INFECTION AND IMMUNITY, May 1989, p. 1445–1451
0019-9567/89/051445-07$02.00/0

Vol. 57, No. 5

Defects in Sera from Acquired Immunodeficiency Syndrome (AIDS) Patients and from Non-AIDS Patients with *Mycobacterium avium* Infection Which Decrease Macrophage Resistance to *M. avium*

ALFRED J. CROWLE,[1]* DAVID L. COHN,[2,3] AND PAT POCHE[1]

Webb-Waring Lung Institute and Departments of Microbiology and Immunology[1] and Medicine (Division of Infectious Diseases) and Preventive Medicine,[2] University of Colorado Health Sciences Center, and Denver Disease Control Service, Denver Department of Health and Hospitals,[3] Denver, Colorado 80262

Received 12 December 1988/Accepted 1 February 1989

Some characteristics of the sera and macrophages (MP) of human immunodeficiency virus (HIV)-infected patients which might contribute to their unusual susceptibility to *Mycobacterium avium* infection were studied. Cultures of patient peripheral blood MP in medium supplemented with their sera or normal subject sera were infected with *M. avium* and compared with similar cultures of normal MP. Intracellular mycobacterial replication was measured in the infected MP by CFU counts of the bacteria made from lysed samples of the MP at 0, 4, and 7 days after MP infection. Sera from patients with chronic granulomatous infection with *M. avium*, but no HIV infection, also were studied. The sera from all of the patients with chronic granulomatous infection and from several HIV-infected patients were deficient or lacking in an inhibitor that in normal serum acts within normal MP to suppress intracellular growth of *M. avium*. Most of the HIV-infected patients also had MP that were abnormally permissive for *M. avium* because they responded poorly to the serum inhibitor. Elucidation of these associated defects in native defenses against *M. avium* may result in better prevention and therapy of *M. avium* infections.

Patients with the acquired immunodeficiency syndrome (AIDS) are unusually susceptible to *Mycobacterium avium*. Up to half develop generalized, frequently fulminant, infection with this bacterium (11–14). They are also abnormally susceptible to tuberculosis (8, 14). Their cellular immunity is severely depressed, partly by $CD4^+$ T-lymphocyte destruction by the human immunodeficiency virus (HIV) (1). Their abnormal susceptibility to intracellular parasites like *M. avium* and *Mycobacterium tuberculosis* consequently has been attributed to deficiencies in T-lymphocyte-produced lymphokines that activate macrophages (MP) (1). However, their MP also become infected with HIV (10, 11) and consequently might become inherently abnormally susceptible to mycobacteria.

Recently, normal human serum was found to contain a potent inhibitor of *M. avium* growth in human MP (4). Lack of this inhibitor in the sera of AIDS patients might be an alternate cause of increased susceptibility to *M. avium*.

Using an in vitro model of human *M. avium* infection (6), we looked for deficiencies in both MP and sera of several patients in different stages of HIV infection and in sera of some patients with chronic granulomatous *M. avium-Mycobacterium intracellulare* but not HIV infection. The data presented here show that sera from both kinds of patients frequently are poor or lacking in the natural inhibitor and that AIDS MP are also uncommonly permissive for *M. avium* at least partly because the inhibitor is ineffective in them.

MATERIALS AND METHODS

Bacteria and infections. These materials and procedures have been described in detail (3, 4, 6). The bacteria used were *M. tuberculosis* Erdman and two strains of *M. avium*—TMC 724 serovar 2 and 7497 serovar 4. For both strains of *M. avium*, only virulent smooth and transparent colony cultures derived from picked single colonies were used (4, 6). As used, the bacteria infected approximately 10% of the cultured MP with 1 to 2 bacteria each (2, 7).

Human subjects. These experiments were done with sera and MP from 8 normal subjects, 13 AIDS patients (4 with disseminated *M. avium* and 2 with disseminated tuberculosis), 2 patients with HIV infection but not AIDS (generalized lymphadenopathy syndrome and AIDS-related complex), and 4 non-AIDS patients with chronic granulomatous *M. avium* (*M. avium-M. intracellulare*) infection. The normal subjects were 4 males and 4 females, all tuberculin negative, 3 of whom were young and 5 of whom were middle aged. These are referred to in presentation of the data with the code letter N for normal. Demographic and clinical characteristics of the HIV-infected subjects are listed in Table 1. These patients are referred to by the following code letters: AIN for AIDS patients without *M. avium-M. intracellulare* infection, AIM for AIDS patients with *M. avium-M. intracellulare* infection, AIT for AIDS patients with tuberculosis, and P for AIDS-prodromal patients. Three of the patients with chronic granulomatous *M. avium* infection (code letter G) were young men, and one was a 9-year-old boy. All had long-lasting infections and were being treated with antimycobacterial drugs. Sera from patients G1, G2, and G3 had been submitted to this laboratory for study because these patients were not responding to treatment. *M. avium-M. intracellulare* infection in the boy (G4) was regressing in response to treatment.

MP isolation and culture. MP were isolated and cultured as previously described (3, 4, 6). Briefly, adherent blood monocytes from subjects or patients were incubated in 35-mm plastic petri dishes for 7 days to allow them to mature to MP. Patient MP were incubated in standardized serum from a normal control subject (AB serum), and normal subject MP

* Corresponding author.

