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Mycobacterium haemophilum Infection in a Patient with Acquired Immune Deficiency Syndrome

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Mycobacterium haemophilum **was isolated from wrist and ankle aspirates as the organism responsible for tenosynovitis in a patient with acquired immune deficiency syndrome. Mycobacterium isolates recovered from synovial fluid were identified as hemin requiring by their failure to grow on subculture unless the medium was supplemented with hemin. *M. haemophilum* is of low virulence and rarely associated with infections in humans. This is the first documented case of *M. haemophilum* infection in a patient with acquired immune deficiency syndrome.**

Mycobacterium haemophilum is rarely associated with infections in humans. In 11 of 12 cases reported, patients were receiving immunosuppressive therapy for kidney allografts (4, 15–17, 23) or for lymphomas (15, 20); one case involved an apparently healthy 12-month-old infant (5). These infections were characterized by the development of skin lesions (4, 6, 15, 20, 23), subcutaneous abscesses (15, 16, 20), or lymphadenitis (5) and occasionally involved joints (6, 15). Most isolates appeared resistant to ethambutol, isoniazid, and streptomycin (6, 15, 17, 20) but were susceptible to rifampin (5, 6, 15, 17).

We report a patient with a clinical diagnosis of acquired immune deficiency syndrome who developed tenosynovitis caused by *M. haemophilum*.

In April 1984, a 32-year-old homosexual male was transferred to the Erie County Medical Center (ECMC) with a 3-day history of fever and chills preceded by swollen, painful ankles. A diagnosis of acquired immune deficiency syndrome was suspected from an initial presentation with *Pneumocystis carinii* pneumonia and Kaposi's sarcoma. Later tests indicated the patient had antibody to human T cell lymphotropic virus type III.

On examination, a subcutaneous erythematous, nodular lesion was apparent laterally on the left wrist of the patient. Both ankles and knees were tender, warm, and swollen, but there did not appear to be involvement of the joint spaces. Fluid was aspirated from tendon sheaths of the left wrist, right knee, and both ankles. Acid-fast bacilli (AFB) were initially detected in wrist and ankle aspirates with Ziehl-Neelsen stain (Table 1). On the basis of microscopic findings, antituberculous therapy consisting of isoniazid and rifampin was instituted. Synovial fluid cultures subsequently yielded AFB in Dubos broth (Table 1).

Over the following months, the patient experienced multiple episodes of acute tenosynovitis and recurrent *P. carinii* pneumonia. By September, soft tissue swelling around the left wrist and right ankle was visible on X ray; drainage of serosanguinous material from the wrist was also apparent. Fluid aspirated from areas of the right ankle tendon sheath contained 46,100 leukocytes per mm^3, with 90% polymorphonuclear leukocytes and 10% lymphocytes or monocytes. Multiple synovial fluids collected during this time contained AFB detected by smear and culture. Despite treatment at different times with various combinations of isoniazid, rifampin, ethionamide, ethambutol, and pyrazinamide, the infection persisted until the death of the patient.

Minimal growth of a mycobacterium was obtained from aspirates of the wrist and both ankles on Lowenstein-Jensen medium (BBL Microbiology Systems, Cockeysville, Md.), on 7H10 and 7H11 media (Difco Laboratories, Detroit, Mich.), and in Dubos broth (Difco) within 1 to 3 weeks of incubation at 24 and 37°C. Concentrated specimens required up to 12 weeks of incubation before growth was detected (Table 1). Urine, stool, bone marrow aspirate, sputa, pleural fluid, bronchial washings, and transtracheal biopsy material yielded no growth of mycobacteria after 8 weeks of incubation at 37°C. No mycobacteria were detected in blood (10 ml) cultured using the combined techniques (9) of lysis-centrifugation (Isolator; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) and BACTEC (7H12 medium; Johnston Laboratories, Towson, Md.).

Subcultures of initial growth from clinical material in Dubos broth were nonviable on Lowenstein-Jensen, 7H10, and 7H11 media after 12 weeks of incubation at 24, 32, 37, and 41°C. Subcultures yielded good growth on 7H10 supplemented with lysed erythrocytes or hemin after several weeks of incubation at 32 and 37°C (Table 1).

7H12 medium used in the radiometric detection of mycobacteria supports the growth of *M. haemophilum* if supplemented with iron complexes, such as blood (2). Although *M. haemophilum* was never recovered from the blood of this patient, subsequent testing of a subculture of our isolate (Table 2) demonstrated that this mycobacterium could be detected in seeded blood at concentrations of 5 to 5,000 CFU/ml. Growth was detected radiometrically within 6 to 21 days at 32 and 37°C and was confirmed by AFB film and subculture findings.

