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

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Danish isolates of *Mycobacterium avium* complex were serotyped by the use of seroagglutination. The most prevalent serovars among patients with AIDS (n = 89) were 4 and 6, while among non-AIDS patients the most prevalent serovars were 1, 6, and 4, with no major differences between those in patients with pulmonary disease (n = 65) and those in patients with lymph node infection (n = 58). The results suggest a Scandinavian distribution of serovars with a predominance of serovar 6 and fail to demonstrate any selective protection against different serovars by *Mycobacterium bovis* BCG vaccination.

Before the AIDS epidemic, clinical disease due to *Mycobacterium avium* complex (MAC) was rarely seen and was often confined to older patients with pulmonary disease or to apparently healthy children with cervical lymph node infections. Disseminated disease was rarely encountered (16, 17). Today, MAC is the most common cause of systemic bacterial infection in patients with AIDS in the western world (5). However, the frequencies with which infections are found associated with AIDS range from 10 to 50% in various studies and may be higher in the United States than in Europe (3, 7, 10, 12, 15). The reason why MAC infections are so prevalent in AIDS patients is as yet unknown. The epidemiology of MAC disease is poorly understood with respect to the source and route of infection, but serotyping of MAC may provide important information, particularly in tracing the origin of infection. The distribution of the serovars of MAC appears to vary with regard to geographic location and clinical and immunological status (4, 6). We report here the serovar spectrum obtained from Danish patients during a period of 8 years.

From January 1984 through December 1991 a total of 241 MAC strains were isolated from 65 non-AIDS patients with pulmonary disease, 58 non-AIDS patients who were children with lymph node infections, and 89 AIDS patients. The specimens included sputum, stool, blood, bone marrow, and tissue and were analyzed at the Mycobacteria Department, Statens Seruminstitut, Copenhagen, Denmark. The mycobacteria were isolated by culture on Löwenstein-Jensen medium or in BACTEC medium. MAC was identified with species-specific DNA probes (Genprobe; Accuprobe, San Diego, Calif.) (1).

MAC strains isolated from children from 1984 through 1991 and all MAC strains isolated from 1990 through 1991 were serotyped at the National Veterinary Laboratory, Copenhagen, Denmark. Serotyping was performed as a tube agglutination test using 28 antiserum specimens as described by Jørgensen (8). In case of cross-reactions, an antibody absorption test was performed (13). No further examination was made on strains that were nonagglutinable or showed autoagglutination. All other MAC strains were serotyped at the National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo., by the use of seroagglutination, reaction with monoclonal antibodies, thin-layer chromatography, and gas chromatography (2).

Of the 241 cultures received, 214 (88.8%) were typeable. A wide distribution of serovars was seen with no significant difference in the spectrum of serovars when the periods 1984 to 1987 and 1988 to 1991 for both AIDS patients and non-AIDS patients, respectively, were compared.

A broad spectrum of serovars was isolated from Danish non-AIDS patients with either pulmonary disease or lymph node infection (Table 1). The most prevalent serovars isolated were serovar 1 (16.3%), serovar 6 (15.4%), and serovar 4 (14.6%). Within both groups of patients each of the serovars 1, 6, and 4 appeared with almost equal frequencies, but the joint frequency of these three serovars was higher among children with lymph node infection (56.9%) than among adults with pulmonary disease (36.9%). Nontypeable MAC strains isolated from non-AIDS patients accounted for 15.4% of the strains.

Among AIDS patients, infections due to serovar 4 were found in 36.0% of the patients, while serovar 6, the second most prevalent serovar, accounted for 15.7% of infections (Table 1). The incidence of serovars which cross-reacted when serotyped was high (20.2%), with serovar 4/8 being the third most frequent serotype among these patients (7.9%). Nontypeable MAC strains accounted for 9.0% of the strains. No significant difference in the spectrum of serovars was found among homosexual or bisexual men, intravenous drug users, transfusion recipients, and heterosexuals with human immunodeficiency virus infection. The serovars obtained from AIDS patients were principally (76.4%) isolated from sterile body areas (blood, bone marrow, liver, and spleen). When MAC was isolated from a pulmonary specimen of these patients, it was serovar 4 in 37.0% of the cases, compared to 10.8% for non-AIDS patients.

