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Differences in Phagocytosis and Killing by Alveolar Macrophages from Humans, Rabbits, Rats, and Hamsters

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Phagocytosis and killing by alveolar macrophages from humans, rabbits, rats, and hamsters were compared in vitro. In the absence of serum opsonins, human alveolar macrophages could phagocytize *Staphylococcus aureus* Cowan I (protein A positive), but not *S. aureus* EMS (protein A negative) or *Pseudomonas aeruginosa* MN. In contrast, rabbit, rat, and hamster alveolar macrophages did not phagocytize *S. aureus* Cowan I or other nonopsonized bacteria. Human alveolar macrophages, but not other species, stained positively with fluorescein isothiocyanate-conjugated protein A. When opsonized bacteria were studied, phagocytosis by human, rabbit, and hamster alveolar macrophages was found to be mediated by both Fc and C3 receptors. However, only Fc receptor-mediated phagocytosis of bacteria was demonstrated for rat alveolar macrophages. Differences were also found in the kinetics of bacterial killing by alveolar macrophages from different species. Human and rabbit alveolar macrophages rapidly killed opsonized *S. aureus* Cowan I. However, bacterial killing by hamster alveolar macrophages proceeded at a slower rate, and rat alveolar macrophages completely failed to kill *S. aureus*. These significant differences in the function of alveolar macrophages from four different species emphasize the need to document the appropriateness of animal models before using them to predict the biological activities of human alveolar macrophages.

Alveolar macrophages (AM) constitute an important defense against inhaled microorganisms (4–6, 17). Knowledge regarding the phagocytic and bactericidal mechanisms of AM has been derived largely from studies with animal AM. The validity of extrapolating results from these studies to the functions of human AM has received little attention and needs to be assessed.

Our recent finding that, in contrast to rabbit AM (13), human AM can phagocytize certain bacterial isolates in the absence of opsonins (8a) has prompted this comparative study of the phagocytic and killing functions of AM from humans, rabbits, rats, and hamsters. The results indicate that AM from these species differ in their abilities to recognize, ingest, and subsequently kill bacteria.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. Two strains of *Staphylococcus aureus*, Cowan I and EMS (a protein A-deficient mutant of Cowan I provided by A. Forsgren, Malmö University, Malmö, Sweden) and a strain of *Pseudomonas aeruginosa* MN (a nonmucoid revertant from a mucoid strain recovered from a patient with cystic fibrosis, kindly provided by D. Speert, Children's Hospital, Vancouver, Canada) were used. All strains were kept on tryptic soy agar with 5% sheep blood at 4°C.

For each experiment, bacteria were grown in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) in a 37°C shaking incubator (New Brunswick Scientific Co., New Brunswick, N.J.) for 16 h, washed three times in phosphate-buffered saline (pH 7.4), and suspended to 5×10^8/ml in Hanks balanced salt solution containing 0.1% gelatin (GHBSS). Bacterial counts were determined with a Coleman 295 Spectrophotometer (Perkin-Elmer Corp., Oak Brook, Ill.) and confirmed initially by counting in a Petroff-Hauser Chamber (Hauser, Philadelphia, Pa.). For phagocytosis studies, bacteria were radioactively labeled by adding 20 μCi of [2-^{3}H]adenine (specific activity, 15.5 Ci/mmol; New England Nuclear Corp., Boston, Mass.) to Mueller-Hinton broth, as previously described (15, 19). Radioactively labeled bacteria were grown, washed, and counted as described above.

Isolation of AM. After informed consent, human AM were obtained from normal donors (smokers and nonsmokers) by subsegmental saline lavage of the lingula of the left lung or the middle lobe of the right lung, as previously described (9). This investigation was approved by the Committee on the Use of Human Subjects in Research of the University of Minnesota Health Sciences Center. AM were also obtained from 2- to 2.5-kg wild rabbits, 275- to 325-g Sprague-Dawley

rats, and 100-g Syrian golden hamsters by a standard lung lavage technique (8). After the lavage, AM were washed three times with GHBSS and suspended to a concentration of 5×10^6/ml of GHBSS. Purity of alveolar macrophages was ≥85%, and viability, as determined by trypan blue exclusion, was ≥90% in all cases.

