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Immunity to *Candida albicans* Induced by *Listeria monocytogenes*

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Immunity to *Candida albicans* was studied in Swiss-Webster white female mice. Lethal and sublethal infections with *C. albicans* did not enhance immunity to a subsequent *Listeria monocytogenes* challenge. Mice sensitized to *L. monocytogenes* and then challenged with *C. albicans* intravenously were able to reduce the population of *C. albicans* in their kidneys after being boosted (rechallenged) with *L. monocytogenes*. However, the acquired cellular immunity so induced was very short-lived. Both *C. albicans*-sensitized and nonsensitized control mice showed an immediate-type and delayed-type skin test response to the cell wall antigen(s) of *C. albicans* but not to the cytoplasmic antigen(s). There did not appear to be any correlation between increase in skin test response and progression of the candida infection in mice.

Infection by a viable intracellular parasite provokes an immune response that is usually associated with three features (19, 20): (i) delayed hypersensitivity skin reaction, (ii) a form of acquired resistance that can be passively transferred with sensitized lymphocytes but not with serum, and (iii) a nonspecific enhancement of antibacterial activity of the host's macrophages. The phenomenon has been termed acquired cellular immunity (19, 20). Mackaness (21) showed that acquired cellular immunity depends upon a population of sensitized lymphoid cells and macrophages. There must first be a specific interaction between the sensitized lymphoid cells and the antigen to which they are sensitized before they can activate macrophages (19, 21). It has been suggested that a lymphokine, possibly migration inhibition factor, is responsible for carrying out macrophage activation. These activated macrophages are capable of killing, nonspecifically, a wide range of intracellular pathogens which would normally multiply within nonactivated cells (21).

The nonspecific expression of acquired cell-mediated immunity is also demonstrated in studies where cross-protection is achieved between antigenically unrelated pathogenic bacteria (8, 19, 22). Mice infected with *Brucella abortus* possessed immunity against a subsequent *Listeria monocytogenes* challenge if the latter was given immediately after the peak of the *B. abortus* infection (19). However, mice which were infected first with *L. monocytogenes* and then challenged with *B. abortus* did not show any immunity against the latter unless the mice were reinfected with *L. monocytogenes*. This is because *L. monocytogenes* is eliminated much faster in mice than *B. abortus* and the presence of a critical antigenic mass appears to be an essential step in establishing cellular immunity (9, 10, 19). A sudden increment in antigenic material to which the host is sensitized can recall the nonspecific phase of immunity (10, 19).

In 1968 Ruskin and Remington demonstrated that nonspecific immunity could be induced against facultative bacteria by a prior infection with an intracellular protozoan (25). In another study (9) resistance against *Cryptococcus neoformans* was obtained by infecting mice with either *Toxoplasma gondii*, *Besnoitia jellisoni*, or *L. monocytogenes* prior to challenge with *C. neoformans*. Infections with protozoa, in which the parasites persist in the tissues for the life of the animal, were able to confer remarkable prolonged immunity against *C. neoformans*.

The mechanisms of host resistance against *C. albicans* have not been clearly defined, although there is some evidence suggesting that the role of cells may be more important than humoral antibodies (1, 2, 3, 5, 11, 12). Studies mentioned above have shown that infections with intracellular parasites, e.g., *L. monocytogenes*, can confer resistance against phylogenetically unrelated organisms (9, 25). It has also

been shown that this resistance appears to depend on cellular mechanisms (26). The purpose of this study was, therefore, to see if cross-immunity can be established between *C. albicans* and *L. monocytogenes*, i.e., whether a prior infection with *C. albicans* can induce a nonspecific immunity against a subsequent *L. monocytogenes* challenge and vice versa.

MATERIALS AND METHODS

Organisms and culture media. *C. albicans* strain B311 (type A) was originally obtained from H. F. Hasenclever (National Institutes of Health, Bethesda, Md.); it has been maintained in our laboratory by monthly transfers on Sabouraud dextrose agar (Difco) with 0.4% yeast extract (Difco) slants and stored at 4 C.

The morphological, physiological, and biochemical characteristics of *C. albicans* B311 were verified by the following. (i) Growth on Sabouraud dextrose agar and Trypticase soy agar plates (BBL) showed a consistent, smooth, characteristic colonial morphology. (ii) Germ tube formation was observed after incubation in human serum at 37 C for 2 h. (iii) Chlamydospore formation occurred on cornmeal agar (Difco) with 1% Tween 80. (iv) Sugar fermentation reactions read at 48 h after inoculation were as follows: acid and gas were produced in dextrose and maltose, only acid was produced in sucrose, and there was no fermentation of lactose.

Using the method of Reed and Muench (24) the intravenous (i.v.) mean lethal dose (LD_{50}) (14 days) of *C. albicans* for female mice was found to range from 6.75×10^4 at the beginning to 8.0×10^4 at the end of the study.

L. monocytogenes was obtained as a lyophilized culture from the culture collection of the Department of Medical Microbiology, University of Wisconsin, Madison, Wis. The strain was originally obtained from the Animal Health Laboratory of the Wisconsin State Department of Agriculture, Madison, Wis. Prior to use in this study, the culture was mouse-passaged twice and a fresh isolate was recovered from the spleen of a moribund mouse. The isolate was then used to prepare lyophilized stock cultures. Brain heart infusion agar and broth (Difco) were used as growth media. Using the method of Reed and Muench (24) the i.v. LD_{50} for mice was found to be 8.0×10^4 viable cells.

