Epidem. Inf. (1989), **103**, 293–300
Printed in Great Britain

Immunosuppression and mycobacteria other than *Mycobacterium tuberculosis*: results from patients with and without HIV infection

M. PETERS[1], D. SCHÜRMANN[1], A. C. MAYR[2], R. HETZER[3], H. D. POHLE[1] AND B. RUF[1]

[1] *II. Department of Internal Medicine and* [2] *Department of Oncology, Rudolf Virchow University Hospital (Wedding),* [3] *Freie Universität Berlin; Deutsches Herzzentrum Berlin; FRG*

(*Accepted 24 April 1989*)

SUMMARY

Infections caused by mycobacteria other than *Mycobacterium tuberculosis* (MOTT) have often been described as common in AIDS patients. To evaluate whether infections with MOTT are specific for HIV related immunosuppression or are also frequent in patients with immunosuppression of different aetiology, data on the frequency of isolation from immunosuppressed patients with HIV infection are important. Blood, stool and urine specimens from 134 patients with non-HIV related immunosuppression, and from 55 immunocompetent subjects were examined for mycobacteria. MOTT have been isolated from one immunocompetent person but from none of the immunosuppressed patients. Since in AIDS patients an initial colonization of the gastrointestinal tract (GI-tract) with MOTT is common, GI-tract biopsy specimens from an additional 80 patients were examined microscopically and histologically for mycobacteria. Mycobacteria were not isolated from these specimens.

In the same period of time 72 AIDS patients have been examined: 7 (10%) had infections with *M. tuberculosis* whereas MOTT have been isolated from 16 (22%) of these patients. Mycobacteria have been found only rarely in immunocompetent patients and have not been isolated from patients with non-HIV related immunosuppression. The isolation of MOTT is highly correlated with an HIV-related immunosuppression.

INTRODUCTION

Until the emergence of the acquired immunodeficiency syndrome (AIDS) in 1981/2 (1, 2) mycobacteria other than *Mycobacterium tuberculosis* (MOTT) were rare pathogenic organisms in visceral infection but occasionally causing lymphadenitis or pulmonary infections (3, 4). In immunocompetent individuals more than the half of these atypical mycobacterioses are caused by *M. kansasii* (5), while the rest of these infections commonly is due to the *Mycobacterium avium—Mycobacterium intracellulare* complex (MAI) (6). In normal hosts disseminated mycobacterial infections are rare (7) and generally associated with malignancy or corticosteroid therapy (8–10).

Mycobacteria are now the most common bacterial agents in patients with AIDS (11, 12) (Table 1). Of all mycobacterial isolates from AIDS patients up to 90% are

Table 1. *Infections with* M. tuberculosis *and disseminated MAI infections in AIDS patients*

Year	Author	AIDS patients	MAI (disseminated)		*M. tuberculosis*	
			n	%	*n*	%
1983	Macher (3)	30	8	26·6	—	—
1984	Murray (33)	441	74	17	19	4
1984	Pitchenik (23)	45*	1	2	27	60
1984	Pitchenik (23)	37	2	2·7	1	2·7
1985	Gill (34)	46	13	28	—	—
1985	Winter (35)	30	10	33·3	—	—
1986	Collins (16)	421	122	28·9	46*	11*
1986	Hawkins (25)	366	67	18·7	—	—
1986	Kiehn (15)	400	90	22·5	11	2·5
1986	Pitchenik (24)	65*	—	—	28	43
1986	Pitchenik (24)	65	—	—	29	44·9
1986	Young (12)	300	73	25	2	1
1988	Ruf (35)	183	34	18·6	19	10·4
1985	Kiehn (29)	55†	30	55		
1986	Hawkins (25)	79†	42	53		

* Only Haitian patients examined.
† Autopsy cases.

MAI and about 10% are *M. tuberculosis* (13); other mycobacteria are found rarely (14, 15).