TABLE 1. Demographic and clinical characteristics of study subjects with HIV infection

Patient	Age (yr)	HIV transmission category[a]	Diagnosis[b]	Class[c]	$CD4^+$ cells[d]	Site(s) of mycobacterial isolation
P1	29	Homosexual	Generalized lymphadenopathy	III	1,382	None
P2	35	Homosexual	Oral candidiasis, night sweats	IV-C-2	477	None
AIN1	38	Homosexual	Kaposi's sarcoma	IV-D	347	None
AIN2	33	Homosexual	Kaposi's sarcoma, *Pneumocystis carinii* pneumonia	IV-C-1	17	None
AIN3	50	IV-drug user	*P. carinii* pneumonia	IV-C-1	0	None
AIN4	38	Homosexual	*P. carinii* pneumonia	IV-C-1	23	None
AIN5	39	Homosexual, IV-drug user	*P. carinii* pneumonia, *Campylobacter* infection, perirectal HSV	IV-C-1	51	None
AIN6	28	Homosexual	Kaposi's sarcoma	IV-D	ND	None
AIN7	47	Homosexual	*P. carinii* pneumonia	IV-C-1	154	None
AIM1	30	Homosexual, IV-drug user	Kaposi's sarcoma, *P. carinii* pneumonia, *M. avium-M. intracellulare* infection	IV-C-1	ND	Blood, bone marrow
AIM2	36	IV-drug user	*P. carinii* pneumonia, cryptococcus, *M. avium-M. intracellulare* infection	IV-C-1	20	Blood, sputum
AIM3	25	Homosexual	*P. carinii* pneumonia, *M. avium-M. intracellulare* infection	IC-C-1	13	Blood, bone marrow
AIM4	26	Homosexual	*P. carinii* pneumonia, CMV retinitis, *M. avium-M. intracellulare* infection	IV-C-1	ND	Blood, liver, bone marrow
AIT1	27	Homosexual	Tuberculosis	IV-C-2	ND	Blood, lymph node, bronchial lavage, pleural fluid
AIT2	37	Homosexual	Tuberculosis	IC-C-2	156	Skin abscess

[a] All patients were male. IV, Intravenous.
[b] HSV, Herpes simplex virus; CMV, cytomegalovirus.
[c] Centers for Disease Control classification.
[d] Number of CD4 T cells per mm^3; ND, not done.