Animals. All conventional mice were females, 8 to 9 weeks of age, Swiss-Webster white strain (Rolfsmeyer Rat and Mouse Farm of Wisconsin, Madison, Wis.). Weights of the mice ranged from 23 to 28 g.

Sprague-Dawley germfree rats, 2 months old, of both sexes were used for skin testing. Male rats ranged from 280 to 310 g, and the females ranged from 200 to 250 g. All animals were bred and housed in flexible germfree isolators at the University of Wisconsin Germ-free Laboratory and were fed a crude pelleted L5010 C diet (Ralston Purina Co., St. Louis, Mo.).

Preparation of challenge inoculum. An 18- to 24-h culture of *C. albicans* was grown on Sabouraud dextrose agar, harvested, and washed twice by alternate centrifugation (5,000 rpm for 5 min) and resuspension in saline. The resulting pellet was resuspended in 5 ml of saline. The total cell count in the suspension was determined with a hemacytometer. For i.v. inoculations, 0.1 ml of the appropriate dilution was injected into the tail vein of the animals. Viable cells in the inoculum were enumerated by the plate count dilution method. Colonies on Sabouraud dextrose agar were counted after 24 to 48 h of incubation at 37 C.

For preparation of the *L. monocytogenes* inoculum, an 18- to 24-h-old slant of the culture was washed with 2 ml of saline and added to 75 ml of brain heart infusion broth. The flask was then incubated on a shaker for 2 to 3 h, at which time a sample was removed for optical density reading at 600 nm using a Spectronic 20 colorimeter (Bausch and Lomb, Rochester, N.Y.). The expected total cell concentration was estimated by comparing the optical density with a previously prepared standard curve showing absorbance versus *L. monocytogenes* cell concentration. A 5-ml amount of the broth culture was then washed twice by alternate centrifugation, (7,700 rpm for 20 min) and resuspension in saline. The cell pellet was finally suspended in 5 ml of saline. A volume of 0.1 ml of the desired dilution was injected i.v. into the mice. The plate count dilution method was used to determine the viable challenge dose given. Colonies on brain heart infusion plates were counted 48 h after incubation at 37 C.

Enumeration of viable microorganisms in the organs of challenged animals. Sacrifices were usually performed at intervals of 2 to 4 days. At each sacrifice animals were killed with ether and dissected immediately. The spleen and kidneys of each animal were removed aseptically and homogenized separately in 4.5 ml of saline (homogenizers with glass mortar and pestle were used, Bellco Biological Glassware and Equipment, Vineland, N.J.). The number of viable organisms in each homogenate was then estimated by the plate count dilution method. Colonies were counted after 24 to 48 h of incubation at 37 C. The viable units (VU) of organisms per ml of organ homogenate were expressed as the mean ± standard error. The Student's *t* test was used to assess the significance of the results.

Preparation of skin test antigens. Cells from 18- to 24-h cultures of *C. albicans* were harvested from Sabouraud dextrose agar slants and washed twice by alternate centrifugation (5,000 rpm for 5 min) and resuspension in saline to give a final packed cell volume of about 0.5 ml. A volume of 4.5 ml of saline was then added to the cell pellet and the cell suspension was broken by passing the cells through a modified (23) French pressure cell (Aminco cell press, Carver Laboratory, Summit, N.J.) at 10,000 lb/in^2. The cracked cells were centrifuged at 15,000 rpm for 20 min. The supernatant was decanted from the cell pellet which was resuspended in 4.5 ml of saline. Both the supernatant fraction (S-Ag) and the cell wall fraction (C-Ag) were used for skin tests. A commercial antigen, Hollister-Stier (HS-Ag, Hollister-Stier Co., Downers Grove, Ill.), consisting of the supernatant

extract of *C. albicans* in a dilution of 1:10 was also used in some experiments.

The concentration of protein in the antigens was determined using the method of Lowry et al. (18). Both the S- and C-Ag contained 5.5 to 6.0 mg of protein per ml. The HS-Ag had a protein concentration between 8 and 10 mg/ml.

Skin test. The antigen was injected into one of the hind footpads of the animal—0.05 ml used for mice and 0.1 ml used for germfree rats. Injection of an equal volume of saline into the opposite footpad served as a control. Reactions were read at 4, 24, and 48 h. Thicknesses of the footpads were measured using a Schnelltaster (H. T. Kroplin, 649 Schleuchtern I, Hesser, Germany). The Student's *t* test was used for determining statistical significance of the data.

RESULTS

Growth and survival of C. albicans in female mice after i.v. infection with various challenge doses. This experiment was designed to follow the course of *C. albicans* infection in the kidneys and spleens of female mice after various challenge doses.

Five challenge levels of *C. albicans* were given: 1.4×10^6, 1.2×10^5, 2.5×10^4, 6.0×10^3, and 2.5×10^3 VU, respectively. For the animals which received high challenge doses (10^6 and 10^5 VU) the experiment terminated at 8 and 15 days, respectively, since all the animals succumbed in that time. For the other groups the experiment lasted for a month or longer.

Mice infected with 10^3 to 10^4 VU of *C. albicans* manifested a similar course of infection in the kidneys (Fig. 1). The infection progressed steadily from 10^2 to 10^3 VU of *C. albicans* per ml of kidney homogenate on day 0 (just after challenge), reaching its peak (0.5×10^8 to 1.0×10^8 VU/ml) around day 24. After this there seemed to be a gradual decline in the number of viable organisms. Complete elimination was observed around day 35. During the latter period of decline in the number of *C. albicans* cells there was a great variability in viable counts. *Candida albicans* was being eliminated from the kidneys of some mice while in others the infection remained. Some animals appeared to be unable to totally suppress the infection, even after 50 days.