Many studies have been published on the incidence of MOTT infections in AIDS patients (Table 1) but not much is known about the importance of MOTT in immunosuppressed patients without HIV related immunosuppression. In the present study we investigate the frequency of mycobacterioses in immunosuppressed patients without HIV infection as well as in immunocompetent patients in order to correlate our data with the frequency of mycobacterial isolation in AIDS patients during the same period of time.

MATERIALS AND METHODS

The study was performed between 1 September 1987 and 31 August 1988 at the Rudolf Virchow University Hospital (Wedding), Berlin, FRG.

Patients

The patients enrolled in the study were separated in four groups: those with HIV infection, those with non-HIV related immunodeficiency, those without immunological disorders, and those who had endoscopical biopsies.

HIV infection was established according to criteria of the Centers for Disease Control (CDC) (16). The group of patients with non-HIV related immunosuppression included heart transplant recipients, patients with neoplasia with or without cytotoxic treatment, and patient with other immunosuppressive disorders. Immunocompetent individuals were selected randomly from various wards of the hospital (exclusion criteria were: infectious diseases, pathological lymphocyte counts, neoplasia, immunomodulating therapy). The group of

https://doi.org/10.1017/S095026880003065X Published online by Cambridge University Press

patients with gastrointestinal tract (GI-tract) specimens included immunocompetent patients who had gastroscopy or colonoscopy. GI-tract specimens were obtained from patients who underwent diagnostic endoscopies.

Sample collection and microbiological methods

Blood, normally sterile body fluids and tissues, respiratory secretions, urine, and stool were cultured for mycobacteria as previously described (17). GI-tract specimens were also examined histologically. Processed specimens were inoculated onto Middlebrook 7H10 agar, Löwenstein–Jensen agar, and onto Gottsacker agar. Agars were prepared in our laboratory according to methods described by Vestal (17). For stool, Löwenstein–Jensen media without and with antibiotic supplement (polymyxin B 20 mg/l, nalidixic acid 40 mg/l, trimethoprim 20 mg/l, azlocillin 40 mg/l, and amphotericin B 20 mg/l) were used simultaneously. Specimens were stained for acid-fast bacilli according to Ziehl–Neelsen technique. Cultures were incubated at 35 °C for 10 weeks. Media were inspected for colony growth twice weekly. Standard techniques were performed to speciate the mycobacterial isolates (17). MAI isolates were serotyped at the Forschungsinstitut Borstel, Borstel, FRG, with seroagglutination methods originally described by Schaefer (18).

From every patient full blood count and lymphocyte subsets were examined. From patients with heart transplantation or neoplasia the dosage of immunosuppressive drugs were recorded to reveal possible correlations between mycobacterioses and the stage of immunosuppression. Bronchial washings and sputum samples have not been obtained routinely, the first for ethical reasons, the latter because more than half of sputum specimens from healthy persons are only saliva (19).

RESULTS

Patients with HIV infection

MOTT were found in 16/72 (22 %) AIDS patients. Fourteen of 16 (87·5 %) were identified as MAI, 1/16 (6·3 %) as *M. simiae*, and 1/16 (6·3 %) as *M. xenopi*. Twelve of the 14 MAI isolates (85·7 %) were identified as serovar 8/21, and one each (7·1 %) as serovar 4 and serovar 8, respectively. Dissemination of mycobacteria (MAI, $n = 12$; *M. xenopi*, $n = 1$) was seen in 13 patients. Dissemination was documented by a positive blood culture or positive cultures obtained from at least two different extrapulmonary tissue specimens. Colonization of mycobacteria (MAI, $n = 2$; *M. simiae*, $n = 1$) was seen in three patients and was documented by a positive culture from respiratory secretions, urine or stool specimens and negative cultures and histologic findings from autopsy specimens. At autopsy MOTT was found in 7/14 (50 %) cases, in 3/7 cases being the first documentation of MOTT infection.

M. tuberculosis was found in 7/72 (10 %) of the AIDS patients. Extrapulmonary manifestations (lymph node(s), spleen, liver, bone marrow) was seen in 4/7 (57 %) patients with tuberculosis.