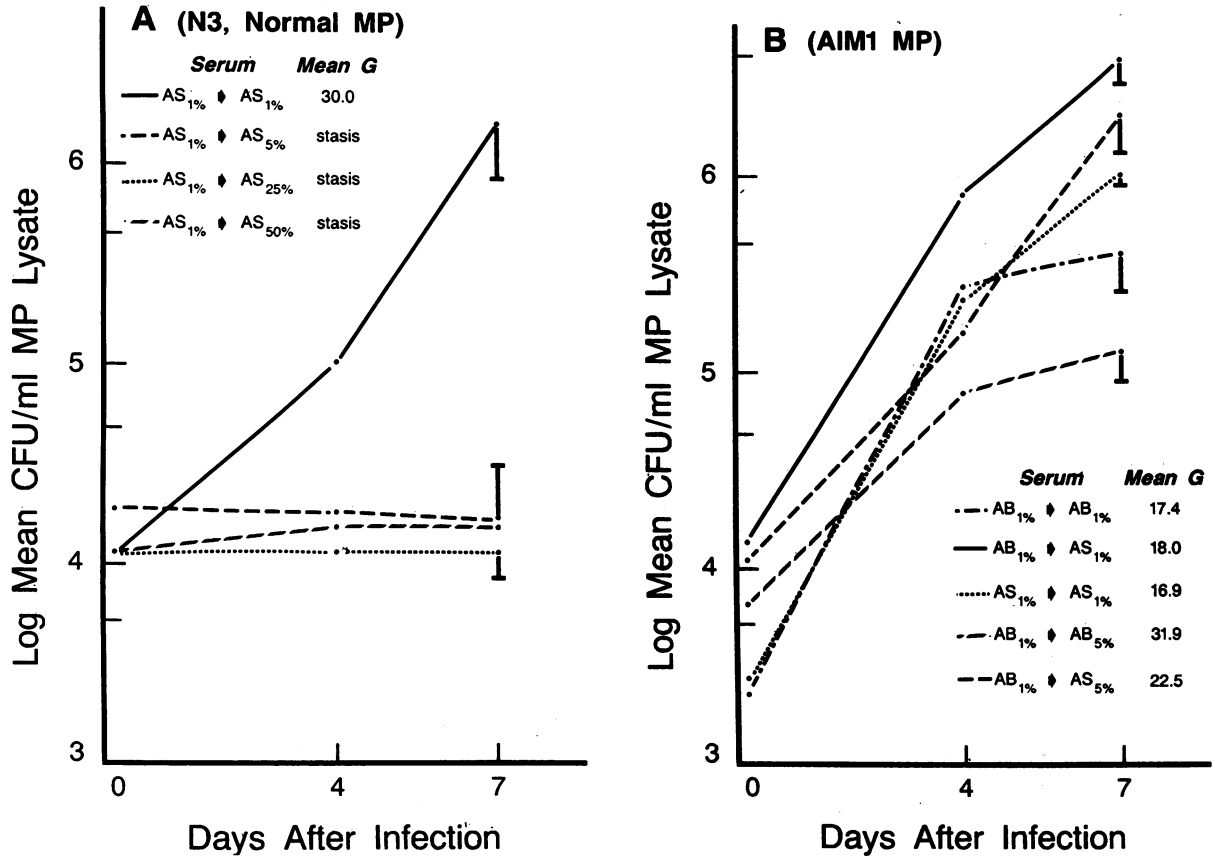

FIG. 1. (A) Growth responses, in CFU per milliliter of MP lysate (approximately 10^5 MP per ml) at 0, 4, and 7 days after infection of the MP, typical of *M. avium* 7497 in normal (subject N3) MP and various concentrations of normal serum (AS). AS was used at 1% before infection (left of arrows) and at 1, 5, 25, and 50% after infection (right of arrows). Mean *G* (in hours) was calculated from the growth curve points (see text). (B) Growth of 7497 in MP from AIDS patient AIM1 in patient AS or in normal AB serum used at 1 and 5%. Datum points are means of 5 values. The bars shown for 7-day CFU data are standard errors of the means.

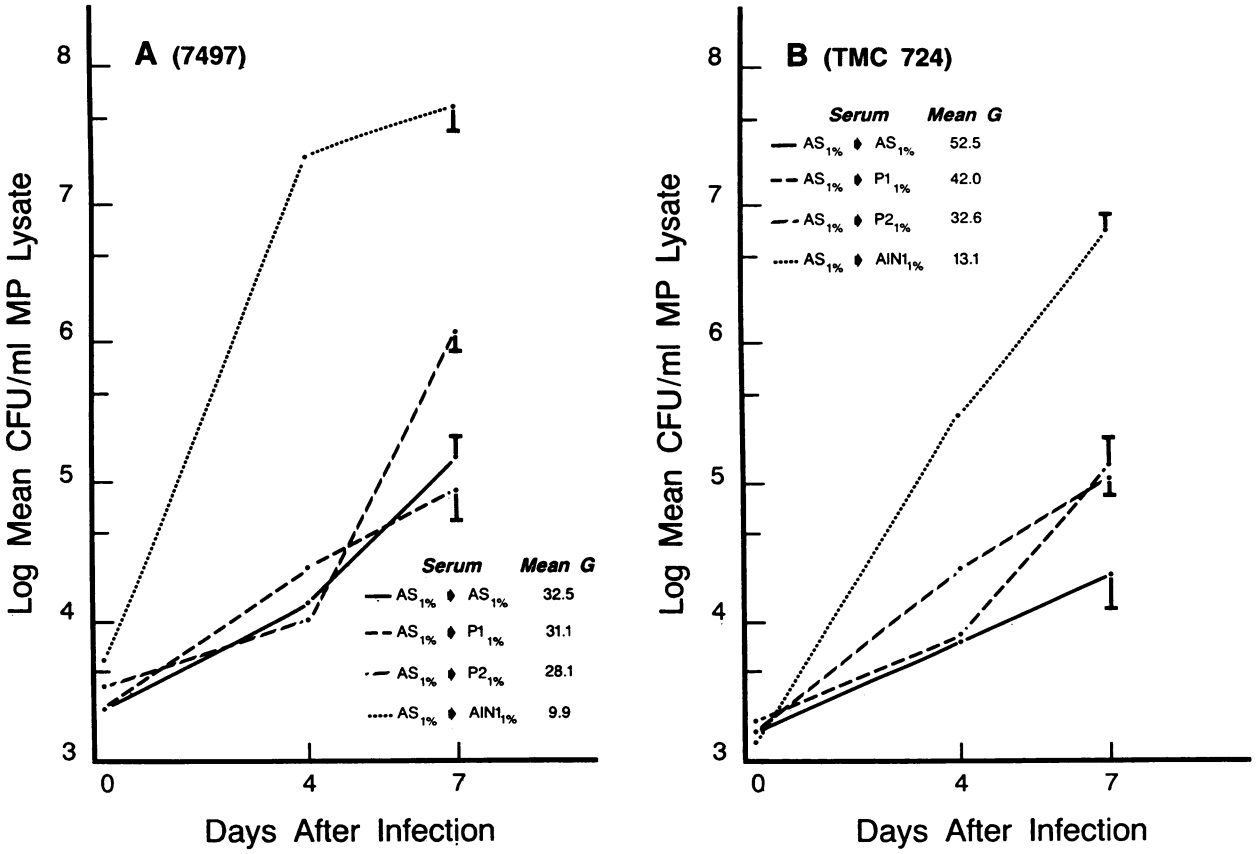

FIG. 2. Simultaneous test of effects of AIDS (AIN1) and prodromal (P1 and P2) sera against serovars 4 (7497 [A]) and 2 (TMC 724 [B]) in normal N1 MP.

were incubated in autologous serum (AS) during that time. Then, the MP were infected by using AB serum, and after infection they were incubated in whatever serum and serum concentration were being tested. These included AS, AB serum, normal subject serum, or a serum substitute (SS). The composition and effects of SS on *M. avium* growth in cultured human MP have been described previously (4, 5).

The numbers and morphologic appearance of MP from the patients were determined during experiments by inverted phase microscopy. Samples were also taken at 0, 4, and 7 days after infection for fixing, staining, and enumeration by conventional microscopy. Except that MP from AIDS patients frequently included cells with two nuclei, patient MP were indistinguishable from normal MP in number and morphologic appearance.