Opsonins and preopsonization of bacteria. Human blood was obtained by venipuncture and allowed to clot, and the serum was recovered by centrifugation, pooled, and frozen at −70°C. Rabbit, rat, and hamster sera obtained by cardiac puncture were similarly prepared. Just before use, pooled human, rabbit, rat, and hamster sera were thawed and diluted to the desired concentration with GHBSS. Heat-inactivated serum was obtained by heating serum in a 56°C water bath for 30 min.

For phagocytosis and killing studies, 0.1 ml of bacteria at 5×10^8/ml were preopsonized in polypropylene vials (Bio-vials; Beckman Instruments, Inc., Chicago, Ill.) with 0.9 ml of opsonin at the desired concentration for 15 min in a 37°C shaking incubator. At the end of incubation, bacteria were centrifuged at $2,000 \times g$ for 15 min (4°C) and resuspended in 1 ml of GHBSS. Nonopsonized bacteria were prepared as described above, except GHBSS was used instead of serum.

Phagocytosis. Phagocytosis of bacteria by AM was assessed by measuring the uptake of radiolabeled bacteria with an assay described in detail previously (14). Briefly, 0.1 ml of nonopsonized bacteria or bacteria opsonized in 10% autologous serum (5×10^7/ml) were mixed with 0.1 ml of AM (5×10^6/ml) in duplicate polypropylene vials, and phagocytosis was allowed to proceed for 15 min in a 37°C shaking incubator. Phagocytosis was stopped by adding 3 ml of cold phosphate-buffered saline to one sample from each pair. This sample was then centrifuged at $160 \times g$ for 5 min (4°C) and washed three more times to remove nonphagocyte-associated bacteria. The final pellet was suspended in scintillation liquid (Aquasol-2; New England Nuclear Corp.) and counted in a liquid scintillation counter (Beckman LS-250). To the other sample, scintillation liquid was added immediately after incubation, and the total number of radiolabeled bacteria added was counted (total counts per minute). The percent uptake was calculated as follows: percent uptake = (counts per minute of washed AM cell pellet [160 g]/total counts per minute) × 100.

Detection of surface immunoglobulins. Surface immunoglobulins on AM were detected by a modified method of a cell surface immunoglobulin assay described previously (3). Briefly, 10^6 phagocytes in phosphate-buffered saline containing 2% fetal calf serum and 15 mg of sodium azide per 100 ml were mixed with fluorescein isothiocyanate-conjugated protein A (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) at 1:20 dilution and incubated at 4°C for 45 min. At the end of incubation, the cells were washed three times with the same phosphate-buffered saline (5 min at 1,200 rpm). The supernatant was then discarded, and a drop from the final pellets was placed on a clean slide and sealed with a cover slip. Slides were read on a Zeiss universal photomicroscope equipped with epifluorescence and phase-contrast systems.

Killing of *S. aureus* Cowan I by AM. Bacterial killing by AM was determined by a microtitration assay which quantitated the number of live *S. aureus* Cowan I cells after 0, 15, and 120 min of incubation with AM from each species. *S. aureus* Cowan I cells were preopsonized in 10% autologous serum as described above. For each incubation time, triplicate samples of 0.1 ml of opsonized bacteria (5×10^7/ml) and 0.1 ml of phagocytes (5×10^6/ml) were incubated at 37°C in a shaking incubator (200 rpm). Bacterial killing was interrupted by adding 2.8 ml of cold, sterile distilled water to the samples. The sample was blended vigorously in a Vortex mixer, and a 1:200 dilution was made in 10 ml of sterile distilled water. Finally, 0.1 ml of this dilution was transferred to a U-shaped well of a 96-well tissue culture plate with cover (Flow Laboratories, Inc., Rockville, Md.) containing 0.1 ml of Mueller-Hinton broth in all wells. Serial twofold dilutions were performed. The microtiter plates were incubated at 37°C overnight, and the next day the number of live bacteria was calculated from the highest dilution that gave visible growth. The percentage of killing was determined for indicated time points as follows: percent killing = 100% − [(bacteria alive at indicated time/bacteria alive at 0 min) × 100].

