INFECTION AND IMMUNITY, Sept. 1988, p. 2223–2227
0019-9567/88/092223-05$02.00/0

Vol. 56, No. 9

Differences in Uptake of Mycobacteria by Human Monocytes: a Role for Complement

RODNEY P. SWARTZ,[1]* DAVID NAAI,[1] CARL-WILHELM VOGEL,[1,2] AND HENRY YEAGER, JR.[1]

Pulmonary Disease Division, Department of Medicine,[1] and Department of Biochemistry,[2] Georgetown University Medical Center, 3800 Reservoir Rd., N.W., Washington, D.C. 20007

Received 7 March 1988/Accepted 26 May 1988

We investigated the influence of serum factors on the uptake of various species of mycobacteria by human peripheral blood monocytes (PBM). On the basis of the percentage of PBM involved during in vitro uptake, the mycobacteria were of two distinct groups. The mycobacteria of one group, which consisted of *Mycobacterium avium* complex and *M. chelonae*, were taken up by many PBM; the other group, consisting of *M. tuberculosis*, *M. kansasii*, *M. fortuitum*, and *M. gordonae*, were taken up by fewer PBM. *M. scrofulaceum* was intermediate to these two groups on the basis of its uptake by PBM. Serum depleted of complement by heating or treatment with cobra venom factor significantly reduced the extent of PBM involvement with *M. avium* complex, indicating that complement is an important serum component mediating the uptake of *M. avium* complex organisms. Preincubation of mycobacteria with serum containing 10 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid] and 10 mM $MgCl_2$ resulted in uptake by a high percentage of PBM, while preincubation in heated serum or serum containing 10 mM EDTA resulted in a significantly reduced percentage of PBM involved in uptake of *M. avium* complex organisms, indicating that these organisms are activators of the alternative pathway of complement. Incubation of *M. avium* complex organisms in human serum consumed 51% of the hemolytic complement activity. Parallel experiments indicated that serum had a lesser effect on the uptake of *M. tuberculosis*. Thus, serum is important in in vitro *M. avium* complex uptake by PBM; complement has a major role in the effect of serum, but this role is less important with *M. tuberculosis*.

Mycobacterial disease is of renewed interest today because of the prominence of *Mycobacterium avium* complex infections in acquired immune deficiency syndrome (AIDS) patients (13, 27, 28) and because of the increase in *Mycobacterium tuberculosis* disease in the United States, in part also due to AIDS (6a). Resistance to infection by mycobacteria entails a complex pattern of involvement by a host's defense system. In recent years, studies have appeared concerning the interaction of mycobacteria with human monocytes (2, 3, 9, 22) but have mainly concentrated on intracellular killing, not on the initial contact with and uptake, of the organisms. In addition, little attention has been focused on the possible role of serum components, especially complement, in the uptake of mycobacteria by mononuclear phagocytes (7, 8, 11). In this work, we report that serum factors, and in particular complement, are of importance in the in vitro uptake by human peripheral blood monocytes (PBM) of several species of mycobacteria, especially of *M. avium* complex organisms.

MATERIALS AND METHODS

Cell donors. PBM were obtained from 19 healthy volunteers (15 males, 4 females), 18 to 40 years old, after informed consent was obtained. All volunteers had normal chest roentgenograms and ventilatory tests. Three males were tuberculin positive, as determined by the Mantoux skin test; all other volunteers were negative. No significant differences were observed in the studies done with PBM from either tuberculin-positive or tuberculin-negative individuals, and the data are combined. The protocol was approved by the Human Research Committee of the Georgetown Medical Center and was carried out by using the principles of the Declaration of Helsinki.

* Corresponding author.

