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Nontuberculous Mycobacteria as Unsuspected Agents of Dermatological Infections: Diagnosis Through Microbiological Parameters

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Nontuberculous mycobacteria were identified as the agents of dermatological lesions in seven patients seen at The Mount Sinai Hospital from 1969 to 1979. Three patients had water-associated cutaneous lesions, three had abscesses at the site of an injection, and one had an erosive nasal lesion. In each of these instances, the mycobacterial etiology was not suspected, and diagnosis was achieved only after careful microbiological studies. These experiences emphasize that a mycobacterial etiology should be sought in chronic cutaneous lesions occurring at traumatized sites.

As early as 1932, Pinner postulated that almost any mycobacterium might invade human tissue and elicit disease characterized by tubercle formation (11). Subsequently, isolations of mycobacteria from subcutaneous abscesses and ulcerative skin disease were reported, respectively, by Freeman in 1938 (5) and MacCallum in 1948 (9). During the last two decades several more reports of so-called atypical mycobacterial species isolated from dermatological lesions have appeared. Most frequently, these reports describe inoculation skin lesions caused especially by *Mycobacterium marinum* and *Mycobacterium ulcerans* and injection or injury abscesses caused by *Mycobacterium fortuitum* and *Mycobacterium chelonei*. Nontuberculous mycobacteria have also been isolated from cutaneous and subcutaneous lesions of patients with disseminated mycobacterial disease (1).

The recent outbreaks of "surgical" mycobacterial infections among patients who underwent cardiac surgery or mammary implantation have reawakened interest on the part of microbiologists and clinicians in these newly appreciated but long-encountered microorganisms (4, 6, 7); yet, despite such reports, the mycobacterial etiology of skin infections is still overlooked.

During the past 10 years we have isolated a variety of mycobacterial species etiologically incriminated in cutaneous and subcutaneous lesions. In most instances, however, their mycobacterial etiology was unsuspected. For the adequate management of such infections, the causative agents must be properly identified. The aim of this report is to highlight our experiences with the microbiological attributes leading to the recognition of unsuspected nontuberculous mycobacterial species as agents of dermatological infections.

MATERIALS AND METHODS

From September 1969 to September 1979, 56 specimens derived from cutaneous, subcutaneous, and mucous membrane sites were submitted to the mycobacteriology laboratory of the Department of Microbiology of The Mount Sinai Hospital. Of these, nine specimens, derived from seven patients, contained acid-fast bacteria. Of the seven patients, three had cutaneous lesions, three had injection abscesses, and one had an erosive nasal lesion.

Water samples collected from the fish tanks of two patients with abrasion-associated skin lesions were also subjected to cultural analysis.

Representative clinicopathological information and the relevant microbiological findings pertaining to the seven patients are presented in Table 1.

Mycobacteriology. Direct and concentrated smears were prepared from all specimens and stained by the Kinyoun method. Specimens free of bacterial contaminants were inoculated directly onto Lowenstein-Jensen and Middlebrook 7H10 or 7H11 agar slants, into modified Dubos liquid medium (3), and onto Trypticase soy agar containing 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.). Nonsterile specimens were decontaminated with filtered and autoclaved 2% sodium hydroxide, washed with sterile saline, and inoculated as above. Cultures were incubated in 5% CO_2 at 31 and 37°C and examined at 7-day intervals for evidence of mycobacterial growth. All isolates were tested for niacin, urease, and catalase production, nitrate and tellurite reduction, and Tween 80 hydrolysis. Tests for chromogenicity, 3-day arylsulfatase production, tolerance to 5% NaCl on Lowenstein-Jensen medium, and growth on MacConkey and glycerol cornmeal agar were performed selectively when indicated by the pigmentation and growth rate of the isolates. All of the above-delineated tests were conducted by standard methods (8).