RESULTS

Phagocytosis of nonopsonized bacteria by AM from different animal species. To determine whether AM from laboratory animals could ingest nonopsonized bacteria in a manner similar to that of human AM, phagocytosis of nonopsonized *S. aureus* Cowan I, *S. aureus* EMS (protein A-deficient mutant of Cowan I), and *P. aeruginosa* MN (Fig. 1) was measured. After 15 min of incubation, there was a significant uptake of nonopsonized *S. aureus* Cowan I by human AM (82%). In sharp contrast, there was little uptake of this strain by AM from rabbits, rats, and hamsters (<15%). Thus, only human AM could recognize and ingest protein A-positive *S. aureus* Cowan I. There was no significant uptake of nonopsonized *S. aureus* EMS or of *P. aeruginosa* MN by AM from all four species (<10%).

In our previous studies with human AM, we have found that the mechanism of nonopsonic recognition of *S. aureus* Cowan I by human AM was mediated by AM surface immunoglobulins (immunoglobulin G [IgG] class). Protein A-rich strains of *S. aureus*, such as *S. aureus* Cowan I, were bound to AM surface immunoglobulins and efficiently phagocytized by human AM (H. A. Verbrugh, B. T. Nguyen, J. R. Hoidal, and P. K. Peterson, Clin. Res. **29**:398A, 1981). AM from rabbits, rats, and hamsters did not recognize nonopsonized *S. aureus* Cowan I, and the presence of such surface immunoglobulins on these AM was determined by staining with fluorescein isothiocyanate-conjugated protein A. Human AM showed positive fluorescence with fluorescein isothiocyanate-conjugated protein A, but AM from the animals studied lacked surface IgG reactive to protein A.

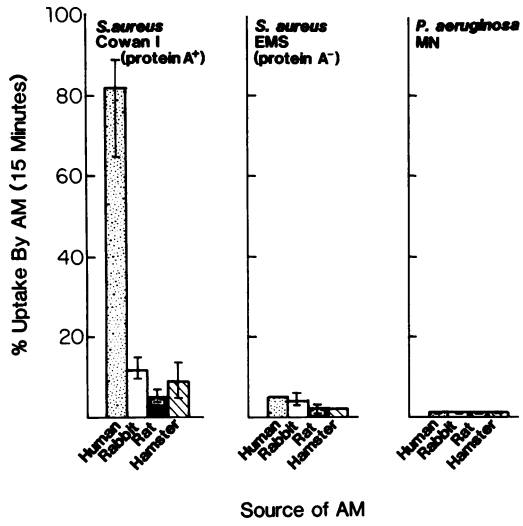

FIG. 1. Phagocytosis of nonopsonized *S. aureus* Cowan I, the protein A-deficient mutant *S. aureus* EMS, and *P. aeruginosa* MN by AM from humans, rabbits, rats, and hamsters. The percent uptake of bacteria by AM was measured after 15 min of incubation. Results represent mean values and ranges of at least three separate experiments.

Phagocytosis of opsonized bacteria by AM from different animal species. We next determined whether uptake of opsonized bacteria by AM from humans, rabbits, rats, and hamsters was similar. *S. aureus* Cowan I, *S. aureus* EMS, and *P. aeruginosa* MN opsonized in 10% autologous serum were used to compare the opsonic recognition by AM from all four species (Fig. 2). After opsonization, all strains were efficiently phagocytized by AM from humans and rabbits. In contrast, less efficient phagocytosis of all strains, especially *P. aeruginosa* MN, was observed with rat AM. Hamster AM, on the other hand, showed good uptake of *S. aureus* Cowan I but phagocytized *S. aureus* EMS and *P. aeruginosa* MN less well. Thus, there appears to be great variation among animal species in the ability of AM to recognize and phagocytize bacteria opsonized in autologous serum.

A single serum pool, 10% pooled human serum (PHS), was also used in the comparison of the efficiency of all four species of AM to phagocytize opsonized *S. aureus* Cowan I, *S. aureus* EMS, and *P. aeruginosa* MN. AM from humans and rabbits efficiently phagocytized all three strains of bacteria (uptake by human and rabbit AM, ≥60 and ≥48%, respectively). Hamster AM were less efficient in phagocytizing the three strains of bacteria opsonized in 10% PHS. The percent uptake of *S. aureus* Cowan I, *S. aureus* EMS, and *P. aeruginosa* MN by hamster AM was 22, 22, and 26%, respectively. No significant uptake of *S. aureus* Cowan I, *S. aureus* EMS, or *P. aeruginosa* MN opsonized in 10% PHS was observed with rat AM (≤8%). Thus, human, rabbit, rat, and hamster AM still differed in their efficiencies to phagocytize opsonized bacteria when PHS was substituted for autologous sera.

