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NOTES

Immunity to Experimental Renal Candidiasis in Rats

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Germfree rats were found to be more susceptible to intravenous challenge with *Candida albicans* than were conventional rats. The resistance of conventional rats could be overcome by increasing the challenge dose. The resistance of the germfree rat was enhanced by vaccination with Formalin-killed *C. albicans* in complete Freund adjuvant, complete Freund adjuvant, or incomplete Freund adjuvant. These results, and histological evidence obtained from infected gnotobiotic rats, provided further information on the mechanism of resistance to the disseminated form of candidiasis.

Several animal models have been used to study the role of the acquired immune system in defense against infection by *Candida albicans* (1, 4, 5, 12, 13, 19), but, since most mammals are colonized with *Candida* (or other similar yeast cells before experimental manipulation), it is difficult to assess the relative roles of acquired and innate immunity in resistance to disseminated candidiasis in such animals (4, 5). For this reason, germfree animals (having no previous immune stimulation by viable yeast or bacteria) were employed to further clarify factors of importance in the hosts' defense against disseminated candidiasis.

The microorganism, laboratory animals, challenge procedure, and the method of enumerating viable organisms in tissues have been described previously (16). Complete Freund adjuvant (CFA), incomplete Freund adjuvant (IFA), or complete Freund adjuvant mixed with Formalin-killed organisms (2×10^6 yeast cells per 2 ml of adjuvant per rat; CA-CFA) were injected by the method of Campbell et al. (3). Tissues chosen for histological examination were removed, placed in either buffered Formalin solution or Hollande Bouins solution, embedded, and sectioned at 5 μm.

Germfree and conventional rats were injected (intravenously, via heart puncture) with 10^5 viable *C. albicans*, and the growth in the kidneys of each experimental group was assessed (Fig. 1). The results indicate that germfree rats are more susceptible than conventional rats to renal infection by *C. albicans* at a challenge dose of 10^5 viable organisms. Conventional rats developed renal candidiasis when they were infected with a higher dose of *C. albicans* (5×10^5 viable units) (Fig. 1).

The greater susceptibility of germfree rats to a *C. albicans* challenge is probably due to their hypoactive innate and acquired immune system (2, 6, 14, 15). Thus, the heightened resistance of conventional rats (as compared with that of germfree rats) to 10^5 viable *C. albicans* is probably due to previous stimulation (by *C. albicans* or other viable yeasts or bacteria) of the innate or acquired immune systems.

When germfree animals were treated with CA-CFA, CFA alone, or IFA 2 weeks before intravenous challenge with viable *C. albicans*, they were just as resistant to renal candidiasis as were conventional rats (Fig. 2). It was interesting that treatment of germfree rats with IFA was capable of providing protection against renal candidiasis, since it is believed that IFA exerts its primary effect on the innate defense system, including stimulation of the reticuloendothelial system (7).

Since conventional rats were susceptible to a challenge of 5×10^5 viable organisms, we wanted to determine whether a higher dose of *C. albicans* would abrogate the apparent resistance of CFA- or IFA-treated germfree animals. Thus, germfree rats, treated with IFA or CFA (with no *C. albicans* antigen included), were challenged with a higher dose of *C. albicans*. The results (Table 1) show that a challenge dose of 3.5×10^5 *C. albicans* established a persistent renal *Candida* infection in the CFA- or IFA-treated germfree rats and that the adjuvant-induced resistance was apparent by day 7 with

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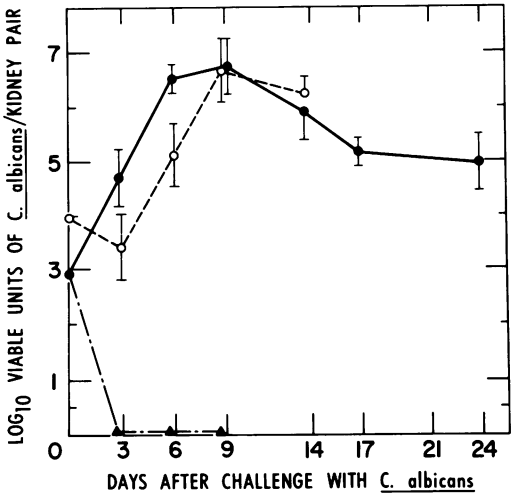

FIG. 1. *Course of an experimental infection by C. albicans in the kidneys of germfree (●) and conventional (△) rats infected with 1 × 10⁵ viable C. albicans and of conventional rats (○) infected with 5 × 10⁵ viable cells. Each point represents a minimum of six rats. The bars represent the standard error of the mean.*

IFA and perhaps as early as day 3 with CFA.

