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Serovars of *Mycobacterium avium* Complex Isolated from Patients in Sweden

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The serovars of clinical isolates of *Mycobacterium avium* complex from 24 acquired immunodeficiency syndrome (AIDS) and 140 non-AIDS patients in Sweden were studied by using type-specific antisera. A wide distribution of serovars was seen. Serovar 6 was predominant in both groups of patients, isolated from 33 and 16% of the AIDS and non-AIDS patients, respectively. The results indicate geographical as well as disease-related differences in the distribution of *M. avium* complex serovars of clinical importance.

Infections with members of the *Mycobacterium avium* complex (MAC) are being increasingly recognized (26). In Sweden, the number of clinical isolates of MAC has steadily increased during the past decade (25). Severe opportunistic MAC infections are common in patients with acquired immunodeficiency syndrome (AIDS) (15, 28). In the United States, certain serovars of MAC occur more frequently in patients with AIDS than in those with MAC infections but without AIDS (12, 15). In Sweden, approximately 10% of AIDS patients are infected with MAC (13).

By elucidating the nature of the glycopeptidolipid antigens of the dominant MAC serovars (2, 17), a detailed epidemiological analysis may be made, since tools such as monoclonal antibodies and gas chromatography of the distinct sugar epitopes may be applied to serodiagnosis and to tracing the origin of these bacteria.

We have collected strains of MAC isolated from patients with and without AIDS in Sweden from 1983 to 1988 and now present information on serovar designation and its relation to the clinical status of patients.

MATERIALS AND METHODS

All MAC isolates derived from patients from the Stockholm area or central Sweden were examined in the period from 1983 to 1986. From 140 non-AIDS patients, 181 MAC isolates were obtained. Also, 35 MAC isolates, collected from 1985 to mid-1988 from the first 24 patients in Sweden with AIDS and culture-verified MAC infections, were included in this study.

The mycobacteria were isolated by culture on Löwenstein-Jensen medium or by BACTEC radiorespirometry: the BACTEC method was used as a complement to conventional culturing on solid medium mainly for extrapulmonary specimens and direct smear-positive samples (9). All clinical isolates were identified as MAC strains by standard methods (24). The MAC isolates were serotyped at the National Jewish Center for Immunology and Respiratory Medicine by the standard Schaefer method, as described by McClatchy, using serovar-specific rabbit antisera (16). When the results of serotyping were inconclusive, isolates were further evaluated by thin-layer chromatography of the typing antigens (3) or by enzyme-linked immunosorbent assay of these isolated antigens against the type-specific antiserum (27).

RESULTS

The 181 MAC isolates obtained from non-AIDS patients consisted of 127 isolates from 89 patients with pulmonary MAC infections, 45 isolates from 44 patients with lymph node infections, and 9 isolates from another 7 patients with other types of MAC infections. The application of a variety of protocols for serotyping (seroagglutination, enzyme-linked immunosorbent assay, and thin-layer chromatography) demonstrated that a wide distribution of serovars was evident and that most known MAC serovars were represented (Table 1).

The most prevalent serovars isolated from Swedish patients without human immunodeficiency virus infections were serovar 6 (22 patients, 16%), serovar 1 (19 patients, 14%), and serovar 4 (15 patients, 11%). A mixture of serovars, particularly serovars 4 and 8, was also recovered from a sizable number of patients (Table 1).

Serovars 1, 6, 7, 9, 14, and 16 were recovered at higher frequencies from patients with pulmonary infections than from persons with extrapulmonary infections. Serovar 4, on the other hand, was isolated from 9 of 51 patients (18%) with extrapulmonary infections. Seven of the nine extrapulmonary isolates of serovar 4 were derived from lymph nodes of children with lymphadenitis, and the remaining two were isolated from the urinary tract. This information can be compared to the isolation of serovar 4 from only 6 of 89 patients (7%) with pulmonary infections. The two MAC strains isolated from bone marrow were of serovars 3 and 6.

The 35 MAC isolates from the first 24 patients in Sweden with AIDS also represented a broad spectrum of serovars. Again, as among the non-AIDS patients, serovar 6 was the most commonly isolated MAC serovar in this necessarily small group of isolates; it was recognized in 8 of the 24 patients (33%). The incidence of mixed serovars, including mixtures of *M. avium* and *M. scrofulaceum*, was high (Table 2).