In addition, comparison of the above data with results obtained from experiments with 10% autologous serum (Fig. 2) indicates that PHS and rabbit serum can be used interchangeably to study phagocytosis of opsonized bacteria by rabbit AM. However, there appears to be species specificity for opsonins with hamster and rat AM. To avoid potential problems with species specificity for opsonins of the AM, autologous sera were used in the rest of this study.

Detection of receptors for heat-stable and heat-labile opsonins on AM from different animal species. Receptors on human, rabbit, rat, and hamster AM were studied by measuring the uptake of *S. aureus* EMS opsonized in heated serum. The uptake of *S. aureus* EMS opsonized in 10% heat-inactivated autologous serum was 75% for human AM, 34% for rabbit AM, 59% for rat AM, and 16% for hamster AM (Fig. 3). This compared with ≤5% uptake of nonopsonized *S. aureus* EMS by AM from all four species (Fig. 1). These data suggest that AM from all four

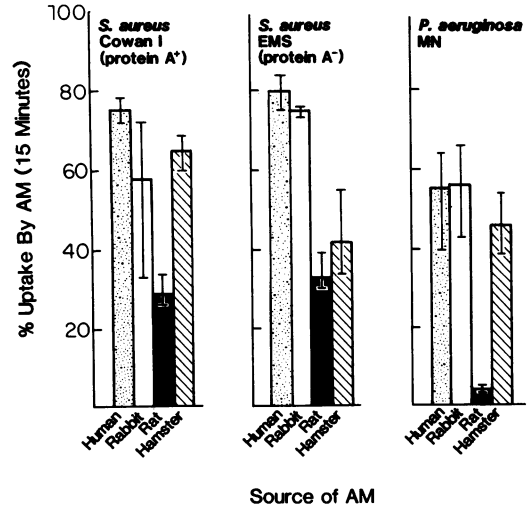

FIG. 2. Phagocytosis of opsonized *S. aureus* Cowan I, *S. aureus* EMS, and *P. aeruginosa* MN by AM from humans, rabbits, rats, and hamsters. Opsonized bacteria were obtained by incubation with 10% autologous serum for 15 min before addition to the AM. Percent uptake of bacteria by AM was determined after 15 min of incubation.

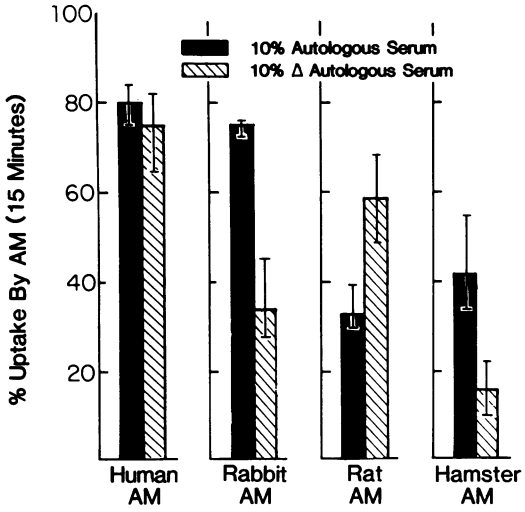

FIG. 3. Phagocytosis of opsonized *S. aureus* EMS by AM from humans, rabbits, rats, and hamsters. Bacteria were opsonized in 10% autologous serum or 10% heat-inactivated (△) autologous serum before being added to AM and measuring uptake at 15 min.

species have Fc receptors for a heat-stable serum opsonin, which can mediate phagocytosis of *S. aureus*.

To investigate the receptors for heat-labile opsonins on the AM from humans, rabbits, rats, and hamsters, the uptake of *S. aureus* EMS opsonized in fresh autologous serum was examined. Enhanced uptake by rabbit and hamster AM was observed when *S. aureus* EMS was opsonized in fresh rabbit and hamster sera, suggesting that these AM possess membrane receptors for heat-labile opsonins. Enhanced uptake was not observed with human or rat AM when *S. aureus* EMS was opsonized in fresh serum. Indeed, depressed uptake of *S. aureus* EMS opsonized in fresh rat serum was observed with rat AM (Fig. 3).