In animals challenged with 10^5 to 10^6 VU of *C. albicans* (Fig. 1) there appeared to be a larger number of viable organisms present in the kidneys and a more rapid rate of increase of *C. albicans* as compared to the kidney infections after lower challenge doses. Further comparisons could not be made since all the animals with the high infecting doses (10^5 to 10^6 VU) died within 1 to 2 weeks.

There appeared to be a steady elimination of *C. albicans* in the spleens of all infected animals (Fig. 2), although clearance was slower in mice infected with higher doses (10^5 and 10^6 VU). In mice infected with lower doses (10^3 and 10^4 VU), elimination was very rapid initially, but it was not complete since further spleen cultures showed sporadic increases of viable cells. However, *C. albicans* did not multiply extensively (never exceeding 5×10^3 VU/ml) in the spleen of any mouse. Complete elimination of the

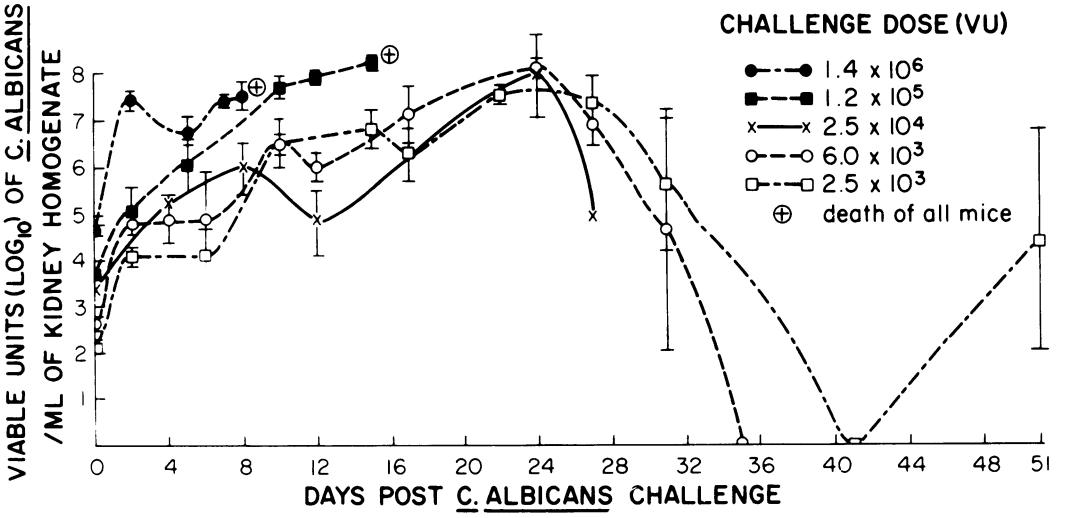

FIG. 1. *Growth curves of C. albicans in the kidneys of female mice after i.v. infection with various challenge doses. (Each data point represents the mean of three to five mice ± standard error.)*

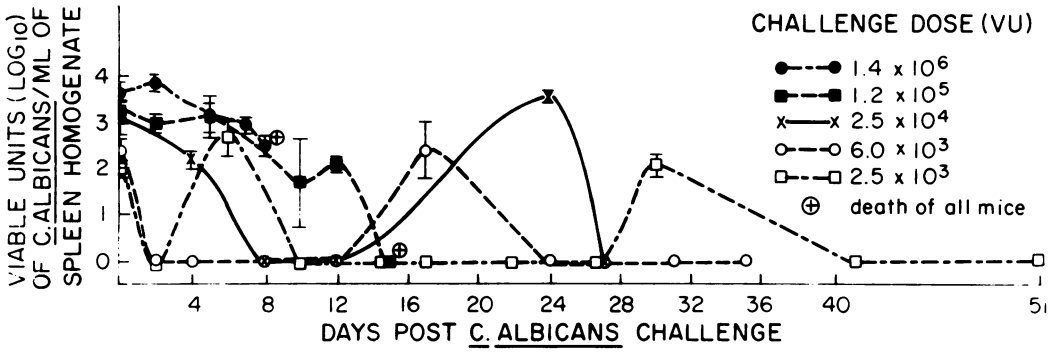

FIG. 2. *Growth curves of C. albicans in the spleens of female mice after i.v. infection with various challenge doses. (Each data point represents the mean of three to five mice ± standard error.)*

infection in the spleens appeared to be reached after day 31.

Course of L. monocytogenes infection in female mice. In this experiment the primary infection course of *L. monocytogenes* in normal female mice was examined. Figure 3 shows that the organism caused an acute infection in the animals. With an i.v. challenge dose of 3×10^4 VU (~1/2 LD_{50}), *L. monocytogenes* multiplied rapidly in the spleen, reaching a peak (~5×10^5 VU/ml) in 48 h. Then the infection gradually regressed, but it was not completely eliminated from the spleen even at 6 days after challenge.

The population of *L. monocytogenes* in the kidneys was much lower, with 10^3 VU/ml at the peak (on day 1). From day 3 on, no more *L. monocytogenes* could be cultured from the kidneys.