The MP culture medium was RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2 mM L-glutamine and unheated AB serum, patient serum (1 or 5%, as noted below), or 10% SS. It contained no antimicrobial drugs. The mycobacteria used in these experiments do not grow significantly in this medium outside of MP (4, 6). Cell cultures were incubated at 37°C in 7% CO_2 in air.

CFU counts. After infection, the MP cultures were incubated for 7 days. Samples were taken at 0, 4, and 7 days following infection for CFU counts, which were made as previously described (3, 4, 6). CFU counts were plotted semilogarithmically for all experiments. Results for several CFU counts are shown in the figures, because they show the kinetics of the intracellular mycobacterial replication. The mean generation time (*G*) for intracellular growth, the most useful single value available for comparing groups with each other (2), is derived from the intersection of a calculated straightened mean curve across a 24-hour period (2, 6). Datum points for the growth curves in the figures are means of 5 values. Standard errors of the means are shown for 7-day values, the largest in each experiment. The statistical significance of differences between means of *G* values in the tables was determined by the *t* test.

Direct antibacterial effects of sera. The direct antibacterial effects of sera were tested as described previously (3). Each serum was added in various concentrations to 0.2-ml volumes of 7H9 broth (Difco Laboratories, Detroit, Mich.) in triplicate in microtiter dishes, after the broth had been inoculated with 7497 or Erdman bacilli. These cultures were incubated for 10 days at 37°C, with readings of bacterial growth at 7 and 10 days. The growth was assessed by bacterial sediment accumulating in the round bottom of each well and recorded relative to the 7H9 broth control wells on a 0 to 4+ scale, with 4+ being the amount of growth in the control broth without serum. The titers of the effects were determined by reciprocals of serum dilutions exhibiting inhibition or enhancement.

RESULTS

Direct effects of patient sera. Sera from all subjects were tested for direct effects on growth of *M. avium* 7497 and *M. tuberculosis* Erdman in 7H9 broth. Sera from HIV-infected patients were tested at ≤4%, and sera from other patients

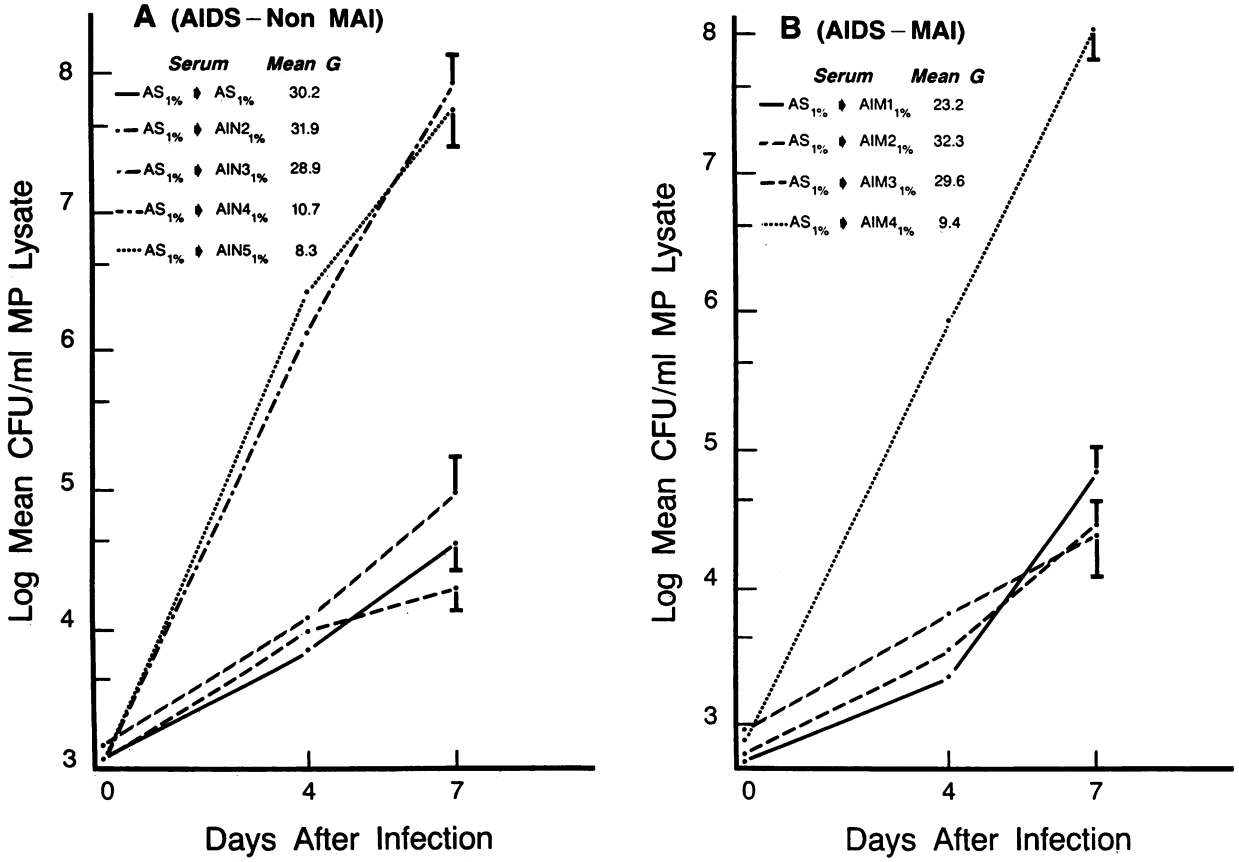

FIG. 3. Simultaneous testing of 4 AIDS-non-*M. avium-M. intracellulare*-infected (MAI) (AIN [A]) and 4 AIDS-*M. avium-M. intracellulare*-infected (AIM [B]) sera on 7497 growth in N1 normal MP.

and normal sera were tested at ≤1%. No serum enhanced or inhibited growth of 7497. Most of the sera also had no effect on Erdman growth, but sera from AIDS patients AIN5, AIN7, and AIM3 enhanced it, and sera from the two AIDS-tuberculosis patients inhibited it.