The equally efficient uptake by human AM of *S. aureus* EMS opsonized in fresh and in heat-inactivated serum precluded any comment on the presence and function of AM receptors for heat-labile opsonins. To investigate further the presence of the receptors for heat-labile opsonins on human AM, *P. aeruginosa* MN opsonized by fresh PHS and heat-inactivated PHS was used (Fig. 4). The importance of heat-labile serum factors in the phagocytosis of these bacteria was apparent at low concentrations of serum (1%). When *P. aeruginosa* MN was opsonized in heat-inactivated serum, the uptake at 5, 15, and 30 min was ≤11%. When fresh serum was used, the uptake at 5, 15, and 30 min was 38, 50, and 69%, respectively. This enhancement of uptake indicates that, like rabbit and hamster AM, human AM possess membrane receptors for heat-labile opsonins that are involved in phagocytosis of *P. aeruginosa*.

The lack of enhanced uptake of *S. aureus* EMS opsonized in fresh rat serum by rat AM (Fig. 3) and the failure of rat AM to phagocytize opsonized *P. aeruginosa* MN (Fig. 2) suggested that either rat AM did not have receptors for heat-labile opsonins or rat serum lacked heat-labile opsonins. The latter possibility was examined by measuring human AM uptake of *P. aeruginosa* MN opsonized in 10% fresh rat serum and 10% heat-inactivated rat serum. After 15 min of incubation, uptake was 35 and 4%, respectively (data not shown), suggesting that rat serum contains heat-labile opsonins but that rat AM do not have receptors for heat-labile opsonins.

Bacterial killing efficiency of AM from different animal species. Having established some of the characteristics of phagocytosis by AM from humans, rabbits, rats, and hamsters, the next goal was to evaluate their bactericidal activity.

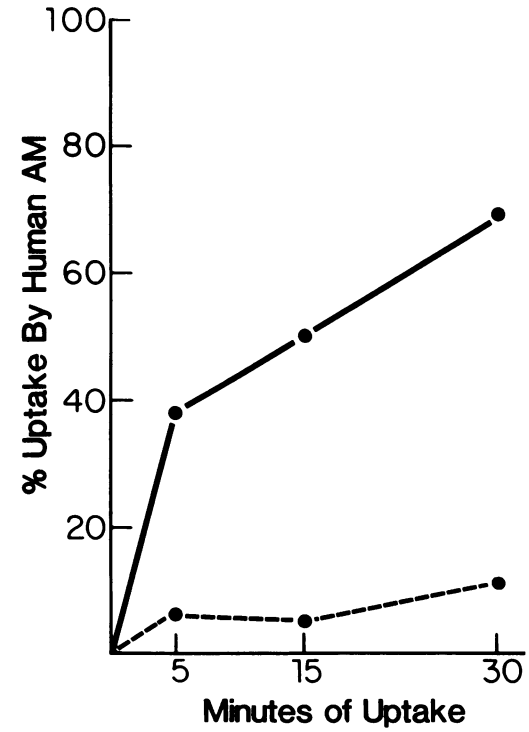

FIG. 4. Human AM phagocytosis of *P. aeruginosa* MN opsonized in 1% serum (●—●) and 1% heat-inactivated (△) serum (●---●). Percent uptake was determined after 5, 15, and 30 min of incubation with AM.

This was done by using *S. aureus* Cowan I, which was phagocytized equally by AM from all four species when opsonized in 10% autologous serum. After 15 min of incubation, human and rabbit AM killed 66 and 62%, respectively, of opsonized *S. aureus* Cowan I, whereas there was no detectable killing by either rat or hamster AM (Table 1). After 120 min of incubation, human, rabbit, rat, and hamster AM killed 75, 88, 0, and 63%, respectively, of opsonized *S. aureus* Cowan I. Thus, killing by hamster AM proceeded at a slower rate than that by human and rabbit AM; rat AM completely failed to kill bacteria.

TABLE 1. Killing of opsonized *S. aureus* Cowan I by AM from humans, rabbits, rats, and hamsters[a]

AM source	% Killing of opsonized *S. aureus* Cowan I at min[b]:	
	15	120
Humans	66 ± 3	75 ± 8
Rabbits	62 ± 8	88 ± 9
Rats	0	0
Hamsters	0	63 ± 9

[a] *S. aureus* Cowan I was preopsonized in 10% autologous serum before being added to AM, and the percentage of bacteria that were killed was determined after 15 and 120 min of incubation, as described in the text.