The results obtained with thin-layer chromatography and enzyme-linked immunosorbent assay techniques were in very good agreement with serotyping results obtained with type-specific antisera. For 14 isolates untypeable by seroagglutination, the serovars could be determined by thin-layer chromatography or enzyme-linked immunosorbent assay techniques.

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TABLE 1. Serovars of *M. avium* complex isolates obtained from 140 non-AIDS patients in Sweden in the period from 1983 to 1986[a]

Serovar no.	No. of patients	No. of isolates		
		Pulmonary	Lymph node	Other[b]
1	19	16	3	
2	6	4	2	
3	1			1
4	15	6	7	2
6	22	17	3	2
7	4	4		
8	7	4	2	1
9	11	8	2	1
11	4		4	
12	2	2		
14	5	5		
15	1	1		
16	10	7	2	1
17	2	2		
18	1	1		
20	1	1		
25	1	1		
42	2	1	1	
43	1	1		
1/4[c]	1	1		
1/6	1	1		
1/14	1	1		
1/2/8	1	1		
1/4/8	1	1		
2/22	1	1		
4/8	10	4	6	
7/12	1	1		
7/13	1		1	
8/42	1		1	
UNT/SA[d]		27	16	11

[a] When different serovars were isolated from a patient, each serovar was included once. When the same serovar was repeatedly isolated from the same site in a patient, it was included once.
[b] Bone marrow, bone, synovial fluid, and urine.
[c] Mixed serovars.
[d] UNT/SA, Untypeable/spontaneous agglutination.

TABLE 2. Serovars of *M. avium* complex isolated from the first 24 patients in Sweden with both AIDS and culture-verified *M. avium* complex infection[a]

Serovar no.	No. of patients	No. of isolates			
		Disseminated infection[b]	Pulmonary[c]	Lymph node	Stool
1	1	1			
2	1		1		
4	3		2		1
6	8	5	2	1	3
9	3	2			2
43	3	1			2
4/6[d]	1		1		
4/8	1		1		
1/43	1				1
4/43	1		1		
6/41	1		1		
UNT/SA[e]	5	1	2		3
Total (isolates/ patients)		10/8	11/11	1/1	12/11

[a] When different serovars were isolated from a patient, each serovar was included once. When the same serovar was repeatedly isolated from the same site in a patient, it was included once.
[b] Blood, bone marrow, and liver.
[c] Sputum, gastric wash, lung tissue, pleural biopsy, bronchial aspiration, and bronchial lavage.
[d] Mixed serovars.
[e] UNT/SA, Untypeable/spontaneous agglutination.

DISCUSSION

Apart from a preliminary report on serovars of MAC isolated from AIDS patients in Sweden (10), nothing has been reported regarding the spectrum of MAC serovars isolated from AIDS patients in relation to non-AIDS patients in Sweden or, to our knowledge, in any other European country.

The identities of the serovars of the *M. avium* complex isolated from the 140 Swedish non-AIDS patients differed from those of earlier studies, mainly from the United States. Serovar 6 (isolated from 16% of the patients) was the most common MAC serovar encountered in the present study. This finding is more in accord with results from Australia, where serovar 6 was found in 11 of 95 sputum samples (21). In the United States, serovars 8 (13%), 16 (10%), and 4 (7%) have been reported (16) to be the three most common MAC isolates from non-AIDS patients; serovar 6 was only rarely (4%) encountered.

Differences in serovars of isolates from different sites of infection were seen; thus, strains of serovar 4 were mainly recovered from extrapulmonary sites, while a high percentage of serovars 1 and 6 was found among pulmonary isolates.

Also, among the isolates from patients with AIDS in Sweden, a spectrum of MAC serovars different from that encountered in the United States was observed. Infections with MAC have been seen in up to 50% of U.S. patients with AIDS (1). Eighteen of 23 MAC isolates (78%) from AIDS patients with disseminated mycobacterioses at the Memorial Sloan-Kettering Cancer Center were serovar 4 (15) and so were 21 of 52 isolates (40%) from AIDS patients from several areas of the United States (12). These studies indicate that patients with AIDS were significantly more likely to have an isolate identified as serovar 4 than were patients without AIDS. According to analysis by geographic origin, AIDS patients from New York City were more likely to have serovar 4 isolates than were AIDS patients from other states. In a recent study from Canada, MAC serovar 8 was demonstrated in 7 of 11 human immunodeficiency virus-infected patients (11). In the present study, a high percentage of isolates from AIDS patients were serovar 6, while only a few were serovar 4. The high frequency of serovar 4 in AIDS patients seen in the United States is thought to be due to a common source of infection, and the high frequency of serovar 4 and 8 among patients with disseminated infection has been suggested to indicate that these serovars are particularly virulent (5). However, none of the 10 isolates from the blood, bone marrow, or liver of AIDS patients in Sweden and none of the two bone marrow-derived isolates from non-AIDS patients were of serovar 4 or 8.