Comparative responses of AIDS and normal MP to infection with 7497. Figure 1 shows typical responses of normal and AIDS MP to infection with 7497 and the respective effects on such infection that of normal and AIDS sera. Growth of 7497 in the normal MP was exponential but slow, with a mean *G* of 30 h, in medium supplemented with 1% normal AS (Fig. 1A). At a concentration of 5, 25, or 50%, the normal serum in normal MP was bacteriostatic, as reported previously (4). In AIDS MP (Fig. 1B) incubated in 1% normal or AIDS serum, 7497 also grew exponentially, but significantly ($P < 0.001$) much more rapidly (mean $G = 17.4$ h). Furthermore, in these AIDS MP neither the AIDS serum nor normal serum at 5% was bacteriostatic. These results, indicating both serum and MP defects in AIDS patients, are confirmed by results from the several experiments summarized below (see Tables 2 and 3).

Phagocytosis of 7497 by the patient MP (zero time CFU) was similar to phagocytosis in the normal MP.

Comparison of prodromal AIDS sera for inhibition of two different serovars of *M. avium* in normal MP. The experiment summarized in Fig. 2 used normal MP infected with 7497 serovar 4 (Fig. 2A) or TMC 724 serovar 2 (Fig. 2B) to test whether the inhibitory effect of serum was peculiar to the usually used serovar 4. The MP were incubated in three patient sera compared with the normal AS and AB sera. Patients P1 and P2 were prodromal, presenting with generalized lymphadenopathy and AIDS-related complex, respectively. Patient AIN1 presented with Kaposi's sarcoma but no detectable *M. avium-M. intracellulare* infection. The AIDS serum, AIN1, lacked inhibitor and permitted abnormally rapid growth in the normal MP of both serovars, while the prodromal sera inhibited growth nearly as well as the normal serum. Where inhibition occurred, it was proportionally the same for both serovars.

Comparison of AIDS sera for inhibitor of *M. avium* 7497 in normal MP. The experiment shown in Fig. 3 simultaneously compared four AIDS-non-*M. avium-M. intracellulare*-infected patient sera with four AIDS-*M. avium-M. intracellulare*-infected patient sera for ability to inhibit 7497 growth in MP from a normal subject. Two of the AIDS-non-*M. avium-M. intracellulare*-infected sera and one of the AIDS-*M. avium-M. intracellulare*-infected sera lacked inhibitor. The other five sera appeared to inhibit like normal serum does.

Combinations of MP and serum deficiency in AIDS patients. Data in Fig. 3, for which normal MP were used, suggested that five of eight AIDS sera could inhibit 7497 normally. However, data in Fig. 1 suggested that if AIDS MP are used, or if the serum concentration is raised to 5%, the underlying serum deficiency will become apparent. This is confirmed by data from several experiments with AIDS MP summarized in Table 2 compared with data from normal MP in Table 3.

Table 2 shows the mean *G* of *M. avium* 7497 in MP from individual AIDS patients, each tested in a separate experiment, incubated in medium supplemented with 1 or 5% AS (patient) or AB (normal) serum. Table 3 shows similar data

TABLE 2. *G* of *M. avium* in AIDS patient MP incubated in AS or AB serum

Condition and patient	Chemotherapy	*G*[a] in: 1% AB serum	1% AS	5% AB serum	5% AS
AIDS, non-*M. avium-M. intracellulare* infected					
AIN2	None[b]	26.8	39.4	195	101
AIN4	None	15.6	12.2	319	71.3
AIN5	None	25.9	13.3	37.1	46.9
AIN6	None		23.2		
AIN7	None	24.0	17.3	52.5	38.4
Mean		23.1	21.1	150.9	64.4
SEM		2.6	5.0	66.4	14.0
AIDS, *M. avium-M. intracellulare* infected					
AIM1	None	17.4	17.5	31.9	22.5
AIM2	None	28.5	35.3	26.3	94.7
AIM3	None	11.6	13.5	41.3	99.4
AIM4	Anti-*M. avium-M. intracellulare*		20.3		
		19.2	21.7	33.2	72.2
		3.7	4.7	5.7	19.5
AIDS, TB[c]					
AIT1	Anti-TB	22.1	20.6		
AIT2	Anti-TB	36.0	40.1		
Mean		29.1	30.4		
SEM		6.9	9.8		
Mean for all values		23.1	22.9	100.4	67.7
SEM for all values		2.5	3.1	42.7	12.1

[a] Intramacrophage *G* (in hours) of *M. avium* 7497 in patient MP incubated in medium supplemented with 1% or 5% AB serum or AS.
[b] No antimycobacterial drugs.
[c] TB, Tuberculosis.