PBM. Peripheral blood was obtained by venipuncture by using heparinized VACUTAINER tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.). The heparinized blood was mixed with an equal volume of room temperature Hanks balanced salt solution (HBSS), pH 7.4, and 9 ml of the mixture was carefully layered over 6 ml of room temperature Lymphocyte Separation Medium (Litton Bionetics, Inc., Charleston, S.C.). The preparation was centrifuged for 30 min at 400 × *g* at room temperature, and the interface band containing mononuclear cells was removed. The cells were washed three times in ice-cold HBSS and suspended in either RPMI 1640 medium containing 100 units of penicillin G per ml (RPMI-P) or KC2000 medium (Hazleton Research Products, Denver, Pa.), a serumless medium containing 100 units of penicillin G per ml (technical information bulletin no. 24, 1987; KC Biologicals, Lenexa, Kans.). Viable cells were counted by using trypan blue dye and were adjusted to approximately 5×10^6 viable cells per ml. A 0.2-ml amount of the cell suspension was dispensed into each well of plastic 24-well tissue culture plates (Costar, Cambridge, Mass.) into which had been placed a 12-mm-diameter sterile circular glass coverslip. The PBM were allowed to adhere to the coverslips by incubation for 1 h at 37°C in 5% humidified CO_2. The nonadherent cells were removed by vigorous washing of the coverslips with warm HBSS. A 1.5-ml amount of RPMI-P supplemented with 5% sterile autologous human serum (RPMI-PS) or 1.5 ml of KC2000 medium was added to the cultures. This protocol produced enriched cultures consisting of approximately 90 to 95% PBM, as determined visually by using DIFF-Quick Stain (Dade Diagnostics, Inc., Aquada, P.R.) to yield a final average number of 2.0×10^5 PBM per cover slip, as determined by counting all monocytes in 10 oil immersion fields and multiplying that number by a conversion factor (the area [in square millimeters] of cover slip divided by the area [in square millimeters]

of the 10 oil fields). Uptake assays were performed in 5% CO_2 at 37°C either immediately or after a 24-h incubation.

Serum. Fresh autologous human serum was obtained from peripheral whole blood by using nonheparinized VACUTAINER tubes. The blood was allowed to clot at room temperature for 20 min and then was placed on ice for 30 min. After centrifugation for 1 h at room temperature at 1,500 × *g*, the serum was removed. All sera were passed through a 0.45-μm-pore-size membrane filter (Millipore Corp., Bedford, Mass.).

Mycobacteria. Three clinical isolates were obtained from the microbiology laboratory at Georgetown University Hospital: *M. tuberculosis*, *M. gordonae*, and an *M. avium* complex organism, serotype 3. *M. avium* ATCC 15679 serotype 1, *M. intracellulare* ATCC 13950, *M. kansasii* ATCC 12478, *M. fortuitum* ATCC 6841, *M. scrofulaceum* ATCC 19981, and *M. chelonae* ATCC 14472 were purchased from the American Type Culture Collection, Rockville, Md. All mycobacteria were initially cultured on Middlebrook 7H10 agar plates (REMEL, Lenexa, Kans.) prior to growth in Middlebrook 7H9 broth supplemented with OADC enrichment (Difco Laboratories, Detroit, Mich.) and 0.02% Tween 80. Broth cultures were allowed to grow to logarithmic phase and were then divided into 5-ml fractions. All fractions were stored frozen at −20°C until needed. Prior to use, the mycobacteria were thawed and then repeatedly passed through a 31-gauge needle, resulting in suspensions of mainly single mycobacteria, with some clusters of two to four mycobacteria (assessed by visual inspection by using acid-fast stain). The concentration of mycobacteria in each culture was determined by direct count by using a Petroff-Hausser counting chamber (Hausser Scientific, Blue Bell, Pa.) under phase-contrast illumination.

CVF. Cobra venom factor (CVF) was purified from lyophilized venom of the cobra *Naja naja siamensis* (Serpentarium Laboratories, Salt Lake City, Utah) as previously described (25). This isolation procedure resulted in a product free of phospholipase A_2. For decomplementation of serum, CVF was added to the serum to a final concentration of 50 μg of CVF per 1.0 ml of serum. The serum was incubated at 37°C for 1 h in a water bath. This procedure effectively decomplements the serum (25).