It is possible that all three serovars, 4, 6, and 8, represent organisms that are common among particularly virulent MAC strains. We and others have shown that strains belonging to certain clones of *Escherichia coli* are especially virulent and that such strains belong to certain serovars (20, 23). Similarly, it has recently been suggested that there may exist certain pathogenic MAC clones (8). Thus, the risk of developing disease with a certain serovar of MAC may, apart from host factors, depend on both environmental factors (6) and bacterial virulence factors which are so far unknown. Host factors are certainly of utmost importance,

and thus AIDS patients may be particularly susceptible to pathogenic MAC strains in the environment.

The serovars of MAC isolated from slaughter pigs and wild animals in Sweden showed a different spectrum of serovars, dominated by serovar 2 (unpublished observations), which is in accordance with earlier characterizations of MAC isolated from animals in both Denmark (22) and Germany (18). This result demonstrates that the source of infection differs between a human population and animals living in the same area.

It has been suggested that *Mycobacterium bovis* BCG vaccination protects against infections with MAC (4, 7, 14, 19). In Sweden only about 10% of AIDS patients were infected with MAC (13). It is possible that this relatively low incidence is due to cross-protection after BCG vaccination (13). Since all Swedes up to 1975 were BCG vaccinated at birth, most pulmonary patients in this study were in fact vaccinated and represent a BCG-vaccinated population. On the contrary, most of the 44 patients with lymph node isolates were unvaccinated children. Among the children, the most common serovar was serovar 4 (7 patients, 16%), a figure close to what is seen in the non-AIDS patients in United States (not BCG vaccinated). Accordingly, one explanation for the differences seen in MAC serovars among Swedish patients, with or without AIDS, is that the protective effect of BCG vaccination differs for different serovars, which is reflected by a different spectrum of MAC serovars giving rise to infection.