TABLE 3. *G* of *M. avium* in normal subject MP incubated in AS, AB serum, or SS

Expt	Subject	*G*[a] in: 1% AB serum	1% AS	10% SS
P-47	N4	19.0	26.6	
P-48	N5	39.0		11.3
P-49	N1	24.0	26.8	11.3
P-49	N1		35.6	
P-50	N1	20.3		
P-53	N1		32.5	
P-56	N2	31.9		
P-61	N2		18.8	
P-62	N1		30.2	
P-70	N1		48.8	
P-71	N1		35.6	
P-74	N1		39.8	
	N2		31.9	
P-78	N5	43.5		
P-80	N6	56.3		
P-81	N7		37.9	
Mean		33.4	33.1	11.3
SEM		5.2	2.4	

[a] *G* (in hours) of 7497 in MP.

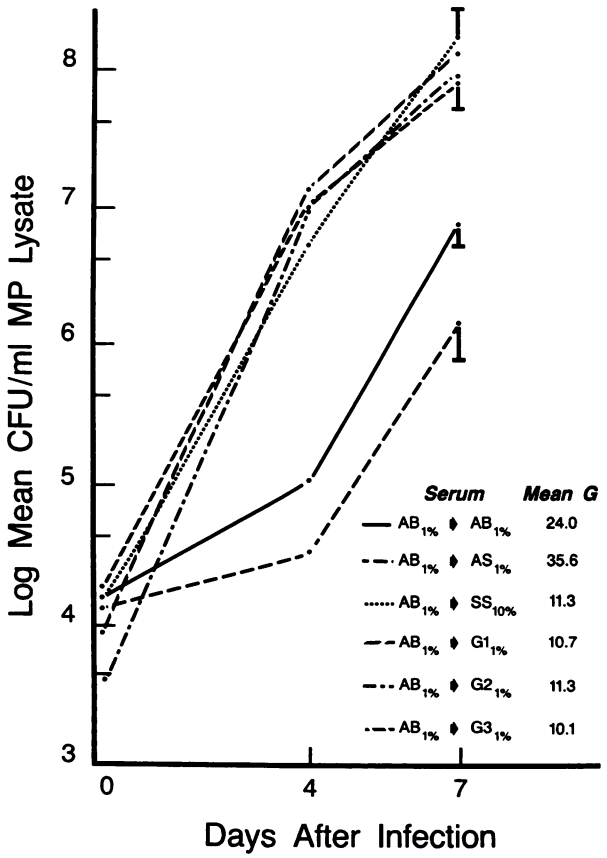

FIG. 4. Comparison of effects of three non-AIDS granulomatous *M. avium-M. intracellulare*-infected patient (G1, G2, and G3) sera with AB serum, AS (subject N1), and 10% SS on 7497 growth in N1 normal MP.

from 14 experiments using MP from 6 different normal subjects and normal AS or AB serum. In the two tables, the means of *G* for all the experiments are underlined for easy comparison. The means for AIDS MP in either 1% AS (i.e., AIDS) (mean = 22.9) or 1% AB (mean = 23.1) serum (Table 2) were consistently and statistically significantly ($P < 0.001$) lower than the means for normal MP (means of 33.1 and 33.4, respectively; Table 3). This confirms that AIDS MP have a defective response to the serum inhibitor. Means for 5% AB serum (normal) and 5% AS (patient) in AIDS MP of 100.4 and 67.7, respectively, are statistically significantly different ($P < 0.001$) from each other and further confirm the serum defect in AIDS. In normal MP, 5% AB serum stops growth of *M. avium* (4; Fig. 1A). Table 2 shows that 5% AB serum was unable to stop growth in MP from five of the eight AIDS patients (AIN5, AIN7, AIM1, AIM2, and AIM3). Only MP from patient AIN4 responded normally with 5% AB serum to cause bacteriostasis.

Defect in sera from non-AIDS *M. avium-M. intracellulare*-infected patients. Sera from non-AIDS *M. avium-M. intracellulare*-infected patients tended to show the same lack of inhibitor of intracellular growth of *M. avium* in normal MP as AIDS sera did. This is illustrated by the experiment in Fig. 4 comparing sera from the three treatment failure patients with AB, normal serum, and 10% SS. G values for *M. avium* 7497 in the three patient sera were statistically the same as in SS. SS completely lacks inhibitor and permits maximum and

TABLE 4. Generation times of *M. avium* in normal (subject N1) macrophages incubated in non-AIDS *M. avium-M. intracellulare*-infected sera

Expt	Serum	G[a] for:	
		7497	Erdman
P-49	N1	35.6	26.6
	AB	24.0	24.8
	G1	10.7	19.1
	G2	11.3	18.4
	G3	10.1	15.6
	$G4_1$[b]	17.3	22.8
	$G4_2$	18.7	22.5
	SS	11.3	25.9
P-70	N1	48.8	34.1
	G1	11.3	22.9
	G2	19.3	24.0
	G3	10.9	19.3
	$G4_2$	22.5	22.7
Mean for all G sera		14.7	20.8
SEM		1.6	0.9
Mean for normal sera		36.1	28.5
SEM		7.2	2.9

[a] Intramacrophage *G* (in hours) for 7497 or Erdman bacilli.
[b] Two different bleedings for patient G4 were being tested.

exponential growth of *M. avium* in cultured human MP (4). AB serum and normal AS significantly inhibited *M. avium* growth, more during the first 4 than the last 3 days, making the growth nonexponential.