[b] Results represent mean ± standard error of the mean of three or four experiments.

DISCUSSION

Results of the current investigation indicate that AM from humans, rabbits, rats, and hamsters differ significantly in their abilities to phagocytize and kill bacteria. Only human AM have surface IgG which mediates phagocytosis of nonopsonized protein A-rich *S. aureus*. Human, rabbit, and hamster AM have Fc and C3 receptors facilitating the uptake of opsonized bacteria. Only Fc receptor-mediated phagocytosis could be demonstrated for rat AM. Although hamster AM can phagocytize opsonized *S. aureus* Cowan I as efficiently as human rabbit AM, the rate of bacterial killing by hamster cells is considerably slower than that of human and rabbit AM.

This investigation indicates that uptake of nonopsonized bacteria by human AM is dependent on the presence of bacterial protein A. Protein A-positive *S. aureus* Cowan I could be recognized and ingested efficiently in the absence of opsonins. However, efficient uptake of its protein A-deficient mutant, *S. aureus* EMS, and *P. aeruginosa* MN required an opsonic source. Reports from previous studies have shown that efficient uptake of nonopsonized bacteria by human AM is dependent on the species of bacteria (8a). Hoidal et al. (8a) have shown that efficient uptake of *S. aureus* 502A, which has surface protein A, by human AM does not require opsonins, whereas effective uptake of an *Escherichia coli* strain is dependent on heat-labile opsonins, presumably complement. Other investigators have reported that bacterial species such as *Streptococcus pneumoniae* (7) and *P. aeruginosa* (16) require either complement or specific antibodies for efficient uptake by human AM.

It is well known that protein A binds to the Fc fragment of IgG in a pseudoimmune reaction (2, 11). Also, the presence of surface or cytophilic antibodies that can bind to macrophages via their Fc region has been demonstrated previously (1, 18). Verbrugh et al. (H. A. Verbrugh et al., Clin. Res. **29**:398A, 1981) have demonstrated that only protein A-positive staphylococci such as Cowan I can be phagocytized by human AM in the absence of opsonins via a protein A receptor on human AM. This protein A receptor was found to be surface IgG antibodies present on the membrane of human AM from normal donors.

The inefficiency of rabbit, rat, and hamster AM in phagocytizing nonopsonized *S. aureus* and the lack of interaction between these phagocytes and fluorescein isothiocyanate-conjugated protein A suggest that AM from these species do not possess a mechanism for recognition of nonopsonized bacteria. Several previous investigations support our finding that rat AM are inefficient in phagocytizing *S. aureus* (10, 12) and that complement and antibodies are needed to promote efficient uptake of *S. aureus* and *P. aeruginosa* by rabbit AM (13).

The absence of a mechanism for nonopsonic recognition may be due either to the absence of surface IgG on these AM or lack of anti-protein A reactivity of these immunoglobulins. It has been reported that human IgG3 lacks anti-protein A reactivity, whereas IgG1, IgG2, and IgG4 give a precipitin reaction with protein A (11). Whether rabbit, rat, and hamster AM possess surface immunoglobulins that do not bind to protein A requires further studies.

The current investigation also demonstrates that, although human, rabbit, and hamster AM are similar in their abilities to phagocytize *S. aureus* Cowan I opsonized in autologous serum, they differ in their capacities for killing *S. aureus*. In comparison with human and rabbit AM, the rate of killing of *S. aureus* Cowan I by hamster AM was slow in spite of normal phagocytosis. Inefficiency of the rat AM in killing opsonized *S. aureus* was probably a reflection of poor phagocytosis of this bacteria by rat AM and might be due, in part, to a rather unique need of these cells for alveolar lining material (10, 12).

In summary, the marked qualitative and quantitative differences in bacterial phagocytosis and killing by AM from humans, rabbits, rats, and hamsters underscore the importance of documenting the appropriateness of animal models before using them to predict the biological activities of human AM.