Gross mycobacterial colonial morphology was observed additionally on 5% sheep blood agar. The microscopic aspect of colonies of rapidly growing mycobacterial species was also studied on Wolin-Bevis agar

TABLE 1. *Clinical and microbiological data for patients with nontuberculous mycobacteria isolated from dermatological infections*

Patient no.	Sex	Age (yr)	Clinical synopsis	Source of isolate	Species
1	M	16	Nine-month-old left elbow skin lesion acquired after frequent swimming in homemade pool	Skin biopsy	*M. marinum*
2	M	15	One-month-old sporotrichoid lesions of right hand acquired after manipulation of fish tank	Pus from finger	*M. marinum*
				Fish tank water	*M. marinum* and *M. avium-M. intracellulare*
3	F	16	Several-week-old finger lesion acquired after manipulation of fish tank	Skin biopsy	Acid-fast bacilli seen on smears[a]
				Fish tank water	*M. fortuitum* and *M. chelonei* subsp. *abscessus*
4	F	37	Sarcoidosis, nasal perforation	Swab from nasal perforation	*M. fortuitum*
5	F	47	Diabetic, injection abscess of the arm	Pus from abscess	*M. chelonei* subsp. *abscessus*
6	M	52	Metastatic melanoma treated with intranodular injections of BCG vaccine	Pus from wound postinjection	*M. bovis* BCG
7	F	48	Leukemia, treated with BCG vaccine	Pus from abscess	*M. bovis* BCG

[a] Failed to grow.

(14). This medium provided more conspicuous filaments in a clearer background than did cornmeal agar.

RESULTS

Acid-fast bacilli were observed in the direct smears of the skin biopsies (patients 1, 2, and 3) and in pus from the injection abscesses (patients 5, 6, and 7). In the case of *M. marinum* (patient 2), the acid-fast bacilli were somewhat swollen, with rounded ends, and stained irregularly. Smears prepared from the nasal specimen (patient 4) and from the water samples (patients 2 and 3) did not reveal mycobacteria.

Culturally, 10 mycobacterial isolates were recovered from the nine clinical specimens and the two water samples; 3 were *M. marinum* (patients 1 and 2 and fish tank water of patient 2), 2 were *M. fortuitum* (fish tank water of patient 3 and nasal perforation of patient 4), 2 were *M. chelonei* subsp. *abscessus* (fish tank water of patient 3 and abscess of patient 5), 1 was *Mycobacterium avium-Mycobacterium intracellulare* (fish tank water of patient 2), and 2 were *Mycobacterium bovis* BCG (patients 6 and 7).

M. marinum isolates developed on slants in 7 to 13 days exclusively at 31°C. However, in the liquid medium, growth also occurred at 37°C. On slanted media, smooth nonpigmented colonies were observed which, upon brief exposure to light and subsequent incubation, became yellow. With prolonged illumination, pigmentation intensified to orange.

Smears prepared from colonies displayed long, thick, cross-banded, intensely stained acid-fast bacilli. Biochemically, Tween 80 was hydrolyzed within 24 h, but nitrate was not reduced. Growth was inhibited on 5% NaCl-containing media. Semiquantitative catalase was less than 45 mm at room temperature, and catalase production at 68°C was present.

M. fortuitum developed abundantly on all media, including 5% sheep blood agar. Growth occurred at 31 and 37°C in 5 to 7 days as smooth and rough, nonchromogenic colonies which became green on Lowenstein-Jensen medium after 3 weeks. In the modified Dubos liquid medium, a thick pellicle formed, portions of which settled to the bottom of the flask. Smears of early growth from the solid media showed filamentous, partially acid-fast bacilli. About 50% of these organisms stained intensely, whereas the remainder displayed the same filamentous morphology but lacked acid fastness. As the culture aged, however, more cells became uniformly acid fast. When colonies were observed by direct microscopy (10×) on Wolin-Bevis agar or were emulsified in saline, filamentous extensions were readily visible. These isolates were niacin negative and presented positive tests for nitrate and tellurite reduction, semiquantitative and 68°C catalase activity, Tween 80 hydrolysis, tolerance to 5% NaCl, and 3-day arylsulfatase and urease production. The isolates grew on MacConkey agar with decolorization of the medium.

M. chelonei subsp. *abscessus* shared all of the characteristics described for *M. fortuitum* but differed by the absence of colonial filamentous extensions and nitrate reduction.

M. avium-M. intracellulare developed primarily in 3 weeks at 31°C and in subcultures at both 31 and 37°C as smooth, nonchromogenic colonies, which appeared domed on Lowenstein-Jensen and thin and transparent on 7H11 media. Smears from these colonies showed short, uniformly pale-staining acid-fast bacilli. Tests for niacin and urease production, Tween 80 hydrolysis, arylsulfatase production, tolerance to 5% NaCl, and nitrate reduction were all negative, whereas the test for tellurite reduction was positive within 3 days. Semiquantitative catalase

was less than 45 mm at room temperature, and catalase production at 68°C was present.