Effect of a sublethal C. albicans infection on the growth of L. monocytogenes in the spleen of female mice. The objective of this study was to find out if acquired cell-mediated immunity could be induced in female mice by a low i.v. challenge dose of *C. albicans*. A low infecting dosage was preferred since other experiments showed that, at this challenge level, there would be a constant antigenic mass over a period of time. If chronic infection with *C. albicans* induces a cell-mediated immunity response, a more rapid elimination of a subsequent *L. monocytogenes* challenge would be realized through the nonspecific nature of acquired cell-mediated immunity.

A large group of mice (84 mice) was challenged i.v. with 10^3 VU of *C. albicans*. Seven days after challenge with *C. albicans*, 21 of the mice were also infected with *L. monocytogenes* (~3.4 to 4.0 × 10^4 VU, i.v.). Three mice were sacrificed daily for the next 6 days and the spleens were assayed for viable *L. monocytogenes*. The same procedure was repeated on days 14, 21, and 28 after the initial *C. albicans* challenge. *L. monocytogenes* infection in a group of mice not challenged previously with *C. albicans* (control group) was also recorded.

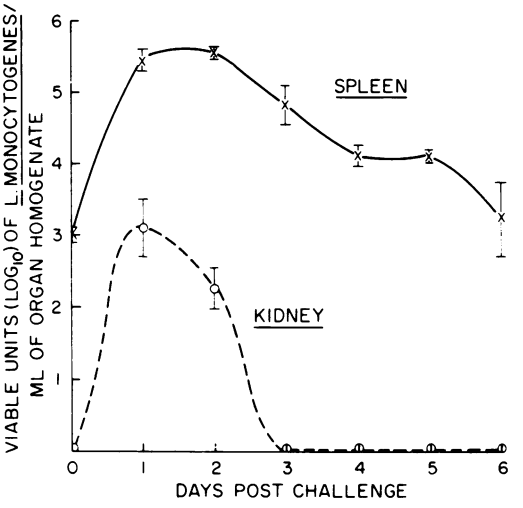

FIG. 3. *Growth curve of L. monocytogenes in the kidneys and spleens of normal female mice. (Each point represents the mean of five mice ± standard error.)*

Figure 4 shows the results. The curve in the upper half of Fig. 4 represents the course of infection in the kidneys of mice challenged with 10^3 VU of *C. albicans* only. In the lower half, counts of *L. monocytogenes* in the spleens were compared between the control group and the group prechallenged with *C. albicans*, i.e., mice which were challenged with *C. albicans* and superinfected with *L. monocytogenes* at different times. There did not seem to be any significant differences in the capacity of the two groups to handle *L. monocytogenes* at any stage

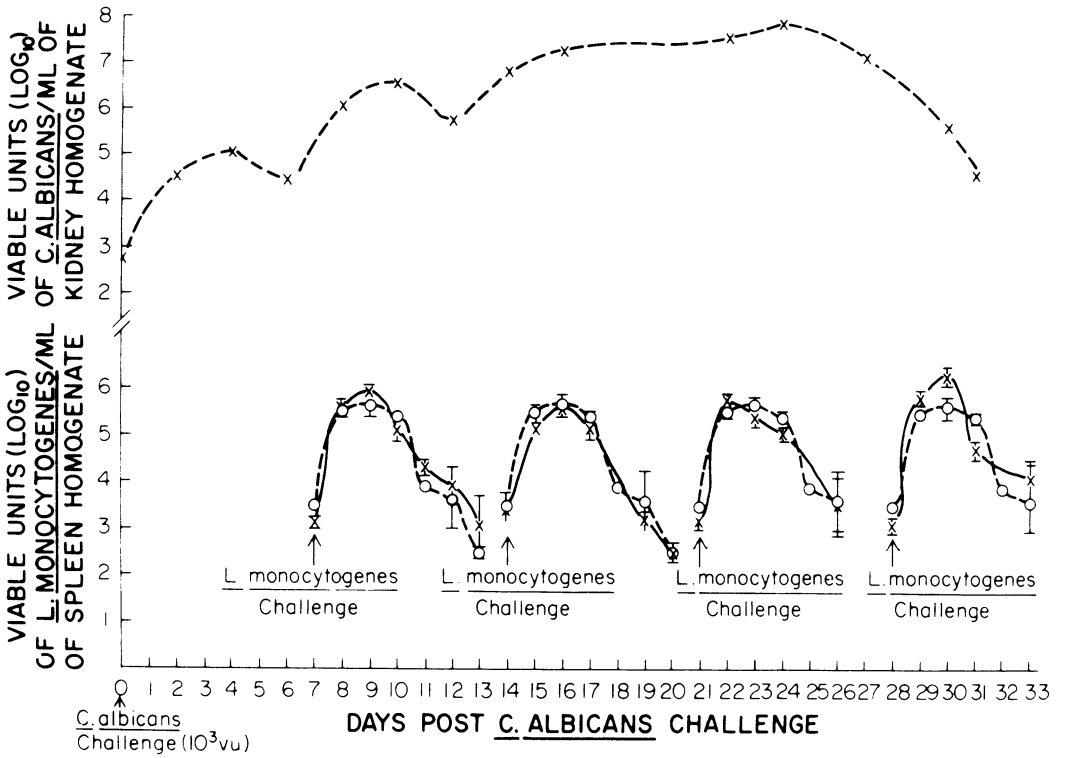

FIG. 4. *(Top) Growth curve of C. albicans in the kidneys of normal female mice* (×- - -×). *(Bottom) Growth curves of L. monocytogenes: (i) injected at various stages (arrow) of a chronic C. albicans infection* (×——×) *and (ii) in a control group not challenged with C. albicans* (O- - -O). *(Each point represents the mean of three mice ± standard error.)*

of the *C. albicans* infection, even though *C. albicans* was being eliminated from the kidneys gradually after day 24, as shown in the upper curve. In fact, mice which were infected with *L. monocytogenes* 28 days after *C. albicans* challenge showed a slightly higher peak of viable *L. monocytogenes* where the difference was significant ($P < 0.05$).