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LITERATURE CITED

1. **Berken, A., and B. Benacerraf.** 1966. Properties of antibodies cytophilic for macrophages. J. Exp. Med. **123**:119–144.
2. **Forsgren, A., and J. Sjoquist.** 1966. "Protein A" from *S. aureus*. I. Pseudoimmune reaction with human γ-globulin. J. Immunol. **97**:822–827.
3. **Gajl-Peczalska, K. J., J. A. Hansen, C. D. Bloomfield, and R. A. Good.** 1973. B lymphocytes in untreated patients with malignant lymphoma and Hodgkin's disease. J. Clin. Invest. **52**:3064–3073.
4. **Golde, D. W.** 1977. Kinetics and function of the human alveolar macrophage. RES J. Reticuloendothel. Soc. **22**:223–230.
5. **Goldstein, E., W. Lippert, and D. Warshauer.** 1974. Pulmonary alveolar macrophage: defender against bacterial infection of the lung. J. Clin. Invest. **54**:519–528.
6. **Hocking, W. G., and D. W. Golde.** 1979. The pulmonary-alveolar macrophage. N. Engl. J. Med. **301**:580–587.
7. **Hof, D. G., J. E. Repine, P. K. Peterson, and J. R. Hoidal.** 1980. Phagocytosis by human alveolar macrophages and neutrophils: qualitative differences in the opsonic requirements for uptake of *Staphylococcus aureus* and *Streptococcus pneumonia in vitro*. Am. Rev. Respir. Dis. **121**:65–71.
8. **Hoidal, J. R., G. D. Beall, F. L. Rasp, Jr., B. Holmes, J. G. White, and J. E. Repine.** 1978. Comparison of the metabolism of alveolar macrophages from humans, rats, and rabbits: response to heat-killed bacteria or phorbol myristate acetate. J. Lab. Clin. Med. **92**:787–794.

8a. **Hoidal, J. R., D. Schmeling, and P. K. Peterson.** 1981. Phagocytosis, bacterial killing, and metabolism by purified human polymorphonuclear leukocytes, monocytes, and pulmonary alveolar macrophages. J. Infect. Dis. **144**:61–71.

9. **Hoidal, J. R., J. G. White, and J. E. Repine.** 1979. Influence of cationic local anesthetics on the metabolism and ultrastructure of human alveolar macrophages. J. Lab. Clin. Med. **93**:857–863.
10. **Juers, J. A., R. M. Rogers, J. B. McCurdy, and W. W. Cook.** 1976. Enhancement of bactericidal capacity of alveolar macrophages by human alveolar lining material. J. Clin. Invest. **58**:271–275.
11. **Kronvall, G., and R. C. Williams, Jr.** 1969. Differences in anti-protein A activity among IgG sub-groups. J. Immunol. **103**:828–833.
12. **LaForce, F. M., W. J. Kelly, and G. L. Huber.** 1973. Inactivation of staphylococci by alveolar macrophages with preliminary observations on the importance of alveolar lining material. Am. Rev. Respir. Dis. **108**:784–790.
13. **Murphy, J. A., R. K. Root, and A. D. Schreiber.** 1979. The role of antibody and complement in phagocytosis by rabbit alveolar macrophages. J. Infect. Dis. **140**:896–903.
14. **Peterson, P. K., J. Verhoef, D. Schmeling, and P. G. Quie.** 1977. Kinetics of phagocytosis and bacterial killing by human polymorphonuclear leukocytes and monocytes. J. Infect. Dis. **135**:502–509.
15. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, S. D. Douglas, P. G. Quie, and J. Verhoef.** 1978. The key role of peptidoglycan in the opsonization of *Staphylococcus aureus*. J. Clin. Invest. **61**:597–609.
16. **Reynolds, H. Y., J. A. Kazmierowski, and H. H. Newball.** 1975. Specificity of opsonic antibodies to enhance phagocytosis of *Pseudomonas aeruginosa* by human alveolar macrophages. J. Clin. Invest. **56**:376–385.
17. **Territo, M. C., and D. W. Golde.** 1979. The function of human alveolar macrophages. RES J. Reticuloendothel. Soc. **25**:111–120.
18. **Tizard, I. R.** 1971. Macrophage-cytophilic antibodies and the functions of macrophage-bound immunoglobulins. Bacteriol. Rev. **35**:365–378.
19. **Verhoef, J., P. K. Peterson, and P. G. Quie.** 1977. Kinetics of staphylococcal opsonization, attachment, ingestion and killing by human polymorphonuclear leukocytes: a quantitative assay using ^{3}H-thymidine labeled bacteria. J. Immunol. Methods **14**:303–311.