Evaluation of Reticuloendothelial System Phagocytic Activity During Systemic *Candida albicans* Infection in Mice (34401)

DAN C. BIRD AND JOHN N. SHEAGREN
(Introduced by S. M. Wolff)

Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Cellular defense mechanisms are thought to be important in combating infections in both experimental animals and man (1). The ability of the fixed macrophages of the reticuloendothelial system (RES) to clear bacteria from the blood stream has been correlated with host protection in several experimental models (2), and in addition, infections *per se* have been shown to affect RES phagocytosis (3, 4). To date, however, there has been no evaluation of RES phagocytic function in experimental systemic mycotic infections. Therefore, we have evaluated the phagocytic function of the RES during systemic candida infections in mice.

Materials and Methods. Male Cedar Farm white (CFW) mice, 18–22 g, were used in all studies. *Candida albicans*, strain B311, originally isolated from the blood of a patient with fatal candidiasis, was the infecting agent (kindly provided by Dr. Herbert Hasenclever). Inocula were prepared from organisms grown on Sabouraud's agar slants for 24 hr at 24° and harvested with 0.9% sterile, pyrogen-free saline. The organisms were counted directly in a hemocytometer and plated in serial tenfold dilutions to determine viability. Appropriate dilutions were made so that each mouse received its total dose of organisms in 0.2 ml iv. Killed organisms were prepared by suspending washings of the 24-hr colonies in 1% buffered formalin for 24 hr at room temperature with intermittent agitation. These organisms were washed in 0.9% sterile pyrogen-free saline, centrifuged and resuspended three times. Counts were made in a hemocytometer and the optical densities of appropriate dilutions were compared with those of viable organisms in a Beckman DU spectrophotometer at 650 nm; optical densitites were comparable and hence, average particle size for the two inocula were the same. The formalin-treated organisms did not grow when plated on Sabouraud's agar.

The mice were divided into groups which received varying inocula of *C. albicans*: groups received 10^6 dead organisms, 10^6, 10^5, or 10^3 live organisms/mouse. Control animals received no injections of any kind. The RES clearance studies were done on four or more animals in each study group at each point in time. The two inoculated groups receiving 10^6 live or dead organisms were studied 5 hr after injections to determine if there was any acute effect of a large number of organisms on the clearing capability of the RES as compared with control animals. Thereafter, animals which had received 10^6 dead organisms, those which had received 10^5 live organisms (representing a lethally infected group), and those which received 10^3 live organisms (representing a sublethally infected group) were studied on days 2 or 3, days 6 or 7, day 10, and day 14 (10^6 dead and 10^3 live groups only, see Table I).

The RES clearance studies with carbon were performed as previously described (5, 6). Briefly, India ink (Pelikan, Guenther-Wagner, Lot C11-1431 A) was diluted 2:1 with normal saline. Each animal was given 0.01 ml/g of body weight, a dose which was empirically found to give half-times of carbon clearance in normal mice of about 10 min. Blood samples were collected from the retroorbital plexus into 20-μl pipettes at 3, 6, 9, and 12 min, following injection of the colloid and lysed in 3.0 ml of 0.1 N Na_2CO_3.

TABLE I. Half-Times of Clearance ($T_{½}$) of Microaggregated Human Serum Albumin (AA) and Carbon in Groups of Mice Inoculated with *C. albicans*.

Test substance / Group	Dose of *C. albicans* per mouse	Half-times of clearance ($T_{½}$) at various times after inoculation with *C. albicans* (min)				
		5 hr	(days): 2–3	6–7	10	14
AA						
Controls	None	5.6 ± 0.3[a]				
Inoculated	10^6 live	6.4 ± 0.4				
	10^6 dead	6.4 ± 0.4	6.0 ± 0.1	5.7 ± 0.2	5.7 ± 0.3	
	10^5 live		5.8 ± 0.3	5.8 ± 0.3	6.3 ± 0.5	
	10^3 live		5.8 ± 0.2	6.0 ± 0.6	5.5 ± 0.2	
Carbon						
Controls	None	9.1 ± 0.5				
Inoculated	10^6 live	9.1 ± 0.6				
	10^6 dead	9.5 ± 0.6	9.2 ± 0.7	8.7 ± 0.6	8.2 ± 0.6	9.0 ± 0.8
	10^5 live		4.9 ± 0.5[b]	10.0 ± 1.3	7.7 ± 0.4	
	10^3 live		9.8 ± 0.9	5.7 ± 1.0[c]	5.2 ± 0.3[b]	10.9 ± 1.6

[a] All values expressed as mean (± SE) of at least four animals at each time.
[b] $p < 0.01$, t test vs. control.
[c] $p < 0.05$, t test vs. control.