The deficiency of inhibitor for non-AIDS *M. avium-M. intracellulare*-infected sera shown in Fig. 4 has been confirmed in several experiments. Two which included G4, the patient responding to treatments, are summarized in Table 4. MP from one normal donor were infected with *M. avium* 7497 or *M. tuberculosis* Erdman and incubated with 1% sera from patients G1, G2, G3, and G4 or normal serum (AS or AB serum). Mean *G*s for 7497 in MP in the patient sera were all abnormally short, compared with the mean *G* for normal serum (14.7 versus 36.1 h; P for difference < 0.001). Sera from the three treatment failure patients, as in Fig. 4, permitted growth of *M. avium* at the same rapid rate as inhibitor-free SS. The two samples of serum from the successfully treated patient, G4, appeared to be deficient but not lacking in the inhibitor. Sera from these patients also modestly increased MP permissiveness for Erdman tubercle bacilli in the normal MP (difference between patient mean of 20.8 and normal mean of 28.5 significant at $P < 0.001$).

Deficiency of non-AIDS *M. avium-M. intracellulare*-infected sera not dependent on donor MP. The deficiency of inhibitor in non-AIDS *M. avium-M. intracellulare*-infected sera was further confirmed and also shown to be independent of which normal donor MP were used to study it by the experiment summarized in Fig. 5. In this experiment, N1 and N2 MP were separately but simultaneously infected with 7497 and then incubated in G1 or G3 serum or N1 or N2 serum. The figure shows that both normal sera inhibited growth of 7497 normally in MP from both donors, while G1 and G3 failed to inhibit similarly in MP from both donors.

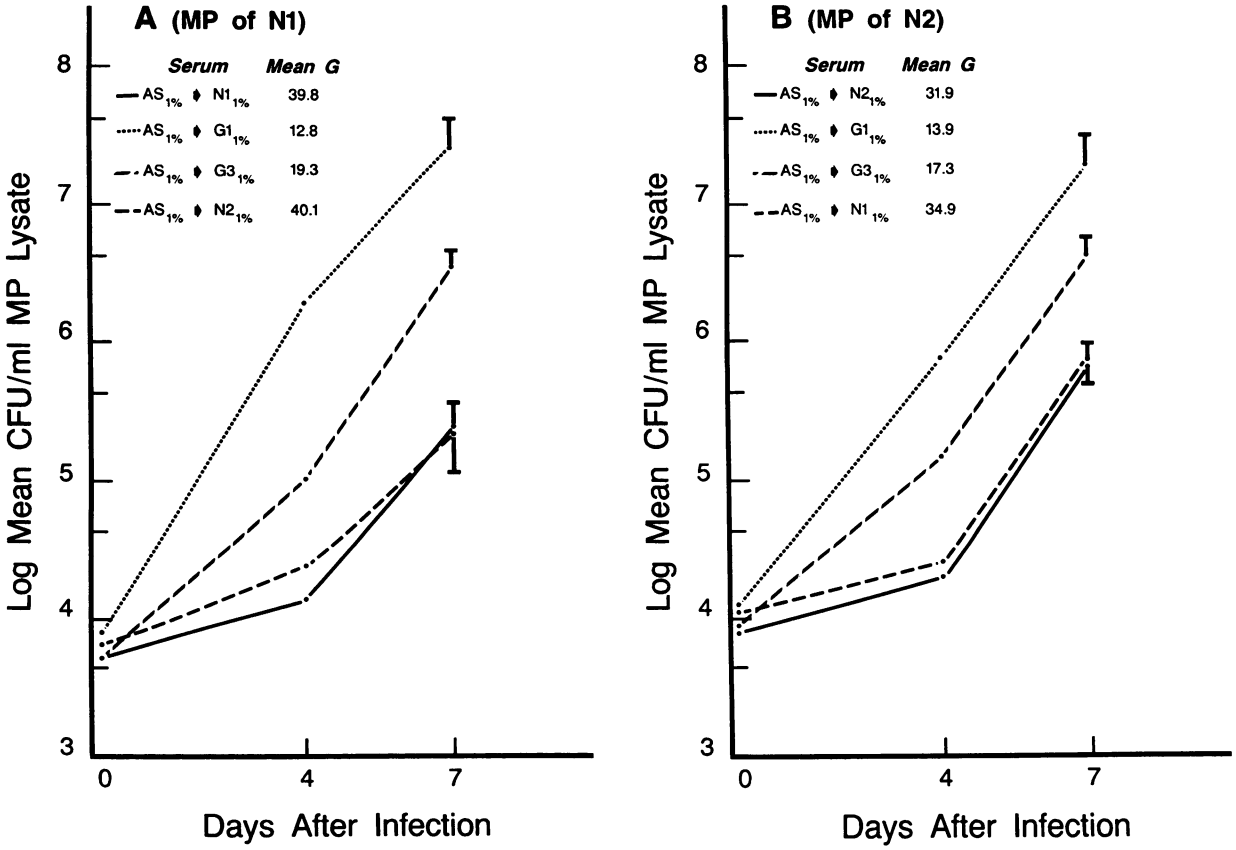

FIG. 5. Double experiment comparing deficiency in inhibitor for 7497 in non-AIDS *M. avium-M. intracellulare*-infected sera G1 and G3 simultaneously by using normal MP from donors N1 (A) and N2 (B) and reciprocal testing of the normal sera from these two donors.