Patients with non-HIV related immunosuppression

One hundred and thirty-four patients were included in this group. Seventy

Table 2. *Peripheral blood lymphocyte counts in immunosuppressed patients*

	Patients (S.D.)			Lymphocytes/μl (S.D.)		
	n	M/F*	age†	$CD4^+$	$CD8^+$	Ratio‡
Group						
Heart transplantation	70	58/12	46 (11)	250 (194)	397 (493)	1·0 (0·8)
Neoplasia	49	17/32	54 (13)	491 (354)	266 (184)	2·2 (1·5)
Other immunosuppression	15	6/9	45 (20)	739 (438)	756 (851)	1·7 (1·7)
HIV infection	72	66/8	38 (14)	178 (118)	698 (465)	0·6 (0·5)

* Male/female ratio.
† Mean age.
‡ Ratio of $CD4^+/CD8^+$ cells.

Table 3. *Peripheral blood lymphocyte counts of patients with gastrointestinal examinations*

	Patients (S.D.)			Lymphocytes/μl (S.D.)		
	n	M/F*	age†	$CD4^+$	$CD8^+$	ratio‡
Group						
Gastroscopy	40	15/25	30 (12)	1314 (561)	609 (279)	2·4 (1·0)
Colonoscopy	40	23/17	48 (13)	1378 (565)	726 (418)	2·3 (1·3)

* Male/female ratio.
† Mean age.
‡ Ratio of $CD4^+/CD8^+$ cells.

patients had orthotopic heart transplantation between January 1984 and February 1988. Every patient with heart transplantation received cyclosporine (serum levels of 200–750 ng/ml); 69/70 patients azathioprin (50–400 mg/d orally); and 60/70 patients prednisolone (5–12·5 mg/d orally). Forty-nine patients had neoplastic malignancies (breast carcinoma $n = 10$; ovarian carcinoma $n = 10$; haemoblastosis $n = 11$; unknown primary tumour $n = 8$; other neoplasia $n = 10$). Fifteen patients had immunosuppression of different aetiology (patients with cirrhosis of the liver $n = 3$, with immunoglobulin deficiency syndrome $n = 1$, with aplastic anaemia $n = 1$, and patients with long-lasting steroid therapy because of sarcoidosis $n = 4$, bronchial asthma $n = 1$, rheumatoid arthritis $n = 1$, and systemic lupus erythematosus $n = 1$).

Mycobacteria have not been isolated from any of the patients with non-HIV related immunodeficiency. Age, male/female ratio and the results of routine peripheral blood lymphocyte (PBL) counts of the patients are shown in Table 2.

Patients who had gastrointestinal examination

Gastroscopy and colonoscopy were each performed in 40 patients. Mycobacteria have not been cultured or seen in histological examination in any of the specimens. Age, male/female ratio and routine PBL counts of these patients are demonstrated in Table 3.

https://doi.org/10.1017/S095026880003065X Published online by Cambridge University Press

Patients without immunosuppression

MOTT have been isolated from one stool specimen. The patient had 3800/μl white blood cells, 720/μl $CD4^+$ cells, 513/μl $CD8^+$ cells, and a $CD4^+/CD8^+$ ratio of 1·2. Control cultures of this patient have not been obtained, due to death of the patient prior to the growth of mycobacteria. Autopsy was refused.

DISCUSSION

Visceral infections with MOTT were infrequent before the AIDS epidemic and are still rare in persons without immunodeficiency. In immunocompetent individuals mycobacterial infections are generally caused by *M. tuberculosis*, but an increasing part of these infections is due to MOTT (5–10%) (13). In Germany between 1969 and 1977 1·4% of all mycobacterial infections were caused by MOTT; from 1978 through 1985 as much as 5·4% have been caused by MOTT (20).

By contrast, up to 45% of HIV infected patients become infected with MOTT; 75% of the isolated mycobacteria are classified as MAI (13). The reasons for the predominance of MAI have not yet been elucidated: one would expect that other MOTT species (*M. kansasii*, *M. gordonae*) would be found more frequently in AIDS patients, since these species are just as widely distributed in the environment.