The kidneys of *C. albicans*-infected conventional and germfree rats were examined histologically. Typical sections of germfree rat kidneys are shown in Fig. 3 and 4. The early inflammatory reaction in *Candida*-challenged gnotobiotic rats was characteristically weak, predominantly polymorphonuclear, and limited to the glomeruli, the pelvic space, and the renal tubules (Fig. 3A). A very weak polymorphonuclear infiltrate was also observed in the tissues of conventional rats. The kidneys of monoassociated (*Candida*-infected) rats sacrificed 7, 14, and 21 days after challenge showed large numbers of *C. albicans* in the pelvic region and occasionally

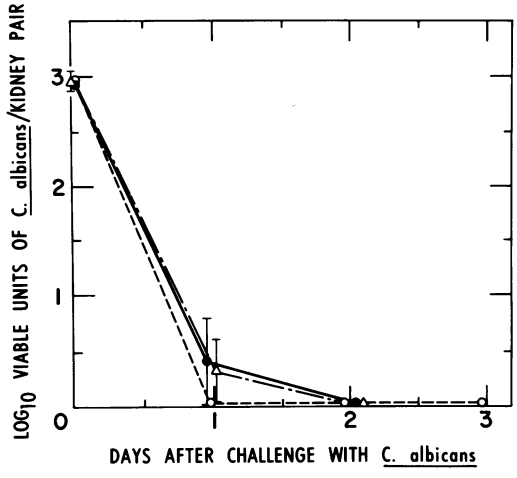

FIG. 2. *Course of an experimental infection by C. albicans in the kidneys of IFA-vaccinated (●), CFA-vaccinated (△), and CA-CFA-vaccinated (○) germfree rats infected with 10⁵ viable C. albicans. Each point represents a minimum of five rats. The bars represent the standard error of the mean.*

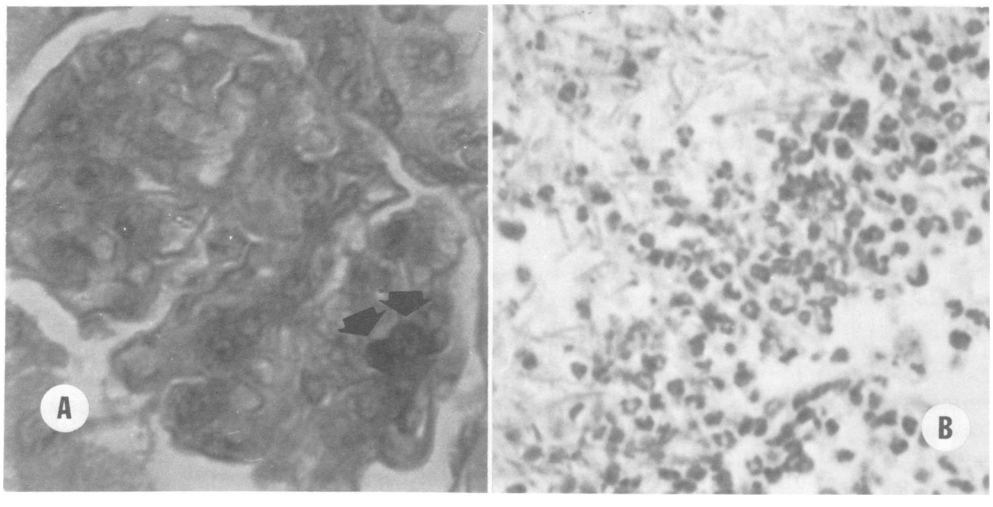

FIG. 3. *(A) Kidney tissue from a monoassociated rat sacrificed 3 days after a systemic challenge with C. albicans. The tissue was stained with periodic acid Schiff; two yeastlike C. albicans cells can be seen in the glomerulus (arrows). The surrounding tissue was relatively free from cellular infiltrate. ×800. (B) Kidney tissue from a monoassociated rat sacrificed 7 days after a systemic challenge with C. albicans. The tissue, stained with azure-eosin B, shows an inflammatory infiltration of the pelvis space, composed primarily of polymorphonuclear leukocytes. ×400.*