Optical density was then read at 650 nm on a Beckman DU spectrophotometer. The optical density was plotted against time on semilog paper to permit graphic determination of the $T_{½}$. Clearance studies were also performed with microaggregated human serum albumin (AA) labeled with ^{125}I [kindly supplied by Mr. W. H. Briner; prepared as previously described (7)]. Each mouse received a solution containing 2.5 mg/ml of AA with an activity of approximately 1 μCi/ml of ^{125}I. The material was injected into a tail vein in a dose of 0.01 ml/g weight of each mouse (*ie.*, 0.2 ml for a 20-g mouse). Blood samples were collected exactly as for the carbon clearance but were diluted in 2 ml of distilled water prior to counting in a gamma spectrometer (Nuclear Chicago, model no. 4336). The radioactivity was plotted against time on semilog paper and the $T_{½}$ graphically determined.

Results. The survival rates of the mice given various doses of *C. albicans* B311 are as follows: 100% of the mice given 10^6 organisms/mouse were dead by 8 days; animals receiving 10^5 organisms exhibited a 70% mortality rate over a 10-day period at which time the observation period was terminated; and animals receiving a dose of 10^4 or less organisms experienced no mortality.

When AA was used as the test colloid, there were no statistically significant changes from control animals in the $T_{½}$'s of any group of infected animals throughout the entire course of the infection, regardless of the size or viability of the original inoculum (Table I, upper part).

In contrast, accelerated clearance during the infection was readily demonstrable with carbon in the groups infected with viable *C. albicans* (Table I, lower part). The group receiving the lethal inoculum (10^5 *C. albicans*/mouse) showed marked acceleration on days 2–3 ($p<0.01$, t test), while studies repeated at days 6–7 and day 10, when the animals were dying at a rapid rate, showed that clearance of carbon had returned to the normal range. With the sublethal inoculum of *C. albicans* (10^3 organisms/mouse), acceleration of the clearance rate was not seen on day 3 but was present on days 6–7, and persisted at least through day 10. Return to normal was demonstrated by day 14. The animals inoculated with 10^3 organisms/mouse appeared well at all times during the study. No changes in the clearance of carbon were

seen acutely (*i.e.*, at 5 hr) in the groups given the inocula of either 10^6 living or 10^6 dead organisms. Mice receiving 10^6 formalin-killed *C. albicans* exhibited no changes in the clearance of carbon at any time during the study.

Discussion. These studies showed several alterations of RES phagocytic function in mice infected with *C. albicans*. Acceleration of the clearance rate of colloidal carbon occurred within the first 3–7 days after infection, and persisted either until the animals began to die of overwhelming disease or began to recover from a sublethal infection. The time of onset of acceleration seemed to be dependent upon the size of the original inoculum; that is, enhanced carbon clearance was seen by day 3 in the 10^5 group but not until day 6 in the 10^3 group. Other investigators have studied the clearance of colloidal carbon in experimental bacterial (3, 8) and parasitic (6, 9) infections. In general, all of these studies showed acceleration of the carbon clearance rate during infection with return to normal as the infection terminates. Thus, our findings, albeit to our knowledge the first such studies in an experimental mycotic infection, fit the pattern of the previously described investigations. The return to normal of the rate of carbon clearance as seen in the moribund group of animals which received 10^5 *C. albicans* could most probably be attributed to decrease perfusion of the RES (*i.e.*, liver blood flow) rather than termination of the hyperphagocytic state *per se*. On the other hand, the slowing seen in the sublethally inoculated (10^3 *C. albicans*) group was probably due to control of the infection by the animals' host defense mechanisms.

The lack of change in the carbon clearance rate in the animals given formalin-killed *C. albicans* in a dose of 10^6 organisms/mouse indicates that living organisms are necessary for stimulation of the RES. The reason for this finding is not apparent from the present studies and warrants further investigation.

