besides strains Harrison and Ranchod, strain Corey 2540 was an aetiological agent of a tuberculosis-like disease. Whereas the former two caused lym-
phadenitis, the latter was the aetiological agent of progressive pulmonary disease. Although strains Dossier 2430, Waters 2778, and Clark 2502 also were from sputum of persons with pulmonary tuberculosis-like disease, the bacteriological histories of these people suggested that the causative agents were MAIS organisms of other serotypes.

Although strains of the same serotypes as strains Harrison, Corey 2540, and Cox 1994 might be encountered at the TSLMP only once or twice a year, this differs little from the frequency with which we isolate strains belonging to Schaefer's serotypes 1, 2, 3, IIIa, IIIb, IV, V, and Scrofulaceum.

As the nomenclature system with which Wolinsky and Schaefer (7) propose to replace Schaefer's present open-ended system will accommodate only another 27 MAIS serotypes, we wonder, in the light of our findings, whether adoption of such a scheme might not be short sighted.

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