Complement activation by mycobacteria. *M. avium* complex, *M. tuberculosis*, or *M. scrofulaceum* bacteria were suspended in fresh human serum (2.5×10^9/ml). The suspensions were incubated at 37°C for 1 h and centrifuged, and the serum was removed from the mycobacterial pellet. Complement activity was measured in the serum samples by hemolytic titration by using sheep erythrocytes sensitized with a 1:500 dilution of a rabbit anti-sheep erythrocyte antiserum (16). Duplicate samples of twofold dilutions of sera (20 μl) were incubated with 10^8 sensitized erythrocytes (in 20 μl of 3.5 mM Veronal-acetate buffer, pH 7.4, containing 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 0.1% [wt/vol] gelatin) for 20 min at 37°C in a water bath. The reaction was stopped by the addition of 1 ml of Veronal buffer (without metals and gelatin), vortexing, and centrifugation. The released hemoglobin was measured spectrophotometrically at 412 nm. The dilution of serum that gave 50% hemolysis in this assay was determined for every serum sample. The percentage of complement activity was derived by comparison with untreated human serum.

Reagents. EDTA and EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid] were purchased from Fisher Scientific Co., Pittsburgh, Pa. Stock solutions were made with HBSS (Ca^{2+} and Mg^{2+} free) (Hazleton Research Products) as follows: (i) 100 mM EDTA, pH 7.4 (hereafter designated EDTA), or (ii) 100 mM EGTA–100 mM $MgCl_2$, pH 7.4 (hereafter designated EGTA-Mg).

Preincubation of mycobacteria in serum preparations. Four serum preparations were made, as follows. (i) A 0.1-ml amount of EDTA or EGTA-Mg stock solution was added to each 1.0 ml of serum, mixed well, and incubated for 1 h at 37°C, resulting in a final concentration of 10 mM. (ii) Serum was heated to 56°C for 1 h in a water bath. (iii) CVF was added to the serum to a final concentration of 50 μg/1.0 ml of serum and incubated at 37°C for 1 h. (iv) A 0.1-ml amount of HBSS was added to each 1.0 ml of serum, mixed well, and incubated for 1 h at 37°C (serum treatment control) (12, 14). *M. avium* complex or *M. tuberculosis* bacteria were suspended in each serum preparation or in HBSS (2.5×10^9/ml). The suspensions were incubated at 37°C for 1 h and centrifuged, and the serum was removed from the mycobacterial pellet. The pellets were washed three times in HBSS, suspended in RPMI-P, and adjusted to 2.0×10^5 mycobacteria per 10 μl.

Uptake assay. The ability of PBM to take up the various mycobacteria was evaluated. All monocyte-enriched cultures, attached to cover slips as previously described, were washed in warm HBSS and placed into wells containing 0.2 ml of either KC2000, RPMI-P, or RPMI-PS medium. Each mycobacterial suspension was thawed, passed through a 31-gauge needle to produce single-cell suspensions, and adjusted to 2.0×10^5 mycobacteria per 10 μl with HBSS. Ten microliters was added to appropriate wells (approximately one mycobacterium per macrophage). The cultures were incubated at 37°C in 5% humidified CO_2. At 30, 60, 90, and 120 min after addition of mycobacteria, cover slips were removed, rinsed in warm HBSS to remove free mycobacteria, fixed in methanol for 1 min, and stained with acid-fast bacillus (AFB) stain (Fisher Scientific Co., Pittsburgh, Pa.). The number of macrophages with one or more AFB was determined by visual assessment of 200 macrophages per cover slip and recorded as the percentage of macrophages with ≥1 AFB. Our data for uptake represent total cell-associated mycobacteria, i.e., both those attached to and those ingested by monocytes.

Statistical analysis. All experiments were repeated at least three times. The significance of the difference between means was determined by using Student's *t* test and the analysis of variance (5).

RESULTS

The percentage of PBM with ≥1 mycobacterium at various times after the addition of the nine mycobacterial specimens is shown in Fig. 1. After 30 min, there was a significant ($P < 0.05$) difference between one group of mycobacterial species (*M. avium*, *M. intracellulare*, *M. chelonae*, and an *M. avium* complex isolate), which was taken up by a high percentage of monocytes (HP group), and a second group (*M. tuberculosis*, *M. kansasii*, *M. fortuitum*, *M. gordonae*, and *M. scrofulaceum*), which was taken up by a much lower percentage of monocytes (LP group). The difference between the two groups continued to increase during the subsequent incubation period until the HP group was taken up by two to three times more monocytes than was the LP group. *M. scrofulaceum* appeared to be taken up at an intermediate level, significantly different from the level of uptake of both of the other groups at each time point assayed. These experiments utilized 24-h PBM cultures. Preliminary experiments with freshly explanted PBM gave similar results (data not shown).