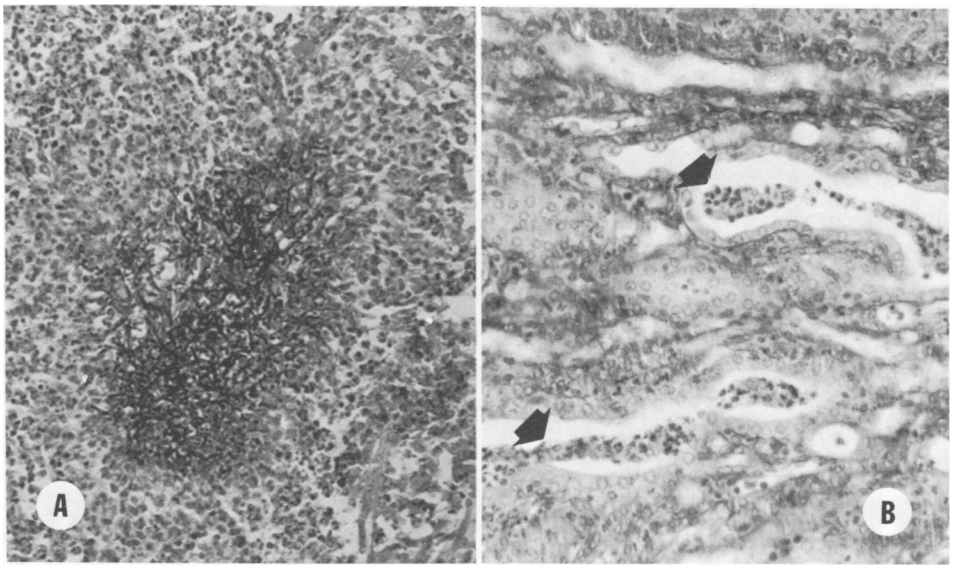

FIG. 4. *Kidney tissue characteristic of monoassociated rats sacrificed 14 days after a systemic challenge with C. albicans (A). The tissue, stained with periodic acid Schiff, shows a microabscess in the renal medulla. There are intact mycelia present, with a cellular infiltrate of both intact and degenerating polymorphonuclear leukocytes. ×160. (B) The tissue, stained with azure-eosin B, shows extensive hydropic degeneration and infiltration (arrows) by inflammatory cells (almost exclusively polymorphonuclear leukocytes at 14 days). The distal convoluted tubules are distended, and there is marked extracellular edema. ×160.*

TABLE 1. *Effect of treatment with CFA or IFA on the resistance of germfree rats to a challenge of 3.5 × 10^5 viable C. albicans*

Treatment	Days after vaccination	Mean $\log_{10}$ viable *C. albicans* per kidney pair[a]	SE[b]	*P*[c]
	0	5.13	0.23	
IFA	3	4.79	0.58	
CFA	3	4.03	0.64	<0.05
IFA	7	3.23	0.89	<0.02
CFA	7	4.25	0.67	
IFA	14	2.67	1.12	<0.01
CFA	14	3.82	1.03	<0.05

[a] Rats were sacrificed 3 days after challenge, and the number of viable *C. albicans* was determined for each pair of rat kidneys.

[b] Standard error of the mean.

[c] Probability values calculated from Student's *t* test.

smaller numbers of fungi in the renal medulla (Fig. 3B and 4). In both cases, the cellular infiltrate was composed of intact and degenerating polymorphonuclear leukocytes and only occasional mononuclear cells. If acquired cell-mediated immunity is an important defense against renal candidiasis, as is the case for mucocutaneous candidiasis (8, 9, 20), one would have expected a larger proportion of the inflammatory cell infiltrate to be mononuclear, especially at the later sacrifice intervals (14 to 21 days).

In conclusion, we attempted to enhance our understanding of the mechanisms by which conventional and gnotobiotic rats clear *C. albicans* from their kidneys. Based on differences in the resistance of germfree and conventional rats to renal candidiasis, the enhanced resistance imparted to germfree rats by adjuvant treatment, and histological evidence, it appears that acquired immunity may not be the main requirement for defense against disseminated candidiasis. The results of these experiments support recent reports from this laboratory (12, 16, 17, 18) and others (10, 11) which indicate that the innate defense system may play a primary role in defense against the disseminated form of candidiasis.

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