Effect of a lethal C. albicans infection on the growth of L. monocytogenes in the spleens of female mice. This experiment was essentially the same as the previous one, except that the mice were prechallenged (i.v.) with a higher dose of *C. albicans* to insure maximum sensitization. Since mortality rate at this challenge level (10^6 VU) was very rapid, and also since the *C. albicans* population reached a high level in the kidneys very early in the infection, the mice were infected (i.v.) with *L. monocytogenes* on the third day after *C. albicans* challenge. The candida-infected group showed a significantly lower count of *L. monocytogenes* ($P < 0.05$) on the first day after *L. monocytogenes* infection (Fig. 5). However, there were no significant differences in the pattern of spleen infections by *L. monocytogenes*, between the control and the *C. albicans*-infected groups, on days 2, 3, and 4 after *L. monocytogenes* challenge (Fig. 5). The lower count 1 day after *L. monocytogenes* challenge was probably due to the lower *L. monocytogenes* challenge dose of 2×10^4 VU which this group received as compared to the control group which received 4×10^4 VU.

Effect of L. monocytogenes infections on the growth of C. albicans in the kidneys and spleens of female mice. The objective of this experiment was to see if the nonspecific nature of acquired cell-mediated immunity stimulated by a *L. monocytogenes* challenge (19) would enhance the elimination of a *C. albicans* infection. All the mice were given a primary (i.v.) *L. monocytogenes* challenge ($\sim 5.5 \times 10^4$ VU) 3 days before they were superinfected (i.v.) with *C. albicans* (2.5×10^3 VU). At intervals of 1 to 3 days, the kidneys and spleens of four mice were assayed for viable *C. albicans* and the spleens were also cultured for *L. monocytogenes*. Since

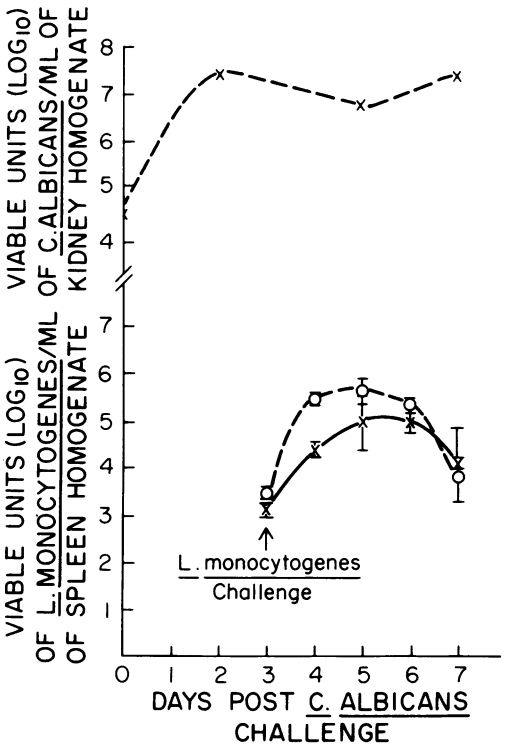

FIG. 5. *(Top) Course of a lethal C. albicans infection (10⁶ VU) in the kidneys of female mice* (×---×). *(Bottom) Growth curves of L. monocytogenes in the spleens of control mice not challenged with C. albicans* (O---O) *and in mice infected 3 days previously with a lethal dose of C. albicans* (×——×). *(Each point represents the mean of three mice ± standard error.)*

nonspecific immunity to *L. monocytogenes* is relatively short-lived (19), mice were given two boosters of *L. monocytogenes* (3×10^4 to 4×10^4 VU, i.v.) on days 7 and 14 after the initial *L. monocytogenes* challenge.

Assays of *L. monocytogenes* in the spleens demonstrated that mice challenged with *L. monocytogenes* showed a strong secondary response when they were reinfected with the homologous organism. Figure 6, which presents the averaged results of two experiments, showed distinctly that *L. monocytogenes* was eliminated much more rapidly from sensitized mice than from nonsensitized mice.

Mice infected with 2.5×10^3 VU of *C. albicans* only, i.e., not prechallenged with *L. monocytogenes*, showed a drop in the viable yeast counts in the spleen immediately after infection (Fig. 7). However, in the spleens of the latter mice, there were recurrences of the yeast growth as seen on the 6th and 17th day after infection (Fig. 7). In mice prechallenged with *L. monocytogenes*, the population of *C. albicans* in the spleen was not eliminated initially as rapidly as it was in the controls, but there was a steady drop in the number of viable cells and *C. albicans* was cleared from the spleen by the 7th day after infection. Also, for the duration of the 17-day experiment (Fig. 7), there was no recurrence of candida in the spleen of the mice which were prechallenged with *L. monocytogenes*.

