Fate of *Candida albicans* in Neonatally Thymectomized Rats[1] (34405)

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Defenses against *Candida albicans* are poorly understood, but some evidence indicates that cell-mediated immune processes play an important role in immunity against this fungus. Mice thymectomized at birth, as well as children having lymphopenic immunological deficiencies, are inordinately susceptible to infections with Candida (1, 2). A syndrome of chronic mucocutaneous candidiasis is characterized by specific deficit in cell-mediated immune function (3). Nonetheless, the true significance of the Candida infections and their pathogenetic basis in lymphopenic hypogammaglobulinemic syndromes of man remains unknown.

As part of a broad investigation of reticuloendothelial function, we chose to determine whether neonatal thymectomy in rats impairs either recognition or subsequent engulfment of *C. albicans* by the reticuloendothelial system (RES). A defect in either of those mechanisms could explain the susceptibility to these microorganisms by humans in whom the thymus develops poorly, or in neonatally thymectomized animals.

Methods. Lewis rats (Grand Island Biological) were thymectomized within 24 hr after birth by adaptation of a standard technique for neonatal thymectomy in mice (4). Completion of neonatal thymectomy was established by inspection and macroscopic examination of the thoracic contents and those animals not completely thymectomized were discarded. As demonstrated in previous experiments (10), thymectomized Lewis rats have significant lymphopenia and a significantly diminished *in vitro* lymphocyte proliferation in response to mitogenic stimuli.

The *C. albicans* group A organisms were isolated from a patient and subcultured on Sabouraud's agar. For clearance studies, organisms were washed three times in Hanks' basic salt solution and killed by heating at 56° for 45 min. Stock suspensions of these organisms were diluted in Tyrode's solution to yield a preparation of 1.5×10^8 microorganisms/ml. A portion of these preparations was labeled with ^{131}I (sodium iodide in 0.2 *N* NaOH from Abbott Laboratories, Chicago) according to a method described by Talmage and Claman (5). Our method was simply to take a suspension of Candida organisms in a concentrate of 1.5×10^8 organisms/ml and to expose these organisms to the iodine ^{131}I exactly as the exposure to and labeling of protein was accomplished by Talmage and Claman. Radioactivity fell in the range of 2×10^6 cpm/ml and approximately 10% of the organisms were labeled, free iodide being less than 1% of the total radioactivity.

The experiments were performed in thymectomized and normal rats of the same litters. They were anesthetized with Nembutal (6 mg/100 g of body wt). After a midline laparotomy, the inferior vena cava was liberated from fat and injected with the above described *C. albicans* preparation so that each animal received 1.5×10^7 microorganisms/100 g of body weight in one group, and 1.5×10^8 microorganisms/100 g of body weight in another group. For study of the clearance rate, blood samples (0.5 ml) were taken from the inferior vena cava at 1, 5, 20, 40, and 60 min following iv injection, the first minute sample being taken as the 100% value. Sixty min after injection, the liver,

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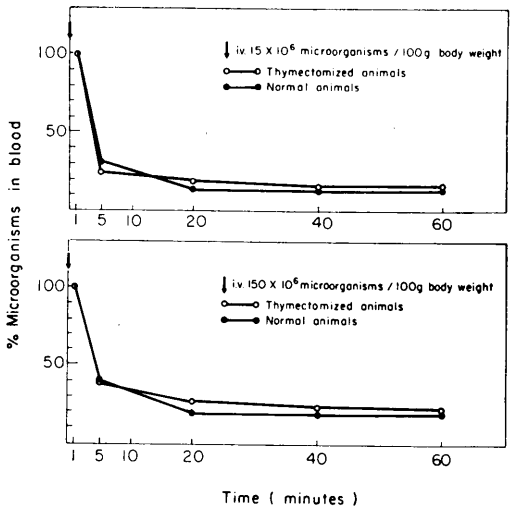

FIG. 1. *In vivo* clearance of *C. albicans*. After iv injection on one dose of ^{131}I *C. albicans*, blood clearance rate was followed by analyzing blood samples (0.5 ml) taken at intervals during 1 hr.

spleen, lungs, kidneys, and muscle were removed, washed thoroughly, and assayed for their radioactivity as a reflection of phagocytic function. Radioactivity per 100 g of wet weight was measured and compared with the specific activity in the last sample of blood taken at 60 min. Blood and organ samples (3 samples for each organ) were weighed on an open Mettler balance, assayed in a scintillation counter (RIDL with 2-in sodium iodide crystal) and the data were recorded as cp/min/100 mg minus background. Clearance rate of fungi from the blood was finally expressed as percentage of the injected radioactivity. The ratio between activity per 100 mg of each organ and 100 mg of blood provided a basis for comparison of relative uptake by the several tissues.

Results. Figure 1 summarizes blood clearance rate of *C. albicans* in 10-week-old rats. Animals thymectomized at birth and control animals of the same litter showed identical clearance rates. Two different doses of microorganisms gave similar results.