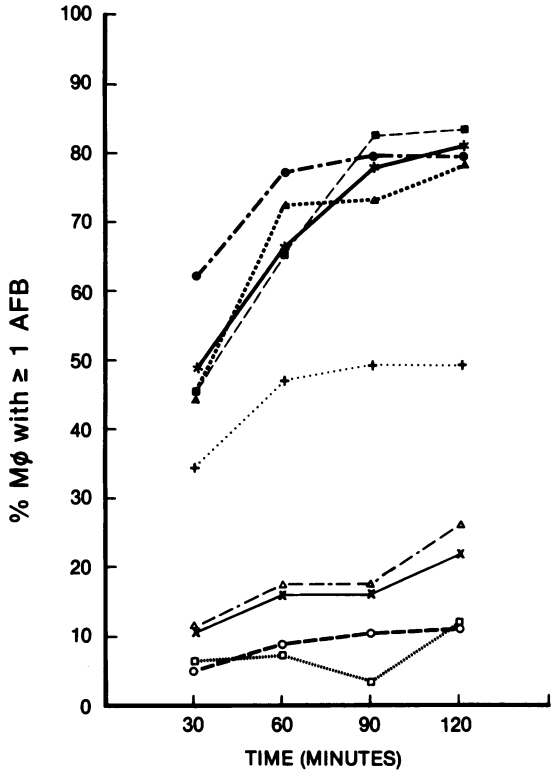

FIG. 1. Uptake of different mycobacteria by human PBM shown as the percentage of PBM with ≥1 AFB. The assay was performed with PBM cultured for 24 h in the presence of 5% autologous serum and in the presence of 5% serum during the uptake period (described in Materials and Methods). The mycobacterium-to-macrophage ratio was 1:1. Values are means; $n = 7$. Symbols: X, *M. tuberculosis*; ○, *M. kansasii*; □, *M. fortuitum*; △, *M. gordonae*; +, *M. scrofulaceum*; ●, *M. avium* complex isolate; ■, *M. intracellulare*; ▲, *M. chelonae*; *, *M. avium*.

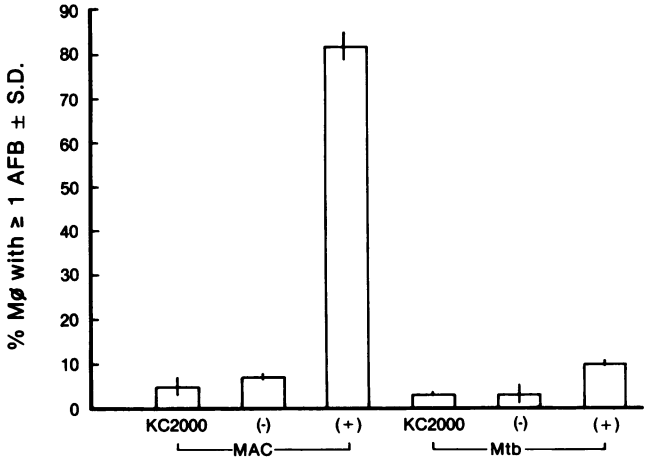

FIG. 2. Effect of the presence (+) or absence (−) of 5% serum during the uptake period. MAC, *M. avium* complex isolate; Mtb, *M. tuberculosis*; KC2000, serumless medium. The mycobacterium-to-macrophage ratio was 1:1.