Of the 89 AIDS patients, 25 had MAC strains isolated from different body sites. Two patients had identical MAC serotypes isolated from blood, lungs, and stools; 12 patients had identical MAC serotypes isolated from blood and stools; and 9 patients had identical MAC serotypes isolated from blood and the lungs. Two patients presented different serovars from different body sites: serovars 4 and 42 from lymph node and lungs, respectively, and serovars 22 and 4/8 from the lungs and blood, respectively.

MAC serovars 1, 6, and 4 were the isolates encountered

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TABLE 1. Serovars of MAC isolates from 123 non-AIDS patients with pulmonary disease or lymph node infection and 89 AIDS patients in Denmark from 1984 to 1991

Serovar(s)	No. of:		
	Non-AIDS patients with:		AIDS patients (n = 89)
	Pulmonary infection (n = 65)	Lymph node infection (n = 58)	
1	10	10	4
2	3	1	
3	1		1
4	7	11	32
6	7	12	14
7	2		
8	1	3	3
9	5	3	5
11		1	
12	1	1	
14	3		
15		2	1
16	4	2	
17			1
18	1	1	
20			1
27	1		
41	2		
1/8			1
1/9			2
2/3		2	5
3/6		1	
3/9			2
4/8		1	6
6/25	1		
7/13	1		
9/16	1		
10/11			1
13/41	1		
20/21		1	
4 and 42			1
4/8 and 22			1
Nontypeable	13	6	8

most frequently in Danish non-AIDS patients. This distribution differs from results obtained in the United States, where serovars 8, 4, and 1 have been reported to be the most predominant serovars and where serovar 6 only rarely has been encountered (6, 11). In Sweden, however, serovar 6 has also been found the most prevalent serovar in non-AIDS patients, but almost exclusively in patients with pulmonary involvement and seldom in the *Mycobacterium bovis* BCG-unvaccinated patient population, namely, children with lymph node infections (4). This is in contrast to our findings, as no differences in the major serovars of isolates obtained from pulmonary or extrapulmonary sites were found. As in the Swedish study, the Danish pulmonary patients represented the BCG-vaccinated population and the children with lymph node infections represented the unvaccinated population (until 1979, Denmark had a nationwide BCG vaccination program, including all children starting primary school). Accordingly, a selective protection against different serovars by the BCG vaccination as suggested by Hoffner et al. (4) could not be demonstrated in this study. The finding of the same predominant serovars found in children with lymph node infections and pulmonary patients is interesting in the light of the different routes of acquisition considered for these two groups of patients: the oral route versus the respiratory route, respectively.

The Danish serovar pattern among AIDS patients differs from the patterns found in the United States, where serovars 4, 8, and 1 most frequently have been isolated (6, 9, 14, 18), but the Danish pattern also differs from that found in Sweden (4), where serovar 4 was found in only 12.5% of isolates from 24 AIDS patients and serovar 6 predominated (33.3%). We found that serovar 4 predominated (36.1%) with a frequency of occurrence comparable to frequencies seen in the United States while serovar 6 was found to be the second most prevalent serovar (15.7%). The prevalence of serovar 6 is striking, involving both patients with AIDS and patients without AIDS, and it suggests a geographical variation of serovar distribution in Scandinavia compared with that in the United States. The high frequency of serovar 4 found in Danish AIDS patients (all BCG vaccinated) stresses the role of this pathogen also in Scandinavia and fails to demonstrate cross-protection against this serovar after BCG vaccination as suggested by Hoffner et al. (4). The marked differences in the distribution of serovars obtained from our AIDS patients compared with those from our non-AIDS patients must be explained by differences in immunological status and bacterial virulence factors, as the environment must be considered the same for both groups of patients. It should be noted that the incidence of MAC infection among Danish AIDS patients is approximately 3,500 times higher than that among the general population. It also should be noted that as in Sweden, only about 10% of Danish AIDS patients (all BCG vaccinated) had MAC infection.

Most of the AIDS patients had isolates obtained from blood. In nearly all cases with a second isolate from another specimen, such as sputum or stool, the serovars were identical (90.0%), indicating the disseminated nature of the infection, either secondary to pulmonary infection or secondary to gastrointestinal invasion. Our data also revealed two patients in whom presumably profound immunological deficiency allowed colonization with more than one serovar to lead to mixed infection.

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