There was a clear-cut difference in the effect of *C. albicans* on the clearance of colloidal carbon and the clearance of colloidal ^{125}I labeled microaggregated human serum albumin (AA). At each point in time where carbon clearance was significantly accelerated, no change in the clearance of AA was demonstrable. The two colloidal particles must be handled in a very different fashion such that *C. albicans* infection has an influence on those factors specific for the clearance of carbon but not on the clearance of AA. Such a difference could reside in separate cell systems responsible for the phagocytosis of different particles; *i.e.*, cellular hyperphagocytosis could be occurring among the carbon-clearing cell system and not among the AA-clearing cell system. In support of this hypothesis, it has previously been shown that the RES clearing phenomenon may be particle (or carrier) specific with regard to RES blockade (10). On the other hand, the differences in clearance might be due to alterations in serum factors rather than the phagocytic cells *per se*. That is, the *C. albicans* infection might be associated with increases in serum factors (opsonins) promoting carbon phagocytosis and not AA phagocytosis. Indeed, using an *in vitro* mouse liver slice system for evaluation of opsonins necessary for the promotion of phagocytosis by the fixed macrophage system of the RES, Saba and DiLuzio (11) classified carbon as a substance dependent on nonspecific serum factors for its phagocytosis, while microaggregated albumin does not require such factors. The differential effect of the infection on the clearance of carbon and AA as described in our experiments could be explained through such a mechanism.

We recently found that several human systemic mycotic infections are associated with enhanced RES phagocytosis (12). Two cases of cryptococcosis, two of histoplasmosis, and one of presumed coccidiodomycosis were studied and had an accelerated clearance rate of ^{125}I-labeled AA; the clearance rate slowed to normal after amphotericin B therapy (12). The clearance of colloidal carbon was not studied. The obvious question arises as to why the clearance of AA is enhanced in these human mycotic infections and not in mice infected with *C. albicans*. Several explanations may be considered. First, none of the patients had systemic candidiasis, and

though unlikely, there may be something unique about an infection with *C. albicans* which does not result in an enhanced clearance of AA. Second, there may be species differences in regard to which test colloid reflects enhanced RES phagocytosis: RES hyperphagocytosis may be associated with more rapid AA clearance in man and more rapid carbon clearance in the mouse. Lastly, it may well be that homologous microaggregated albumin is needed to reflect accelerated particle clearance in any given species. That is, since the clearance of microaggregated *human* albumin was more rapid during infection in man, microaggregated *murine* albumin might show a similar change in mice. Obviously, further investigation will be required to clarify these intriguing but disparate observations.

Summary. Mice infected with *Candida albicans* exhibited enhanced RES phagocytosis as measured by the clearance of colloidal carbon from the blood stream. Production of accelerated carbon clearance required living organisms. The time of onset of acceleration seemed to be dependent upon the size of the infecting inoculum and return to normal of the clearance occurred as the infection terminated. While accelerated clearance of colloidal carbon was produced by the infection, no change was seen at any time in the rate of clearance of microaggregated human serum albumin.

We thank Mr. Joseph Edelin for his expert technical assistance.

1. Mackaness, G. B. and Blanden, R. V., Progr. Allergy **11**, 89 (1967).

2. Rogers, D. E., Bacteriol. Rev. **24**, 50 (1960).

3. Biozzi, G., Halpern, B. N., Benacerraf, B., and Stiffel, C., *in* "Physiopathology of the Reticuloendothelial System" (B. N. Halpern, B. Benacerraf, and J. R. Delafresnaye, eds.), p. 204. Thomas, Springfield, Illinois (1957).

4. Howard, J. G., Scot. Med. J. **6**, 60 (1961).

5. Biozzi, G., Benacerraf, B., and Halpern, B. N., Brit. J. Exptl. Pathol. **34**, 441 (1953).

6. MacGregor, R. R., Sheagren, J. N., and Wolff, S. M., J. Immunol. **102**, 131 (1969).

7. Briner, W. H., J. Nucl. Med. **9**, 482 (1968).

8. Boehme, D. H., Ann. N. Y. Acad. Sci. **88**, (1), 172 (1960).

9. Cox, F. E. G., Bilbey, D. L. J., and Nicol, T., J. Protozool. **11**, 229 (1964).

10. Schapiro, R. L., MacIntyre, W. J., and Schapiro, D. I., J. Lab. Clin. Med. **68**, 286 (1966).

11. Saba, T. M. and DiLuzio, N. R., RES, J. Reticuloendothelial Soc. **2**, 437 (1965).

12. Bird, D. C., Sheagren, J. N., and Bennett, J. E., J. Lab. Clin. Med. **74**, 340 (1969).

Received July 7, 1969. P.S.E.B.M., 1970, Vol. 133.