The incidence of *M. tuberculosis* infections in AIDS patients seemed to vary according to the prevalence of tuberculosis in different geographical areas. In Haitians living in Florida the prevalence of positive tuberculin skin tests ranged from 78·5–91·2% (21) and is much higher than that found in US-born persons (3·8%) (22). The incidence of tuberculosis is 650 cases per 100000 Haitians compared with 11 cases per 100000 US-born persons (23). These figures indicated, that the higher rate of infection with *M. tuberculosis* in Haitian AIDS patients (Table 1) appeared to be influenced by the high incidence of tuberculosis among the Haitian population. However, recent investigations have shown that the proportion of tuberculosis in Haitian and non-Haitian AIDS patients is equal (Table 1) (24).

In contrast to infections with *M. tuberculosis* in AIDS patients, which commonly are characterized by clinical symptomatology, infections, with MOTT often remain asymptomatic: Hawkins described a considerable discrepancy between the diagnosis of a disseminated infection *in vivo* by blood cultures and post mortem by autopsy (25). *In vivo* 18·3% (67 of 366) of the AIDS patients had disseminated infections; post mortem the diagnosis has been made in 53% (42 of 79) of the AIDS patients (25). These figures suggest a high frequency of mycobacterial colonization in AIDS patients who in spite of this do not develop clinical illness. We have augmented these figures, investigating the rate of colonization in immunocompetent individuals as well as in immunosuppressed patients without HIV infection, who were expected to have immunological conditions comparable to those found in AIDS patients.

The patients with heart transplantation ($n = 70$) fulfilled well this assumption. Cyclosporine, due to inhibition of interleukin-2 transcription leads to a suppression of cell mediated immunity (CMI) and caused the lowest mean value

of $CD4^+$ cells/μl (mean: 250 $CD4^+/\mu$l) in this group of patients (Table 2). Surprisingly mycobacteria have been isolated from none of the heart transplant recipients, even if $CD4^+$ cell counts were in the range of those found in patients with HIV infection (Table 2). Therefore, an impaired CMI cannot readily explain the high frequency of isolation of MAI from AIDS patients, since both AIDS patients and non-HIV related immunosuppressed patients had depletion of their CMI but differed concerning the incidence of mycobacterioses.

Infections with MOTT in AIDS patients frequently start with an initial colonization of the GI-tract (7, 26–28), which subsequently leads to a dissemination of the bacteria. The patients who had GI-tract biopsies did not have any immunological disorders. No mycobacteria have been cultured in these patients.

The patients without immunosuppressive conditions served as a control group of normal subjects. One 76-year-old female patient had a positive stool culture, which could be explained as a gastrointestinal colonization rather than an infection, because of the lack of symptoms of the patient.

The high frequency of isolation of MOTT in our AIDS patients confirms the importance of these pathogens in AIDS, which has been described previously (11, 29). Our findings and those of others (10, 29) indicate a predominance of certain serotypes in these patients. Kiehn reported in 1985 that 77% of the MAI strains isolated from AIDS patients with disseminated mycobacterioses were identified as serovar 4. By contrast, 90% of the MAI isolates of our AIDS patients were serovar 8/21. While some authors believe these findings to be attributable to a different pathogenicity of the various serovars (30), in our opinion it is more likely that different serovars have geographical predominances, which may reflect the presence of localized outbreaks. If not, one should expect a higher incidence of serovar 4 in Germany, which has also been isolated, but with a much lower frequency during the past decade (31; Schröder, 1988, personal communication).

AIDS patients almost always have infections with other opportunistic pathogens in addition to MAI, especially in later stages of immunosuppression (32), which renders it difficult to know exactly which aspects of the symptomatology are manifestations of MAI. Nevertheless, the lack of isolation of mycobacteria from patients with non-HIV related immunodeficiency and from GI-tract biopsies of immunocompetent persons underlines the correlation of mycobacterioses to infections with HIV as well as a close relation between GI-tract colonization with MOTT and HIV infection. Tuberculosis in AIDS patients mostly appears to be a reactivation of an infection acquired in childhood. The lack of isolation of MOTT in patients with non-HIV related immunosuppression highlights the likelihood of actually acquired infections with MOTT in AIDS patients.