A right ankle isolate (ECMC strain) was saved for comparison with *M. haemophilum* ATCC 33206 (15) and type strain ATCC 29548 (20). The ECMC strain was acid fast by the Ziehl-Neelsen method and did not stain by the Gram

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TABLE 1. Recovery of mycobacteria from clinical material[a]

Date	Specimen	Smear result	Initial culture result					Subculture result			
			L-J[b]	Dubos broth	7H10	7H11	BACTEC 7H12	L-J	7H10	7H10 plus: Lysed erthrocytes	7H10 plus: Hemin
3 April 1984	Synovial fluid										
	Right knee	Neg	NG	NG							
	Left ankle	3–9 AFB/smear	NG	+				NG	NG		
	Right ankle	Neg	NG	+				NG	NG		
	Left wrist	<1 AFB/field	NG	+				NG	NG		
21 September 1984	Right ankle	<1 AFB/field	+	+	+	+		NG	NG	+ +	+ +
31 October 1984	Left wrist	>100 AFB/field	NG	+	+	+		NG	NG	+ +	+ +
3 April 1984–6 February 1985	Other										
	Respiratory,[c] urine, stool, bone marrow	Neg	NG	NG	NG	NG					
	Blood	Neg	NG	NG	NG	NG	NG				

[a] Neg, Negative for AFB; NG, no growth; +, minimal growth; + +, good growth.
[b] L-J, Lowenstein-Jensen medium.
[c] Respiratory specimens were sputa, pleural fluid, bronchial washings, and transtracheal biopsy material.

method. Microscopic (Ziehl-Neelsen stain) and colonial morphology of the ECMC strain were similar to those of ATCC 33206 and 29548. When stained by the Ziehl-Neelsen method, the ECMC strain and ATCC 33206 consisted of single, short rods that frequently formed loose cords. Type strain ATCC 29548 exhibited moderate cording.

TABLE 2. Growth characteristics of *M. haemophilum* strains[a]

Growth condition(s)	Growth[b]		
	ECMC strain	ATCC 33206	ATCC 29548
Medium (32°C, ambient air)			
7H10 + 39 μg of hemin per ml	4+	4+	4+
7H10 + hemin (no OADC[c])	−	−	2+
7H10 + 3% lysed erythrocytes	4+	4+	4+
7H10 + 2% ferric ammonium citrate	2+	2+	3+
7H10	1+	1+	1+
7H10 subcultured to 7H10	−	−	−
Chocolate agar	1+	1+	2+
5% Sheep blood	1+	1+	1+
Temperature and CO_2			
7H10 + hemin			
24°C without CO_2	2+	2+	2+
32°C with/without 7% CO_2	3+/4+	3+/4+	4+/4+
37°C with/without 7% CO_2	4+/3+	3+/3+	4+/3+
41°C without CO_2	−	−	−
7H10 (ambient air)			
24, 32, and 37°C	1+	1+	1+
Chocolate agar			
32°C with/without 7% CO_2	4+/1+	3+/1+	4+/2+
37°C with/without 7% CO_2	2−/−	1+/−	2+/−
5% Sheep blood			
32°C with/without 7% CO_2	2+/1+	2+/1+	4+/1+
37°C with/without 7% CO_2	1+/−	1+/−	3+/−
7H12 + blood (32 and 37°C; 10% CO_2)	Growth[d]	NT	NT

[a] 7H12 medium was inoculated with 0.1 ml of 5 to 5,000 CFU of a *M. haemophilum* suspension per ml in lysed blood. All other cultures were inoculated with a 10^{-2} dilution of a McFarland 1 saline suspension of *M. haemophilum*.
[b] −, No growth; 1+, minimal growth to 4+, luxuriant growth; NT, not tested.
[c] OADC, Oleate-albumin-dextrose-catalase.
[d] Radiometric detection of growth in 7H12 medium.

On 7H10 plus hemin in ambient air, the ECMC isolate and the other *M. haemophilum* strains produced colonies that were nonchromogenic, rough, and white. After 3 weeks, colonies of the ECMC strain and ATCC 33206 were 0.5 to 1.0 mm in diameter and flat with a raised central area. Type strain colonies were 0.5 to 2 mm in diameter with more vertical growth (many dome-shaped colonies, as well as flat colonies). In the presence of increased CO_2, each strain produced colonies with little or no vertical growth that were larger in diameter than colonies produced by strains grown in ambient air; this was most apparent on chocolate agar (BBL).