There was also a noticeable difference in the kidney infections of those mice that were infected only with *C. albicans* and those which were prechallenged with *L. monocytogenes* and then challenged with candida (Fig. 8). In the latter group the initial *L. monocytogenes* challenge did not appear to affect the early stages of the superimposed *C. albicans* infection (Fig. 8). However, there was a marked drop in the *C. albicans* population after booster doses of *L. monocytogenes* on days 4 and 11 after *C. albicans* challenge. In the first experiment (Fig. 8) the *C. albicans* population was significantly ($P < 0.05$) reduced on the third day after the first *L. monocytogenes* boost, but the candida counts rose sharply again 2 days later. After the second boost with *L. monocytogenes* the effect was more apparent (Fig. 8). The number of

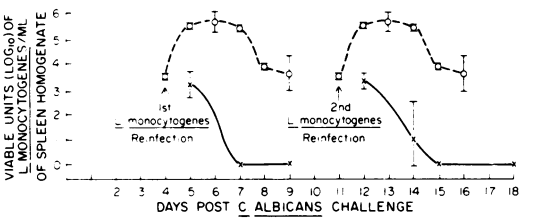

FIG. 6. *Growth curves of L. monocytogenes in the spleens of female mice after a primary challenge* (O---O) *and after reinfection with L. monocytogenes* (×——×). *(Each point represents the mean of four mice ± standard error.)*

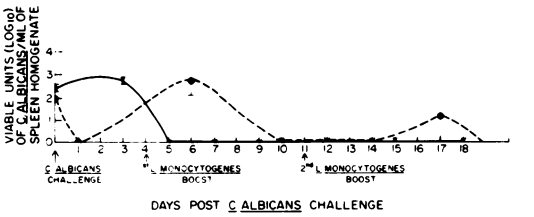

FIG. 7. *Growth curves of C. albicans in the spleens of female mice not challenged with L. monocytogenes* (●---●) *and in those infected previously and rechallenged with L. monocytogenes* (×——×) *(average of two experiments). (Each point represents the mean of four mice ± standard error.)*

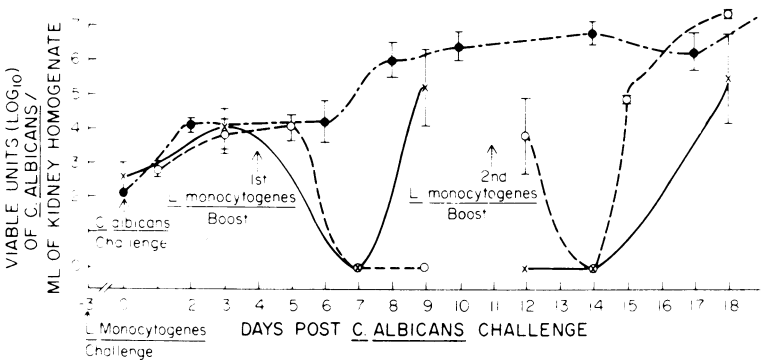

FIG. 8. *Growth curves of C. albicans in the kidneys of female mice not challenged with L. monocytogenes (●———●) and in those infected previously and rechallenged with L. monocytogenes (×——×, experiment 1; O- - -O, experiment 2). (Each point represents the mean of four mice ± standard error.)*

viable *C. albicans* dropped off significantly ($P < 0.05$) on the day after a second booster dose with *L. monocytogenes* and remained so for 3 days before the number of viable yeast cells in the kidneys started upwards again.

Similar effects were observed in a second repeat experiment (Fig. 8). However, the acquired nonspecific resistance to *C. albicans* was more persistent after the first *L. monocytogenes* boost than after the second one.

In both cases the *L. monocytogenes* challenges brought definite but short-lived reduction of the *C. albicans* infection in the kidneys.

Skin tests: delayed hypersensitivity reactions to C. albicans. The objective of this experiment was to determine if conventional mice and germfree rats, after being challenged with *C. albicans*, are able to manifest a delayed hypersensitivity skin reaction to *C. albicans* antigens, as an indication of acquired cell-mediated immunity (20).

Results were expressed as the average (of two or three animals) increase in footpad swelling in response to candida antigen over that of the control footpad (injected with saline). The Student's *t* test was used for determining the significance of (i) increase in swelling between control and antigen-injected footpads and (ii) increase in footpad swelling with progression of the infection.

Table 1 shows the results of skin testing. In conventional female mice there was no significant hypersensitivity to the commercial HS-Ag or the S-Ag in noninfected mice or in mice infected with either 10^3 or 10^6 VU of *C. albicans*. All control and infected mice showed a positive reaction to the C-Ag, as shown by significant increases ($P < 0.05$) in footpad swelling. The reactions to the C-Ag in all mice tested were similar at 4, 24, and 48 h after injection into the footpad. A marked increase ($P < 0.05$) in the response to the C-Ag was observed 30 days after infection with 10^3 VU of *C. albicans*. However, at no other time was there any significant increase in response to the C-Ag with progression of the infection. In fact in mice challenged with 10^6 VU of *C. albicans* there appeared to be an overall decrease in the footpad response to candida antigens (Table 1).

Conventional mice are known to harbor in their gastrointestinal tracts other yeast cells which may cross-react with *C. albicans* antigens (28). Therefore, germfree animals were used as a possibly better model for studying the specific relationship between delayed hypersensitivity and acquired cell-mediated immunity to *C. albicans* infections.

Results (Table 1) with the germfree rats show that there was no response to either fraction of the *C. albicans* antigens before the rats were infected by *C. albicans*. A positive reaction to the S-Ag appeared 4 days after infection whereas a positive reaction to the C-Ag was evident 8 days later. In each case the positive reactions showed significant ($P < 0.05$) increases in footpad swellings. There was also a significant increase ($P < 0.05$) in response to the C-Ag with progression of the infection.