Table I summarizes the ratio of radioactivity present in 100 mg of organ wet weight per 100 mg of blood 60 min after the iv injection of the above-described two doses of *C. albicans*. This ratio reflects the avidity of *C. albicans* for the different tissues considered. Although spleen, liver, and kidneys played an important role in clearance, the most striking concentration with both doses of microorganisms was found in the lung. No differences in organ distribution of these organisms were found between thymectomized and control animals.

Discussion. Prior studies had indicated that nonspecific RES clearance of colloidal materials is not basically altered in neonatally thymectomized animals (1, 6). New evidence indicating that the influence of neonatal thymectomy may be indirect (7–9) and involve, for example, the so-called afferent limb of the immune response made it seem

TABLE I. Organ Avidity for *C. albicans*: Relation between Organs and Blood.[a]

Activity relation (organ/blood)	Doses iv administered	Normal rats (5 rats)	Thymectomized rats (7 rats)
Liver/blood	15×10^6	23.4 ± 10.10	23.5 ± 4.74
Spleen/blood	15	24.6 ± 14.60	23.3 ± 4.24
Lung/blood	15	333 ± 11.5	395 ±34.20
Kidney/blood	15	15.8 ± 2.18	17 ± 5.99
Muscle/blood	15	1.0 ± 0.09	0.60± 0.11 (3 rats)
Liver/blood	150	14.6 ± 3.69	8.7 ± 4.74
Spleen/blood	150	13.8 ± 3.97	10.0 ± 2.61
Lung/blood	150	158 ± 50.4	136 ± 8.3
Kidney/blood	150	8.0 ± 2.06	6.0 ± 2.90
Muscle/blood	150	1.3 ± 0.50	.50± 0.25

[a] Tissue avidity is measured by the relation between activity in 100 mg of tissue/activity in 100 mg of blood. Values indicate the mean ± the standard error of the mean.

wise to evaluate directly RES clearance of *C. albicans* in neonatally thymectomized rats. As shown by studies in our laboratories (10) and by others (12), neonatally thymectomized Lewis rats have striking immunological deficiencies consisting of lymphopenia, decreased ability to exhibit delayed allergic responses, and markedly diminished *in vitro* responses to phytohemagglutinin, pokeweed mitogen, and mitomycin-C-treated allogeneic cells (10). Results of our study show that clearance of Candida 10 weeks following neonatal thymectomy is not different from that of controls. Previous studies from our laboratories also have shown that different bacteria were differently distributed within the several compartments of the RES depending upon the organisms and the presence or absence of antibodies in the circulation. For example, *Salmonella typhosa* organisms and *Brucella melitensis* were avidly phagocytized and cleared from the circulation by the liver both *in vivo* and in isolated perfused liver system (11). *Brucella abortus* in the absence of antibodies seemed not even to be recognized by the liver *in vitro*, but seemed selectively to be removed by spleen *in vivo*. In immunized animals, on the other hand, *S. typhosa* were removed in surprising numbers by the pulmonary RES, while with *B. abortus* organisms, immunization led to more different clearance via the liver. By contrast, pulmonary RES seemed of relatively little importance in clearance of *B. abortus* even in immunized animals. In the present experiments, the lung was a predominant site of phagocytic clearance of Candida in both normal rats and neonatally thymectomized rats, far exceeding the clearance of Candida by either liver or spleen. This predominant role was more striking when a relatively small number of organisms was injected than when a larger dose was used. Consequently it seems that the clearance of *C. albicans* via the lungs is a selective pathway for *C. albicans* and does not reflect an overload of the more conventional pathways, for example, in liver or spleen. It is of course important to realize that these observations relating to clearance of Candida from the circulation do not in any manner argue against the increased killing capacity of phagocytic cells for facultative intracellular bacterial pathogens and by extrapolation perhaps to fungi that is developing from the contributions of Mackaness and Blanden (13, 14). Indeed in earlier work in our laboratory Salvin *et al.* (1) showed that neonatally thymectomized mice, which are unable to generate cell-mediated immunities, are extremely susceptible to persistence and extension of Candida infections. Our present work, however, does argue that defective resistance to Candida of neonatally thymectomized rodents and perhaps of humans lacking a thymic-dependent system does not depend upon alterations of phagocytic clearance of the Candida organisms from the blood stream by fixed cells of the reticuloendothelial system. These studies, taken with our prior investigations, seem to indicate that it may be useful to think of the RE function in terms of separate and distinct compartments, each having distinct factors regulating their role in the clearance of microorganisms and in bodily defense.

Summary. The clearance of *C. albicans* organisms from the blood in Lewis rats is not altered in neonatally thymectomized rats despite the presence of profound immunological disturbances. The selective clearance of *C. albicans* by the lung is also unimpaired in these rats.

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