The ECMC isolate and the other *M. haemophilum* strains demonstrated optimal growth at 32°C on media containing sufficient amounts of hemin (39 μg/ml) or hemoglobin (3% lysed human erythrocytes) (Table 2). Minimal growth on unsupplemented media was attributed to residual hemin, because subculture to fresh, unsupplemented 7H10 medium resulted in no growth (Table 2).

The effect of incubation temperature on the growth of each strain was modified by the growth medium used (Table 2). Incubation at 37°C resulted in good (3+) growth of each strain on 7H10 medium plus hemin but only minimal or no growth on suboptimal media.

A compensatory effect of increased (7%) CO_2 (21) was apparent for each strain when incubated at a suboptimal temperature (37°C) or on suboptimal media (chocolate or 5% sheep blood agar) (Table 2). Growth was significantly greater and sometimes comparable to that achieved under optimal conditions. Chocolate agar proved to be a satisfactory substitute for 7H10 plus hemin when used under increased CO_2 at 32°C.

A comparison of the ECMC isolate with the other *M. haemophilum* strains in biochemical reactivities (22) was done (Table 3). Tests for neutral red binding (12), hydroxylamine susceptibility (10), β-glucosidase activity (3), indole production (20), and phosphatase activity (20) were performed as described elsewhere. Iron uptake was determined from examination of 4-week-old growth on 7H10 plus 2% ferric ammonium citrate. Tests which required more than

TABLE 3. Characteristics of *M. haemophilum* strains (ECMC, ATCC 33206, and ATCC 29548)

Test	Result[a]
Arylsulfatase	
3 day	−
14 day	−
Catalase	
Semiquantitative	<5 mm
pH 7, 68°C	−
β-Glucosidase	−
Hydroxylamine susceptibility (500 μg/ml)	NG
Indole production	−
Iron uptake	−
Neutral red binding	Strong[b]
Niacin synthesis	−
Nitrate reduction	−
Nicotinamidase, 4 day	+
Pyrazinamidase, 4 day	+
Phosphatase	+
5% Sodium chloride tolerance	NG
2TH[c] susceptibility	
5 μg/ml	R
10 μg/ml	R
Tellurite reduction	−
Tween 80 hydrolysis	−
Urease	−

[a] −, Negative; NG, no growth; +, positive; R, resistant.
[b] Binding was moderate with ATCC 29548.
[c] 2TH, 2-Thiophene carboxylic acid hydrazide.

a few hours of incubation used medium substrates with and without added hemin.

The ECMC isolate was similar to the other strains in its strong binding of neutral red and identical to them in all other characteristics. All results, except that of phosphatase (20), conformed to previous descriptions of *M. haemophilum*. Phosphatase activity was detected with the use of a higher (3%) concentration of phenolphthalein diphosphate substrate.

Susceptibility to various antituberculous drugs was determined by the proportion method (22) on quadrant plates of 7H10 medium supplemented with 39 μg of hemin per ml. A susceptible strain, *Mycobacterium tuberculosis* $H_{37}Ra$, was also tested on 7H10 with and without hemin to monitor any effect of iron compounds (6, 8, 15) on drug activity. The ECMC strain was similar to the other *M. haemophilum* strains in its resistance to most antituberculous drugs (Table 4). Only *p*-aminosalicylic acid and rifampin clearly demonstrated significant in vitro activity against the ECMC isolate. A culture of the ECMC strain has been deposited with the American Type Culture Collection (ATCC 43160).

The presence of AFB in wrist and ankle aspirates in this patient was initially thought to represent an infection with *Mycobacterium avium-M. intracellulare*, because disseminated infection (25) with continuous bacteremia (24) by this mycobacterial complex is frequent in patients with acquired immune deficiency syndrome. Infection with mycobacteria other than the tubercle bacilli is one of the hallmarks of an underlying cellular immunodeficiency that characterizes this syndrome (18). Repeated cultures of various repiratory and urine specimens, a bone marrow aspirate, blood, and stool proved negative for *M. avium-M. intracellulare* and other mycobacteria. Normally, these types of specimen are excellent sources for recovery of disseminated *M. avium-M. intracellulare* from such patients (13). Smear and culture findings indicated there was no dissemination of infection beyond this patient's wrist and ankles.