Further studies were done using the two most clinically important species which we studied (*M. tuberculosis* and *M. avium* complex), which exhibited great differences in uptake by monocytes. Fig. 2 indicates the effects of serum on the uptake of *M. avium* complex organisms and *M. tuberculosis* by PBM. When PBM were cultured in a serumless medium exclusively (KC2000), *M. avium* complex organisms were taken up by only 6.5% of the PBM. If 5% (vol/vol) serum was present during the first 24 h but removed during the uptake period, *M. avium* complex organisms were taken up by 8.5% of the PBM (not significantly different from culture in KC2000). However, if 5% (vol/vol) serum was present during the entire PBM culture period and the uptake period, the percentage of PBM with *M. avium* complex organisms was significantly increased (82.5%) ($P < 0.001$). *M. tuberculosis* was taken up by only 2.5% of the PBM cultured in KC2000 medium alone or cultured without serum during the uptake period only. When 5% (vol/vol) serum was present during the uptake period, the percentage of PBM with *M. tuberculosis* increased to 10% ($P < 0.05$).

Fig. 3 shows the results of experiments to elucidate the mechanism(s) involved in the effect of serum when serum was present during the uptake assays. In these experiments, the mycobacteria had been treated prior to the uptake assay by suspension in HBSS (Plain), normal autologous serum (Neat), serum heated to 56°C for 1 h (H-i), serum treated with CVF, serum treated with 10 mM EDTA (EDTA), or serum treated with 10 mM EGTA–10 mM $MgCl_2$ (EGTA-Mg).

The results obtained with *M. avium* complex are presented in Fig. 3a. The *M. avium* complex organisms which had never been exposed to serum (Plain) were taken up by 15% of the PBM. The *M. avium* complex organisms exposed to normal autologous serum (Neat) were taken up by significantly more PBM (89.8%) ($P < 0.001$). Heating the serum or treating it with CVF or EDTA reduced the serum effect (only 47, 60, and 40%, respectively, of the PBM had taken up *M. avium* complex organisms) ($P < 0.05$ compared with Neat). In contrast, treatment of serum with EGTA-Mg had no significant effect on the serum activity.

The results obtained with *M. tuberculosis* are shown in Fig. 3b. *M. tuberculosis* bacteria never exposed to serum (Plain) were taken up by only 2% of the PBM, while exposure of bacteria to normal autologous serum (Neat) increased their uptake by PBM to 9.1%. Heating the serum or treating it with CVF did not significantly affect the uptake of these bacteria by PBM (5 and 9%, respectively). Both EDTA and EGTA-Mg preparations slightly reduced the effect of serum on uptake of *M. tuberculosis* (4 and 3%, respectively).

Experiments were performed to detect whether *M. tuberculosis* and *M. avium* complex organisms could activate complement. Under our experimental conditions, *M. avium* complex organisms reduced hemolytic complement activity by approximately 51.1 ± 18.0%, *M. avium* ATCC 15679 reduced complement activity by 64.7 ± 20.7%, *M. scrofulaceum* reduced complement activity by 70.0 ± 7.4%, and *M. tuberculosis* reduced complement activity by 32.2 ± 8.7%.

DISCUSSION

We demonstrate in this study that major differences exist between mycobacterial species, as evident in the percentages of human PBM involved in the uptake of the mycobacteria. The molecular factors which determine whether a particular species of mycobacterium will be taken up by many or few monocytes are unknown. Most likely, several factors of both host and mycobacterial origin are involved.

The differences in uptake may be due to variation between the number or type of surface molecules on the different