The *M. bovis* BCG isolates were characterized in a previous report (2). Diagnostic features included the following: growth on Lowenstein-Jensen and 7H11 media and in the modified Dubos liquid medium at 37°C; inhibition of growth in the presence of thiophene-2-carboxylic acid hydrazide; negative tests for niacin, catalase production at 68°C, nitrate reduction, and Tween 80 hydrolysis; and a positive urease test.

DISCUSSION

Infections of the skin and subcutaneous tissues with nontuberculous mycobacteria are rare but constantly occurring diseases of which the etiology is seldom suspected. In most instances, requests for cultures are not directed by physicians for the recovery of an acid-fast microorganism from such specimens. In the present study, a nontuberculous mycobacterial species was recovered from three of the nine specimens only after a Kinyoun-stained smear was performed intuitively with specimens of patients 2 and 3 and with the abscess culture isolate of patient 5, which was initially identified as an antibiotic-resistant "diphtheroid-like" bacillus. Moreover, despite discouraging results reported for direct acid-fast smears from skin biopsies (16), with patient 1, the presumptive clinical diagnosis of "swimming pool granuloma" was strengthened through evaluation of a Kinyoun-stained smear and confirmed by the isolation of *M. marinum*.

Patient 2 was suspected of having sporotrichosis as this 15 year old was thought to have sustained injury of a finger while on a hiking trip. When routine cultures failed to reveal a bacterial or mycotic agent to account for the progression of his lesions and antifungal treatment failed to resolve the lesions, acid-fast smears, prepared from surgically obtained aspirated pus, were diagnostic. Subsequent questioning of the patient revealed that the abrasion of his finger occurred while cleaning his fish tank. Cultures of the aspirated pus and of fish tank water both grew *M. marinum*.

The abundant growth of *M. marinum* from the fish tank water of this patient strongly supports the acquisition of the infecting species from this source. *M. marinum*, as is amply documented, is a mycobacterium adapted to aquatic habitats and has a wide host range to include insects, fish, and humans (12, 15). Abrasions acquired in swimming pools, from fish tanks, and while skin diving give rise to granulomatous skin disease. Sporotrichoid forms may occur (13).

The skin biopsy of patient 3 revealed thick acid-fast bacilli. This patient's lesions had undergone three surgical procedures ("unroofings") before the punch biopsy which established an acid-fast etiology. After being questioned intensively, this 16-year-old patient also admitted having contact with fish. Neither *M. marinum* nor another mycobacterial species, however, was recovered from culture of the punch biopsy specimen, because the patient had repeatedly bathed and dressed her hand with an iodine solution before biopsy. The recovery, however, of *M. fortuitum* and *M. chelonei* subsp. *abscessus* from her fish tank water suggests that either of these organisms may have been etiologically responsible for her lesion, especially as cutaneous involvement with organisms of the *M. fortuitum* complex has been described previously (15).

M. fortuitum was isolated from a chronic erosive lesion of the nasal mucosa of patient 4, who had sarcoidosis. The nasal perforation in this patient was thought to be caused either by *Mycobacterium tuberculosis* or by *Mycobacterium leprae*. Although the recovery of *M. fortuitum* from this patient's lesion does not per se establish its etiology, the isolation, however, of nontuberculous mycobacteria from patients with sarcoidosis (10) and from those immunosuppressed by disease, treatment, or both is well known (15).

The mycobacterial etiology of the arm abscess of patient 5, a diabetic, resulted from routine culture of aspirated pus. Inoculation of the pus to blood agar revealed, after 4 days, smooth, white colonies which on Gram-stained preparations showed gram-variable, diphtheroid-like filamentous cells. As this was the only microbial species recovered, the isolate was submitted for antibiotic susceptibility studies, which disclosed inhibition only by gentamicin and kanamycin. As such an antibiogram was highly unusual for a diphtheroid, a mycobacterium was suspected, and Kinyoun-stained smears revealed long, filamentous acid-fast cells. Once its mycobacterial nature was recognized, the isolate was subsequently identified as *M. chelonei* subsp. *abscessus*. After the true etiology of this abscess was established, the patient was recalled, and direct Kinyoun-stained smears of abscess pus revealed acid-fast bacilli, which were again isolated and identified as *M. chelonei* subsp. *abscessus*.