M. haemophilum was first described by Sompolinsky et al. (20). One of their isolates (ATCC 29548), which was recovered from skin lesions of a patient receiving immunosuppressive therapy for Hodgkin's disease, was designated as the type strain for the species *M. haemophilum*. Other cases of infection caused by this species were subsequently reported (4, 5, 15–17, 23). Agglutination and absorption studies (5, 6, 16, 21) indicated that this species is serologically distinct. Differences among isolates have been noted in ability to grow on media containing ferric ammonium citrate, hemin, or hemoglobin (6, 15–17). We found the ECMC strain, ATCC 33206, and type strain ATCC 29548 to be quite similar in their capacity to grow on various hemin (hemoglobin)-supplemented media (Table 2). Not all strains exhibited a

TABLE 4. Susceptibility to antituberculous agents

Antituberculous agent	Concn (μg/ml)	% Resistance				
		M. haemophilum			*M. tuberculosis* $H_{37}Ra$	
		ECMC strain	ATCC 33206	ATCC 29548	Hemin	No hemin
p-Aminosalicyclic acid	2	1	56	0	100	0
	10	0	0	0	44	0
Rifampin	1	0	1	0	0	0
	5	0	0	0	0	0
Ethambutol	5	79	100	97	60	<1
	10	94	100	87	70	<1
Ethionamide	5	78	100	91	56	0
	10	64	44	48	54	0
Isoniazid	0.2	88	85	68	60	6
	1	86	100	80	47	0
Streptomycin	2	100	83	78	50	3
	10	39	35	0	24	2
Pyrazinamide	50	86	100	80	7	1

temperature optimum near 32°C (17). Although *M. haemophilum* is characterized as unable to grow at 37°C (19), minimal growth of *M. haemophilum* from clinical material (4, 5, 15) and on subculture (6, 16, 17) has been observed at temperatures of 35 to 37°C. Our findings indicated a wide variation in growth of *M. haemophilum* strains at 37°C that was dependent on the growth medium used. Good growth of *M. haemophilum* at 37°C was obtained if adequate amounts of hemin were supplied in the medium (Table 2).

Most *M. haemophilum* strains are resistant to the majority of commonly used antituberculous agents. Our patient was treated with various combinations of antituberculous drugs. Later, in vitro susceptibility tests (Table 4) indicated rifampin was the only antituberculous drug prescribed that exhibited significant activity against the isolate of the patient.

The incidence of infection by this organism is rare. In the United States, several reports have described noncultivable AFB associated with skin infections in both immunosuppressed (14) and relatively healthy (7) persons. These may or may not have been infections caused by *M. haemophilum*. Only two cases of infection with this mycobacterium have been documented (4; M. A. Saubolle, P. P. McKellar, and E. S. Merritt, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1081, 1985). Both patients were renal transplant recipients receiving immunosuppressive therapy.

Although most *M. haemophilum* infections were initially diagnosed by the development of visible skin lesions, infection in our patient was almost exclusively confined to synovium-tendon sheath regions. Whether this can be attributed to the growth requirements of our isolate or to other factors is unknown. The propensity of *M. haemophilum* to produce skin lesions has been attributed to its requirement for reduced temperatures near 32°C, the temperature of human skin (17). The detection and persistence of this mycobacterium in synovial fluid was not considered unusual, because body fluids contain iron compounds. The requirement of 39 μg of hemin per ml in bacteriologic media (20, 21) compares favorably with estimated levels of heme compounds in serum, e.g., hemoglobin, which range from 5 to 50 μg/ml (11). The amount of hemoglobin present in body fluids, e.g., synovial fluid, may increase with infection and the subsequent destruction of surrounding tissues (our patient had a significant inflammatory response). Indeed, in a recent case report (16), the lesions progressed after the patient received therapeutic doses of iron and folate for anemia, which subsequently raised the hemoglobin level. Although the route of infection is unknown, a hematogenous spread from a primary site of infection to other areas has been postulated (4), because most patients demonstrated noncontiguous sites of infection. The source of these infections has not been demonstrated; the natural habitat of *M. haemophilum* is unknown.

M. haemophilum is a mycobacterium of low virulence. Animals inoculated with *M. haemophilum* strains do not develop overt infection (1, 16, 20) except when pretreated with steroids (1). Most animals survive after inoculation with large doses of the organism (20). *M. haemophilum* infections in humans are almost always associated with a deficiency in cellular immunity.

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