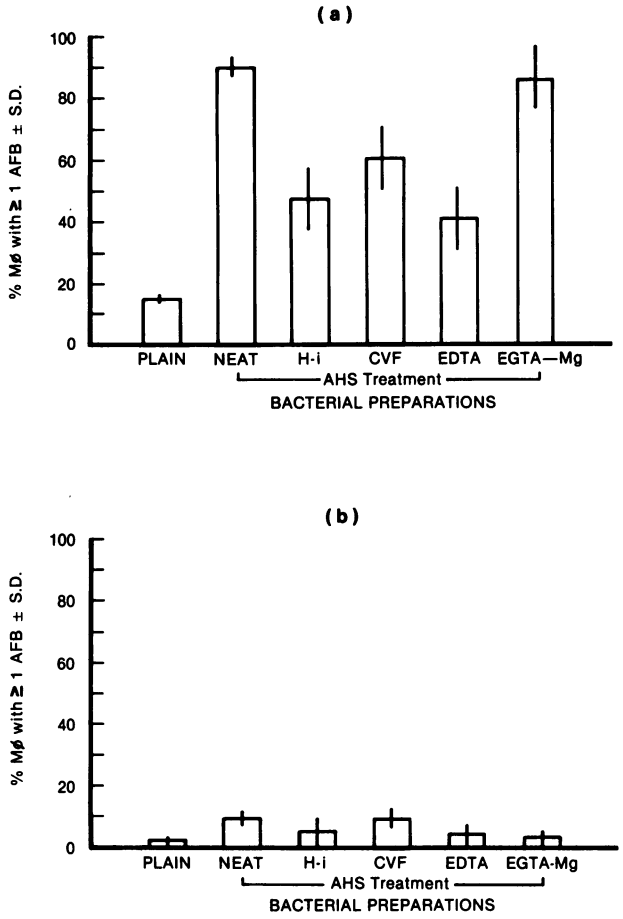

FIG. 3. Effect of preincubation of *M. avium* complex organisms (a) or *M. tuberculosis* (b) with different serum preparations. Mycobacteria were pretreated with HBSS (Plain), normal autologous serum (Neat), serum heated to 56°C for 1 h (H-i), serum treated with cobra venom factor (CVF), serum treated with 10 mM EDTA (EDTA), or serum treated with 10 mM EGTA–10 mM $MgCl_2$ (EGTA-Mg). The mycobacterium-to-macrophage ratio was 1:1.

mycobacteria and corresponding receptors on the PBM which aid ingestion. A wide variety of antigenic structures have been found on the surfaces of mycobacteria (4, 10, 19). In addition, macrophages have been shown to have many different receptors on their surfaces, depending upon the phagocyte population or level of maturity studied (6, 23, 24, 26). Thus, monocyte receptors for carbohydrates and proteins found on the surfaces of mycobacteria may be an initial, nonimmune mechanism by which some of the PBM of the infected host can recognize and phagocytize the infecting mycobacteria. The uptake of *M. avium* complex organisms and of *M. tuberculosis* in KC2000 serumless medium supports this type of interaction.

The data presented here indicate that host serum components, especially complement, affect the initial interactions between the invading mycobacterium and the host's mononuclear phagocytes. This result is in contrast to the result of other work (3) on the phagocytosis and killing of *M. avium* complex organisms by human PBM which involved different experimental conditions, such as much greater multiplicities of infection, pooled human serum, and a 24-h mycobacterium-PBM incubation time. The investigators concluded that serum did not significantly alter phagocytosis of *M. avium* complex organisms. In our study, we used a lower mycobacterium-to-PBM ratio (1:1), a shorter incubation time (2 h), and autologous human serum, conditions which may more closely simulate what is occurring in situ in humans.

Uptake of *M. avium* complex organisms and of *M. tuberculosis* by PBM was enhanced when serum was present during the uptake period (Fig. 2). This effect of serum is mediated by substances opsonizing onto the mycobacterial surface (Fig. 3) rather than by the serum directly activating the PBM, as has been shown to occur in other systems (26). The results obtained after heating the serum to 56°C for 1 h or after treating the serum with CVF indicate that complement is a major constituent of the substances opsonizing onto the *M. avium* complex organism surface. The chelators EDTA and EGTA-Mg were used to elucidate the complement pathway activated by *M. avium* complex organisms. EDTA effectively blocks both the classical pathway and the alternative pathway of complement activation. EGTA-Mg blocks only the classical activation pathway. Treatment of normal human serum with EDTA reduced the effect of serum by 55%, while treatment with EGTA-Mg did not reduce its effect. This pattern of the effect of the chelators on serum is indicative of the alternative pathway activation of complement by *M. avium* complex organisms (15). Uptake of *M. tuberculosis* is much less dependent on serum components, including complement.

The hemolytic assays for complement activity indicate that *M. avium* complex organisms, *M. avium* ATCC 15679, *M. tuberculosis*, and *M. scrofulaceum* activate complement. These observations are in agreement with findings with other species of mycobacteria, such as *Mycobacterium bovis* BCG (Glaxo strain), *M. leprae*, and *M. lepraemurium*, which have been reported to be capable of activating the alternative pathway (20, 21). A recent preliminary study has indicated that *M. tuberculosis* may bind C3 (N. R. Payne, C. G. Bellinger-Kawahara, and M. A. Horwitz, Clin. Res. **35:** 617A, 1987).