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LITERATURE CITED

1. **Armstrong, D., J. W. M. Gold, J. Dryjanski, E. Whimbey, B. Polsky, C. Hawkins, A. E. Brown, E. Bernard, and T. E. Kiehn.** 1985. Treatment of infections in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **103**:738–743.
2. **Brennan, P. J.** 1988. Mycobacteria and other actinomycetes, p. 203–298. *In* C. Ratledge and S. Wilkinson (ed.), Microbial lipids, vol. 1. Academic Press, Inc. (London), Ltd., London.
3. **Brennan, P. J., L. B. Heifets, and B. P. Ullom.** 1982. Thin-layer chromatography of lipid antigens as a means of identifying nontuberculous mycobacteria. J. Clin. Microbiol. **15**:447–455.
4. **Collins, F. M.** 1985. Protection afforded by BCG vaccines against an aerogenic challenge by three mycobacteria of decreasing virulence. Tubercle **66**:267–276.
5. **Collins, F. M.** 1986. Mycobacterium avium-complex infections and development of the acquired immunodeficiency syndrome: causal opportunist or causal cofactor? Int. J. Lepr. **54**:458–474.
6. **duMoulin, G. C., K. D. Stottmeier, P. A. Pelletier, A. Y. Tsang, and J. Hedley-Whyte.** 1988. Concentration of *Mycobacterium avium* by hospital hot water systems. J. Am. Med. Assoc. **260**:1599–1601.
7. **Engbaeck, H. C., and A. Jespersen.** 1966. Effect of BCG-vaccination on experimental infection with M. avium. Acta Pathol. Microbiol. Scand. **67**:505–513.
8. **Hampson, S. J., J. Thompson, M. T. Moss, F. Portaels, E. P. Green, J. Hermon-Taylor, and J. J. McFadden.** 1989. DNA probes demonstrate a single highly conserved strain of *Mycobacterium avium* infecting AIDS patients. Lancet **i**:65–68.
9. **Hoffner, S. E.** 1988. Improved detection of Mycobacterium avium complex with the Bactec radiometric system. Diagn. Microbiol. Infect. Dis. **10**:1–6.
10. **Hoffner, S. E., B. Petrini, P. J. Brennan, A. Y. Tsang, and G. Källenius.** 1989. AIDS and *Mycobacterium avium* serovars in Sweden. Lancet **ii**:336–337.
11. **Horn, R., A. Laszlo, and H. G. Robson.** 1989. Recovery of *Mycobacterium avium-M. intracellulare* from blood specimens by using the routine BACTEC 6B blood culture system. J. Clin. Microbiol. **27**:348–349.
12. **Horsburgh, C. R., Jr., D. L. Cohn, R. B. Roberts, H. Masur, R. A. Miller, A. Y. Tsang, and M. D. Iseman.** 1986. *Mycobacterium avium-M. intracellulare* isolates from patients with or without acquired immunodeficiency syndrome. Antimicrob. Agents Chemother. **30**:955–957.
13. **Källenius, G., S. E. Hoffner, and S. B. Svenson.** 1989. Does vaccination with Bacille Calmette Guerin protect against AIDS? Rev. Infect. Dis. **11**:349–351.
14. **Katila, M. L., E. Brander, and A. Backman.** 1987. Neonatal BCG vaccination and mycobacterial cervical adenitis in childhood. Tubercle **68**:291–296.
15. **Kiehn, T. E., F. F. Edwards, P. J. Brennan, A. Y. Tsang, M. Maio, J. W. Gold, E. Whimbey, B. Wong, K. McClatchy, and D. Armstrong.** 1985. Infections caused by *Mycobacterium avium* complex in immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroagglutination characteristics. J. Clin. Microbiol. **21**:168–173.
16. **McClatchy, J. K.** 1981. The seroagglutination test in the study of nontuberculous mycobacteria. Rev. Infect. Dis. **3**:867–870.
17. **McNeil, M., A. Y. Tsang, and P. J. Brennan.** 1987. Structure and antigenicity of the specific oligosaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serotype 4, the dominant mycobacterium isolated from patients with acquired immune deficiency syndrome. J. Biol. Chem. **262**:2630–2635.
18. **Meissner, G., and W. Anz.** 1977. Sources of Mycobacterium avium complex infection resulting in human disease. Am. Rev. Respir. Dis. **116**:1057–1064.
19. **Orme, I. M., and F. M. Collins.** 1985. Prophylactic effect of BCG vaccination against non-tuberculous mycobacterial infections. Tubercle **66**:117–120.
20. **Örskov, F., and I. Örskov.** 1983. Summary of a workshop on the clone concept in the epidemiology, taxonomy and evaluation of the Enterobacteriaceae and other bacteria. J. Infect. Dis. **148**: 346–357.
21. **Reznikov, M., J. H. Leggo, and D. J. Dawson.** 1971. Investigation by seroagglutination of strains of the Mycobacterium intracellulare-Mycobacterium scrofulaceum group from house dust and sputum in southeastern Queensland. Am. Rev. Respir. Dis. **104**:951–953.
22. **Saitanu, K.** 1977. Studies on mycobacteria isolated from animals, with special reference to the agglutination test. Acta Pathol. Microbiol. Scand. Sect. B **85**:303–307.
23. **Väisänen-Rhen, V., J. Elo, E. Väisanen, A. Siitonen, I. Örskov, F. Örskov, S. B. Svenson, P. H. Mäkelä, and T. K. Korhonen.** 1984. P-fimbriated clones among uropathogenic *Escherichia coli* strains. Infect. Immun. **43**:149–155.
24. **Vestal, A. L.** 1975. Procedures for isolation and identification of mycobacteria. U.S. Department of Health, Education, and Welfare publication no. 75-8230. Center for Disease Control, Atlanta.
25. **Wickman, K.** 1986. Clinical significance of nontuberculous mycobacteria. A bacteriological survey of Swedish strains isolated between 1973 and 1981. Scand. J. Infect. Dis. **18**:337–345.
26. **Woods, G. L., and J. A. Washington II.** 1987. Mycobacteria other than Mycobacterium tuberculosis: review of microbiological and clinical aspects. Rev. Infect. Dis. **9**:275–294.
27. **Yanagihara, D. L., V. L. Barr, C. V. Knisley, A. Y. Tsang, J. K. McClatchy, and P. J. Brennan.** 1985. Enzyme-linked immunosorbent assay of glycolipid antigens for identification of mycobacteria. J. Clin. Microbiol. **21**:569–574.
28. **Young, L. S., C. B. Inderlied, O. G. Berlin, and M. S. Gottlieb.** 1986. Mycobacterial infections in AIDS patients, with an emphasis on the Mycobacterium avium complex. Rev. Infect. Dis. **8**:1024–1033.