DISCUSSION

In all the groups of *C. albicans*-challenged mice the fungus did not multiply extensively in the spleen. This indicated an immediate onset of the host defense mechanisms in the spleen. This goes along with the data of Lehrer and Cline (14, 15), which showed the rapidity with which human neutrophils and monocytes could phagocytize *C. albicans*, and of Salvin and Cheng (27), who demonstrated the increased

TABLE 1. *Increase in footpad thickness after skin testing conventional female mice and germfree rats sensitized to C. albicans*

Animal tested	*C. albicans* i.v. challenge dose (VU)	Antigen (Ag)	Footpad thickness (mm)[a] at 24 h						
			0[b]	6[b]	10[b]	14[b]	17[b]	22[b]	30[b]
Conventional female mice	10^3	S-Ag[c]	0.1[d] ± .2	0.5 ± .15	0.06 ± 0	0.17 ± .12	0.20 ± .03	0.13 ± .06	0.06 ± .06
		C-Ag[e]	2.2 ± .1	2.3 ± .3	2.0 ± .4	2.2 ± .6	2.0 ± .2	1.9 ± .3	3.0 ± .09
			2[b]		5[b]		7[b]		8[b]
Conventional female mice	10^6	S-Ag	0		0		0		0.1 ± .12
		Hs-Ag[f]	0		0		0		0
		C-Ag	1.0 ± .2		0.8 ± .03		1.5 ± .2		1.0 ± .13
			0[b]	4[b]	7[b]	12[b]	20[b]	24[b]	27[b]
Germfree rats	10^3	S-Ag	0	1.0 ± .13	0.8 ± .09	1.0 ± .2	1.4 ± .2		1.7 ± .4
		C-Ag	0	0.1 ± .08	0	1.3 ± .09		1.7 ± .06	

[a] Footpad thickness is measured as the difference between thickness of footpad injected with antigen and the thickness of footpad injected with saline.
[b] Days after challenge with *C. albicans*.
[c] Supernatant fraction.
[d] Each data point represents the mean of two or three animals ± standard error.
[e] Cell wall fraction.
[f] Hollister-Stier (commercial antigen).

capacity of immune macrophages to kill *C. albicans*. Also, increased clearance rates of the reticuloendothelial system after *C. albicans* challenge has been reported by Bird and Sheagrin (3). However, although there was a rapid onset of the control of *C. albicans* in our study, complete elimination from the spleen was not attained in all the mice. A possible explanation of the latter might be that some of the *C. albicans* survived, probably in phagocytic cells, and periodically were able to escape from the phagocytic cells by forming hyphal cells (14, 16) and increasing in numbers.

In all the infected animals the kidney appeared to be the main target of infection. It has been demonstrated that the concentrations of urinary solutes in the kidneys can suppress phagocytosis by human leukocytes (6)—especially functions such as migration, aggregation, and adhesion (4). Such effects may inhibit delivery of leukocytes to the site of infection (4). Louria, Brayton and Finkel (17) observed that the inflammatory response in the kidneys, after *C. albicans* infection, appeared 4 h later than in the other tissues, e.g., spleen, liver, lungs, and heart. This delayed cellular response would be expected to give the pathogen an advantage in establishing itself. They (17) also showed that *C. albicans* was able to break into and proliferate in the renal tubular lumen, within which it could be protected from the inflammatory reaction. In our study the surviving animals appeared to develop an immunity against the infection since *C. albicans* began to be eliminated from the kidneys 24 days after challenge.

Mackaness has demonstrated a definite relationship between delayed hypersensitivity and acquired cellular resistance (19). He showed that in the *L. monocytogenes* infection the peak of delayed hypersensitivity corresponded to the onset of resistance, whereas the peak of resistance in a *B. abortus* infection corresponded to the disappearance of delayed hypersensitivity which was probably due to desensitization because of the excess antigen present (19). In 1971 Salvin and Cheng (27) successfully sensitized guinea pigs to *C. albicans*. The guinea pigs showed delayed hypersensitivity responses to *C. albicans* antigen (commercial HS-Ag was used). However, no correlation was made between the appearance of delayed hypersensitivity and the onset of immunity. In this study normal, i.e.,

not challenged with *C. albicans*, mice reacted to the C-Ag just as well as mice which were chronically infected. Significant increase in footpad swelling was observed 4 h after skin testing and was still present at 48 h, indicating both an immediate type and delayed-type hypersensitivity. Reaction to the C-Ag could be expected since mice are known to harbor yeasts in their gastrointestinal tracts (28). Such yeasts could share antigens with the C-Ag of *C. albicans*. There was a significant increase in response to the C-Ag in female mice at 30 days postinfection (i.v.) with 10^3 VU of *C. albicans*. There was no increase in response to the C-Ag at any other time during the infection. Therefore, we cannot make any definite correlation between the delayed hypersensitivity response to the C-Ag and the onset of resistance to *C. albicans* since the C-Ag resulted in positive delayed-type hypersensitivity reactions in both challenged and control mice.

The S-Ag and HS-Ag are the antigens used for eliciting specific skin test reactions in humans. Our results showed that none of the mice showed significant footpad swelling (as compared to the control footpad) to the S-Ag or HS-Ag, although the animals had chronic kidney infections. It is possible that the mice were not sensitized to *C. albicans* using the i.v. challenge route and at these challenge doses. The conventional mouse does not appear to be a good model for studying skin test responses to *C. albicans*. Also, the failure to induce a delayed hypersensitivity response to the S-Ag or HS-Ag in mice may be related to the concentration of antigen used to elicit the reaction. Kong, Savage, and Kong (13) showed that, in mice vaccinated with *Coccidioides immitis* spherules, a higher percentage of positive skin test reactors was obtained when the concentration of *C. immitis* extract was 100 times greater than that effective in man, guinea pigs, and monkeys.