Monocytes express the complement receptor CR1 (the classical immune adherence receptor with specificity for C3b) and the receptors CR3 and CR4, which bind to the C3b degradation products iC3b, C3dg, and C3d (23). Further work will have to be done to identify the form(s) of C3 present on the mycobacteria since both *M. avium* complex organisms and *M. tuberculosis* activate complement but only the uptake of the *M. avium* complex organisms is dramatically dependent on complement. One might expect that the C3b form is present on the *M. avium* complex, but that is degraded to iC3b, C3dg, and C3d on *M. tuberculosis* in a manner similar to that which has been shown with complement-susceptible and complement-resistant tumor cells (17, 18).

Many healthy persons have been shown to have serum antibodies to mycobacteria (1). It remains to be determined whether some part of the heat-stable serum effect on mycobacterial uptake may in fact be due to the presence of anti-mycobacterium antibody.

In conclusion, serum components, especially complement, are of importance in the in vitro uptake of mycobacteria, in particular of *M. avium* complex organisms, by human PBM. The in vivo significance of our observations remains to be determined, but our results suggest that humoral factors in addition to cellular factors may be involved in the body's defense against mycobacteria.

ACKNOWLEDGMENTS

We thank S. D. Chaparas, G. V. Kadival, and A. Andersen of the Food and Drug Administration for helpful advice; E. Delaha (Georgetown Hospital) for providing mycobacterial cultures; E. C. Petrella (Department of Biochemistry) for performing the hemolytic complement assays; and Anna Y. Tsang, National Jewish Center for Immunology and Respiratory Disease, Denver, Colo., for serotyping the *M. avium* complex isolate.

This work was supported in part by Public Health Service grants CA35525 and AI25849 from the National Institutes of Health. C.-W. Vogel is the recipient of Research Career Development Award CA01039 from the National Cancer Institute.

LITERATURE CITED

1. **Bardana, E. J., Jr., J. K. McClatchy, R. S. Farr, and P. Minden.** 1973. Universal occurrence of antibodies to tubercle bacilli in serum from non-tuberculous and tuberculous individuals. Clin. Exp. Immunol. **13**:65–77.
2. **Bermudez, L. E., and L. S. Young.** 1988. Tumor necrosis factor alone or in combination with IL-2, but not IFN-γ, activates macrophages to kill Mycobacterium avium complex. J. Immunol. **140**:3006–3013.
3. **Bermudez, L. E. M., and L. S. Young.** 1987. Phagocytosis and intracellular kill of *Mycobacterium avium* complex by human and murine macrophages. Brazil J. Med. Biol. Res. **20**:191–201.
4. **Brennan, P. J.** 1984. Antigenic peptidoglycolipids, phospholipids and glycolipids, p. 467–490. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook, vol. 15, part A. Marcel Dekker, Inc., New York.
5. **Brown, W. B., Jr., and M. Hollander.** 1977. Statistics: a biomedical introduction. John Wiley & Sons, Inc., New York.
6. **Bullock, W. E., and S. D. Wright.** 1987. Role of the adherence-promoting receptors, CR3, LFA-1, and p150.95, in binding of *Histoplasma capsulatum* by human macrophages. J. Exp. Med. **165**:195–210.

6a. **Centers for Disease Control.** 1988. Tuberculosis, final data—United States, 1986. Morbid. Mortal. Weekly Rep. **36**:817–820.