DISCUSSION

Several data here, for instance in Fig. 1A, confirm that normal human serum inhibits the growth of *M. avium* in cultured normal human MP (4). Sera from AIDS patients and from non-AIDS patients with chronic granulomatous *M. avium-M. intracellulare* infection tend to be deficient in or lack this inhibitory property. The deficiency was proportionally evident for serovars 2 and 4 of *M. avium* (Fig. 2) and so is not a peculiarity of the serovar 4 strain used in most of these experiments. It may tend to develop as the natural history of HIV infection progresses, in that it was apparently not present in sera from the two prodromal patients with generalized lymphadenopathy or AIDS-related complex (Fig. 2). The deficiency, further, may relate to treatment failure in non-AIDS granulomatous *M. avium-M. intracellulare* infections, since it was marked in treatment failure patients G1, G2, and G3 but only partial in the patient (G4) whose infection had been brought under control.

AIDS patient MP phagocytized *M. avium* normally, confirming the report by Schnittman et al. (13). However, MP from most of the patients were more permissive than normal for *M. avium* growth in the presence of either normal or patient serum (Table 2). This unusual permissiveness of patient MP in normal serum, even where it was used at 5%, which is bacteriostatic in normal MP, suggests that the AIDS patient MP abnormality is an intracellular unresponsiveness to the action of the serum inhibitor. No direct inhibition or enhancement of *M. avium* by sera from normal subjects or patients was seen in the absence of MP. These patients therefore would appear to frequently have a double and interdependent deficiency (MP and serum) in native immunity to *M. avium*.

Nothing is known, yet, about the serum inhibitor or how MP respond to it to suppress *M. avium* infection. Some indirect evidence (4) suggests that the inhibitor might block conversion within MP of a serum pronutrient, for *M. avium*, to an essential nutrient, possibly a fatty acid necessary for division and replication of these bacteria (9). This inhibitor and its anti-*M. avium* effects via human MP probably are worth studying, because defects in this newly discovered defensive system may explain why normally nonpathogenic mycobacteria like *M. avium* can infect AIDS patients, as well as people who develop chronic granulomatous *M. avium-M. intracellulare* infections.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant 1 R01 A121919.

We thank Stuart Henochowicz for the samples of non-*M. avium-M. intracellulare*-infected patient sera studied here.

LITERATURE CITED

1. **Bowen, D. L., H. C. Lane, and A. S. Fauci.** 1985. Immunologic features of AIDS, p. 89–110. *In* V. T. DeVita, Jr., S. Hellman, and S. A. Rosenberg (ed.), AIDS. Etiology, diagnosis, treatment, and prevention. J. B. Lippincott Co., Philadelphia.
2. **Crowle, A. J.** 1988. The tubercle bacillus-human macrophage relationship studied *in vitro*, p. 99–135. *In* M. Bendinelli and H. Friedman (ed.), *Mycobacterium tuberculosis*. Interactions with the immune system. Plenum Publishing Corp., New York.
3. **Crowle, A. J., N. Elkins, and M. H. May.** 1988. Effectiveness of ofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium*, and rifampin against *M. tuberculosis* in cultured human macrophages. Am. Rev. Respir. Dis. **137**:1141–1146.
4. **Crowle, A. J., and P. Poche.** 1989. Inhibition by normal human serum of *Mycobacterium avium* multiplication in cultured human macrophages. Infect. Immun. **57**:1332–1335.
5. **Crowle, A. J., E. J. Ross, and M. H. May.** 1987. Inhibition by $1,25(OH)_2$-vitamin D_3 of the multiplication of virulent tubercle bacilli in cultured human macrophages. Infect. Immun. **55**: 2945–2950.
6. **Crowle, A. J., A. Y. Tsang, A. E. Vatter, and M. H. May.** 1986. Comparison of 15 laboratory and patient-derived strains of *Mycobacterium avium* for ability to infect and multiply in cultured human macrophages. J. Clin. Microbiol. **24**:812–821.
7. **Douvas, G. S., and A. J. Crowle.** 1985. Macrophages. I. Model for studying antimicrobial immunity *in vitro*, p. 81–90. *In* T. Yoshida (ed.), Investigation of cell-mediated immunity. Churchill Livingstone, Ltd., Edinburgh.
8. **Flepp, M., C. Fisberg, and A. Burnens.** 1988. Mykobacteriosen bei Patienten mit HIV-Infektion. Dtsch. Med. Wochenschr. **113**:711–718.
9. **McCarthy, M.** 1988. Reversal of cerulenin inhibition of *Mycobacterium avium* by octanoate. Curr. Microbiol. **17**:121–125.
10. **Orenstein, J. M., M. S. Meltzer, T. Phipps, and H. E. Gendelman.** 1988. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastuctural study. J. Virol. **62**:2578–2586.
11. **Pauza, C. D.** 1988. HIV persistence in monocytes leads to pathogenesis and AIDS. Cell. Immunol. **112**:414–424.
12. **Perfect, J. R.** 1988. *Mycobacterium avium-intracellulare* complex infections in the acquired immunodeficiency syndrome. J. Electron Microsc. Techn. **8**:105–114.
13. **Schnittman, S., H. C. Lane, F. G. Witebsky, L. L. Gosey, M. D. Hoggan, and A. S. Fauci.** 1988. Host defense against *Mycobacterium-avium* complex. J. Clin. Immunol. **8**:234–243.
14. **Snider, D. E., P. C. Hopewell, J. Mills, and L. B. Reichman.** 1987. Mycobacterioses and the acquired immunodeficiency syndrome. Am. Rev. Respir. Dis. **136**:492–496.