M. chelonei subsp. *abscessus*, a subdivision of the *M. fortuitum* complex, is widely distributed in nature and may be found as a commensal of the human skin (12). As demonstrated by this case, this species may produce subcutaneous infections when inoculated through the skin by needle puncture or traumatically by splinters and nails (12). Individual and clustered cases of

patients with infection of artificial heart valves (7), infection of mammary implants (4), and postsurgical infections (6) have been reported recently.

A mycobacterial etiology should always be suspected for lesions developing at traumatized sites which follow a chronic course despite antibiotic therapy and drainage. In each of the above instances, the patients had a clinical entity whose mycobacterial etiology was largely unsuspected and diagnosed only after careful microbiological studies. Continued awareness on the part of microbiologists and especially clinicians of the mycobacterial etiology of skin infections should lead to careful management of patients and reduce unnecessary surgical procedures as the therapeutic approach.

LITERATURE CITED

1. **Chapman, J. S.** 1977. The atypical mycobacteria and human mycobacteriosis, p. 34, 120, and 151-153. Plenum Publishing Corp., New York.
2. **Damsker, B., E. J. Bottone, and S. S. Schneierson.** 1974. Human infections with *Mycobacterium bovis*. Am. Rev. Respir. Dis. **110**:446-449.
3. **Dubos, R. J., and B. D. Davis.** 1946. Factors affecting the growth of tubercle bacilli in liquid media. J. Exp. Med. **83**:409-423.
4. **Foster, M. T., W. E. Sanders, J. L. Baker, C. B. Bass, M. M. Shuster, H. J. Wald, R. M. Yeller, T. R. Kitchens, M. P. Hines, W. E. Barnes, G. W. Johnson, R. J. Wallace, R. W. Wood, J. R. Toranto, T. S. Wilkinson, and C. R. Webb.** 1978. Mycobacterial infections associated with augmentation mammoplasty. Morbid. Mortal. Weekly Rep. **27**:513.
5. **Freeman, G.** 1938. Minimal mycobacterial infections. Am. Rev. Tuberc. **38**:612-623.
6. **Hines, M. P., R. Waggoner, and T. M. Vernon.** 1976. Atypical mycobacteria wound infections. Morbid. Mortal. Weekly Rep. **25**:238-243.
7. **Laskowski, L. F., J. J. Marr, J. A. Curtin, C. Levy, M. E. Levy, R. L. Cohen, R. Field, L. S. Simon, D. Freeman, J. Chin, S. W. Schwartzman, J. D. Smith, R. C. Bartlett, G. O. Carrington, K. Doyle, and J. C. McLaughlin.** 1978. Follow-up on mycobacterial contamination of porcine heart valve prostheses. Morbid. Mortal. Weekly Rep. **27**:92-98.
8. **Lennette, E. H., E. H. Spaulding, and J. P. Truant (ed.).** 1974. Manual of clinical microbiology, 2nd ed., p. 163-166. American Society for Microbiology, Washington, D.C.
9. **MacCallum, P., J. C. Tolhurst, C. Buckle, and H. A. Sissons.** 1948. A new mycobacterial infection in man. J. Pathol. Bacteriol. **60**:93-122.
10. **Mankiewicz, E.** 1963. The relationship of sarcoidosis to anonymous bacteria, p. 68-73. *In* S. Löfgren (ed.), Third International Conference on Sarcoidosis, Stockholm. Norstedts Svenska Bökforlaget, Stockholm.
11. **Pinner, M.** 1932. Atypical acid-fast organisms. Proc. Soc. Exp. Biol. Med. **30**:214-216.
12. **Runyon, E. H.** 1971. Whence mycobacteria and mycobacterioses? Ann. Intern. Med. **75**:467-468.
13. **Van Dyke, J. J., and K. B. Lake.** 1975. Chemotherapy 1381.
14. **Wolin, H. L., M. L. Bevis, and N. Laurora.** 1962. An improved synthetic medium for the rapid production of chlamydospores by *Candida albicans*. Sabouraudia **2**: 96-99.
15. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119**:107-159.
16. **Zeligman, I.** 1972. *Mycobacterium marinum* granuloma. Arch. Dermatol. **106**:26-31.