7. **Chaparas, S. D.** 1982. The immunology of mycobacterial infections. Crit. Rev. Microbiol. **9**:139–179.
8. **Collins, F. M.** 1982. The immunology of tuberculosis. Am. Rev. Respir. Dis. **125**(2):42–49.
9. **Crowle, A. J.** 1986. Studies of antituberculous chemotherapy with an in vitro model of human tuberculosis. Semin. Respir. Infect. **1**:262–264.
10. **Daniel, T. M.** 1984. Soluble mycobacterial antigens, p. 417–466. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook, vol. 15, part A. Marcel Dekker, Inc., New York.
11. **Edwards, D., and C. H. Kirkpatrick.** 1986. The immunology of mycobacterial disease. Am. Rev. Respir. Dis. **134**:1062–1071.
12. **Fine, D. R., S. R. Marney, Jr., D. G. Colley, J. S. Sargent, and R. M. Desprez.** 1972. C3 shunt activation in human serum chelated with EGTA. J. Immunol. **109**:807–811.
13. **Horsburgh, C. R., U. G. Mason III, D. C. Farhi, and M. D. Iseman.** 1985. Disseminated infection with *Mycobacterium avium-intracellulare* infection. Medicine (Baltimore) **64**:36–48.
14. **Kitz, D. J., C. R. Johnson, G. S. Kobayashi, G. Medoff, and J. R. Little.** 1984. Growth inhibition of *Cryptococcus neoformans* by cloned cultured murine macrophages. Cell. Immunol. **88**:489–500.
15. **Lachmann, P. J.** 1976. The immunochemistry of complement, p. 56–75. *In* M. J. Hobart and I. McConnell (ed.), The immune system: a course on the molecular and cellular basis of immunity, 1st ed. Blackwell Scientific Publications, Ltd., Oxford.
16. **Mayer, M. M.** 1961. Complement and complement fixation, p. 133–145. *In* E. A. Kabat and M. M. Mayer (ed.), Experimental immunochemistry, 2nd ed. Charles C Thomas, Publisher, Springfield, Ill.
17. **Panneerselvam, M., R. Bredehorst, and C.-W. Vogel.** 1986. Immobilized doxorubicin increases the complement susceptibility of human melanoma cells by protecting complement component C3b against inactivation. Proc. Natl. Acad. Sci. USA **83**: 9144–9148.
18. **Panneerselvam, M., S. Welt, L. J. Old, and C.-W. Vogel.** 1986. A molecular mechanism of complement resistance of human melanoma cells. J. Immunol. **136**:2534–2541.
19. **Petit, J.-F., and E. Lederer.** 1984. The structure of the mycobacterial cell wall, p. 301–314. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook, vol. 15, part A. Marcel Dekker, Inc., New York.
20. **Ramanathan, V. D., J. Curtis, and J. L. Turk.** 1980. Activation of the alternative pathway of complement by mycobacteria and cord factor. Infect. Immun. **29**:30–35.
21. **Rieger, M., L. Trnka, J. Skvor, and P. Mison.** 1979. Immunoprofile studies in patients with pulmonary tuberculosis. III. Study of haemolytic complement in serum and phagocytic activity of blood neutrophils. Scand. J. Respir. Dis. **60**:172–175.
22. **Rook, G. A. W.** 1987. Progress in the immunology of the mycobacterioses. Clin. Exp. Immunol. **69**:1–9.
23. **Ross, G. D. (ed.).** 1986. Opsonization and membrane complement receptors, p. 87–114. *In* Immunobiology of the complement system. Academic Press, Inc., New York.
24. **Stahl, P., and S. Gordon.** 1982. Expression of a mannosyl-fucosyl receptor for endocytosis on cultured primary macrophages and their hybrids. J. Cell Biol. **93**:49–56.
25. **Vogel, C.-W., and H. J. Muller-Eberhard.** 1984. Cobra venom factor: improved method for purification and biochemical characterization. J. Immunol. Methods **73**:203–220.
26. **Werb, Z.** 1987. Phagocytic cells: Chemotaxis and effector functions of macrophages and granulocytes, p. 96–113. *In* D. P. Stites, J. D. Stobo, and J. V. Wells (ed.), Basic and clinical immunology, 6th edition. Lange Medical Publications, Los Altos, CA.
27. **Young, L. S., C. B. Inderlied, O. G. Berlin, and M. S. Gottlieb.** 1986. Mycobacterial infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. Rev. Infect. Dis. **8**(6):1024–1033.
28. **Zakowski, P., S. Fligiel, O. G. W. Berlin, and B. L. Johnson Jr.** 1982. Disseminated *Mycobacterium avium-intracellulare* infection in homosexual men dying of acquired immunodeficiency. J. A. M. A. **248**:2980–2982.