The MAI infection is established by the CDC as a criterion for the diagnosis of AIDS (16). Since mycobacterial infections do not seem to be detected as frequently in patients with other immunodeficient conditions, as shown in this study, it is reasonable to maintain MAI infection as a diagnostic or staging criterion for AIDS patients.

ACKNOWLEDGEMENTS

We wish to thank the members of the II. Department of Internal Medicine and of the Department of Oncology, Rudolf Virchow University Hospital (Wedding), and the members of the Deutsches Herzzentrum Berlin, who helped us collecting the samples. We thank S. Schwenke, B. Spisla and W. Brehmer for their excellent assistance processing the samples. We are indebted to H. Mauch, Krankenhaus Zehlendorf–Heckeshorn and K.-H. Schröder, Forschungsinstitut Borstel for helping us speciating and serotyping the mycobacterial isolates.

REFERENCES

1. Gottlieb MS, Schrof R, Schanker HM, et al. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. N Engl J Med 1981; **305**: 1425–31.
2. Masur H, Michelis MA, Greene JB, et al. An outbreak of community acquired *Pneumocystis carinii* pneumonia. N Engl J Med 1981; **305**: 1431–8.
3. Macher EC, Kovacs JA, Gill V, et al. Bacteremia due to *Mycobacterium avium-intracellulare* in the acquired immunodeficiency syndrome. Ann Intern Med 1983; **99**: 782–5.
4. Wolinski E. Nontuberculous mycobacteria and associated diseases. Am Rev Respir Dis 1979; **107**: 119–58.
5. Ahn CH, Lowell JR, Onstad GD, Shuford EH, Hurst GA. A demographic study of disease due to *Mycobacterium kansasii* or *M. intracellulare–avium* in Texas. Chest 1979; **75**: 120–5.
6. Young LS. *Mycobacterium avium* complex infection. J Infect Dis 1988; **157**: 863–7.
7. Horsburgh CR, Mason UG, Farhi DC, Iseman MD. Disseminated infections with *Mycobacterium avium-intracellulare*. Medicine 1985; **64**: 36–48.
8. Ortbals DW, Marr J. A comparative study of tuberculous and other mycobacterial infections and their associations with malignancy. Am Rev Respir Dis 1978; **117**: 39–45.
9. Feld R, Bodey GP, Gröschel D. Mycobacteriosis in patients with malignant disease. Arch Intern Med 1976; **136**: 67–70.
10. Horsburgh CR, Cohn DL, Robert RB, et al. *Mycobacterium avium–M. intracellulare* isolates from patients with or without acquired immunodeficiency syndrome. Antimicrob Agents Chemother 1986; **30**: 955–7.
11. Armstrong D, Gold JWM, Dryanski J, et al. Treatment of infections in patients with the acquired immunodeficiency syndrome. Ann Int Med 1985; **103**: 738–43.
12. Young LS, Inderlied CB, Berlin OG, Gottlieb MS. Mycobacterial infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. Rev Infect Dis 1986; **9**: 1024–33.
13. Collins FM. *Mycobacterium avium* complex infections and development of the acquired immunodeficiency syndrome: Casual opportunist or causal cofactor? Int J Lepr 1986: **54**: 458–74.
14. Good RC. Opportunistic mycobacteria infections. Adv Exp Med Biol 1986; **202**: 73–82.
15. Kiehn TE, Cammarata R. Laboratory diagnosis of mycobacterial infections in patients with acquired immunodeficiency syndrome. J Clin Microbiol 1986; **24**: 708–11.
16. Centers for Disease Control. Revision of the CDC surveillance. Case definition for acquired immunodeficiency syndrome. MMWR 1987; **36**: N 1 (Suppl).
17. Vestal AL. Procedures for the isolation of mycobacteria. Atlanta, Georgia: Center for Disease Control, Department of Health, Education and Welfare. 1975 DHEW publication no. 75–8230.
18. Schaefer WB. Serologic identification and classification of the atypical mycobacteria by their agglutination. Am Rev Respir Dis 1965; **92**: (Suppl.): 85–93.
19. Edwards LB, Palmer CE. Isolation of atypical mycobacteria from healthy persons. Am Rev Respir Dis 1959; **80**: 747–9.
20. Radenbach KL. Diagnostische und therapeutische Fortschritte bei nichttuberkulösen Mykobakteriosen. Prax Klin Pneumol 1985; **39**: 43–9.
21. Pitchenik AE, Russel BW, Cleary T, Pejovic I, Cole C, Snider DE. The prevalence of tuberculosis and drug resistance among Haitians. N Engl J Med 1982; **307**: 162–5.

22. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B and histoplasmin in the United States. Am Rev Respir Dis 1969; **99**: 1–18.
23. Pitchenik AE, Cole C, Russel BW, Fischl MA, Spira TJ, Snider DE. Tuberculosis, atypical mycobacteriosis, and the acquired immunodeficiency syndrome among Haitian and non-Haitian patients in south Florida. Ann Intern Med 1984; **101**: 641–5.
24. Pitchenik AE, Burr J, Cole CH. Tuberculin testing for persons with positive serologic studies for HTLV-III. N Engl J Med 1986; **314**: 447.
25. Hawkins CC, Gold JWM, Whimbey E, et al. *Mycobacterium avium* complex infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med 1986; **105**: 184–8.
26. Damsker B, Buttone EJ. *Mycobacterium avium–Mycobacterium intracellulare* from the intestinal tracts of patients with the acquired immunodeficiency syndrome: Concepts regarding acquisition and pathogenesis. J Infect Dis 1985; **151**: 179–81.
27. Dworkin B, Wormser GP, Rosenthal WS, et al. Gastrointestinal manifestations of the acquired immunodeficiency syndrome. Am J Gastroenterol 1985; **80**: 774–8.
28. Kehrein B, Ruf B, Thalmann U, et al. Mykobakteriosen bei AIDS. Patholge 1988; **9**: 218–27.
29. Kiehn TE, Edwards, FF, Brannon P, et al. 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 1985; **21**: 168–73.
30. Gangadharam PRJ, Edwards CK. Release of superoxide anion from resident and activated mouse peritoneal macrophages infected with *Mycobacterium intracellulare*. Am Rev Respir Dis 1984; **151**: 179–81.
31. Meissner G, Anz W. Sources of *Mycobacterium avium* complex infection resulting in human diseases. Ann Rev Respir Dis 1977; **116**: 1057–64.
32. Fauci AS. Acquired immunodeficiency syndrome: epidemiologic, immunologic and therapeutic considerations. Ann Intern Med 1984; **110**: 92–106.
33. Murray JF, Felton CP, Garay SM, et al. Pulmonary complications of the acquired immunodeficiency syndrome. N Engl J Med 1984; **310**: 1682–8.
34. Gill VJ, Park CH, Stock F, Gosey LL, Witebsky FG, Masur H. Use of lysis-centrifugation (Isolator) and Radiometric (BACTEC) blood culture systems for the detection of mycobacteremia. J Clin Microbiol 1985; **22**: 543–5.
35. Winter SM, Bernard EM, Gold JWM, Armstrong D. Humoral response to disseminated infections by *Mycobacterium avium–Mycobacterium intracellulare* in the acquired immunodeficiency syndrome and hairy cell leukemia. J Infect Dis 1985; **151**: 523–7.
36. Ruf B, Schürmann D, Matos-Marques B, et al. Bedeutung und Diagnostik der Mykobakterien bei Patienten mit HIV-Infektion: Ergebnisse einer prospektiven Studie über zwei Jahre (Abstract). Deutscher AIDS-Kongreß. Programm und Abstracts 1988.