The germfree rats presented a nice model for correlating the relationship between delayed hypersensitivity and the *C. albicans* infection course. Any delayed hypersensitivity reaction to the *C. albicans* antigens would be specifically related to the *C. albicans* infection without the possibility of cross-reactions from other viable organisms. On day 0, before the rats were infected with *C. albicans*, they showed no reaction at all to either the S-Ag or C-Ag of *C. albicans*. This would be expected since the animals should be free from any viable organisms and therefore from any sensitized cells that could cross-react with the *C. albicans* antigens. After monocontamination with the yeast, the rats showed cutaneous reactions (significant increase in footpad swelling) at 24 h to the S-Ag 8 days earlier than to the C-Ag. The reason for the delay in response to the C-Ag in this case is not clear. With progress of the infection there appeared to be an increase in the intensity of the skin tests with the C-Ag (cell wall) but not with the S-Ag (cytoplasmic material).

As mentioned in the introduction, nonspecific cross-immunity between phylogenetically unrelated intracellular parasites has been achieved by some investigators (10, 25). In this study mice infected with a low (0^3 VU) dose of *C. albicans* were not protected against a subsequent *L. monocytogenes* challenge at any stage of the *C. albicans* infection. After a high (10^6 VU) dose of *C. albicans* there did appear to be some protection against *L. monocytogenes* 1 day after the *L. monocytogenes* challenge. However, the latter indication of immunity did not persist on days 2, 3, and 4 post *L. monocytogenes* challenge.

Because of the chronic kidney infection with *C. albicans*, there was a persistence of a high level of *C. albicans* antigen which should have caused a constant stimulation for active cellular immunity. However, this did not appear to be the case. A possible speculation is that, even though there was a high concentration of *C. albicans* antigen, it was almost totally localized in the kidneys (probably sequestered in the collecting tubules) and perhaps the *C. albicans* did not come into contact with the lymphoid cells in the reticuloendothelial system. While in the spleen, *C. albicans* appeared to be suppressed almost immediately after infection and, although sporadic increases of *C. albicans* in the spleen were observed, the antigenic load may not have reached the level or persisted long enough to activate macrophages and induce a nonspecific immunity against the *L. monocytogenes* challenge.

On the other hand, infection with *L. monocytogenes* followed by a challenge (i.v.) with *C. albicans* and then a rechallenge with *L. monocytogenes* appeared to confer some increased immunity against *C. albicans*. After each reinfection with *L. monocytogenes*, decreased numbers of *C. albicans* were demonstrable in chronically infected kidneys.

Assays of *L. monocytogenes* in the spleen showed that the *L. monocytogenes*-sensitized mice cleared *L. monocytogenes* reinfections much faster than primary challenged mice. The result verified a previous study by Mackaness (19) and showed that *L. monocytogenes* reinfec-

tions were able to recall immunity in mice previously sensitized to *L. monocytogenes*.

The nonspecific immunity induced in the kidneys of *C. albicans*-infected mice after each booster dose of *L. monocytogenes* was very short-lived (1 to 3 days). The efficiency of recalled immunity appears to be very much dependent upon the persistence of a sufficient antigenic level. This has been demonstrated by Collins, Mackaness, and Blanden (7) with *Salmonella* and by Halliburton and Hinsdill (10) with the *B. abortus* system. An appropriate booster dosage may also be important for recall of immunity. Mackaness (19) showed that a *L. monocytogenes* infection in mice gave protection against *B. abortus* challenge only when the animals were boosted with *L. monocytogenes* on every fourth day and with a high booster dosage (10 LD_{50}). In our experiments the *L. monocytogenes* antigen remaining in the kidneys of the mice on the day of recall (seventh day after infection) was very low and the recall dose we used (0.5 LD_{50}) might have been inadequate. If a strong booster dose was given and at more frequent intervals, the protection achieved might be more persistent. It is also possible that if the mice were reinfected with *L. monocytogenes* via another challenge route, e.g., in the footpads, the *L. monocytogenes* antigen would persist longer in the tissues and could give a more prolonged period of cross-protection to *C. albicans*.

In the spleens of mice which were infected with *L. monocytogenes* and then challenged with *C. albicans*, there appeared to be a slower elimination of the yeast cells early in the infection. However, spleen clearance of *C. albicans* was more complete as compared to mice infected with *C. albicans* only where sporadic increases in *C. albicans* were observed. However, more frequent spleen cultures in the early part of the infection course would have to be made to ascertain whether a *L. monocytogenes* challenge interfered with the control of *C. albicans* in the spleen during the first few days after infection.

Gentry and Remington (9, unpublished data) stated that they failed to find any cross-protection against *C. albicans* after a *L. monocytogenes* infection. This could be due to differences in dosages used, routes of infection, times of challenges, and so forth.

The mechanism of host resistance against *C. albicans* has not been clearly defined, although, as we have mentioned previously, there are data suggesting that the role of cells may be more important than humoral factors. Since cross-protection between intracellular parasites appears to depend on cellular immunity and since it was demonstrated in this study that *L. monocytogenes* infections provided some protection against *C. albicans* challenge, it seems, therefore, that acquired immunity to *C. albicans* does involve cell-mediated immunity.

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