Immunological Responses to *Candida albicans*  

II. Amyloidosis in Mice Induced by Candidiasis

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*Candida albicans* infection in the mouse thigh rapidly induces amyloidosis in mice of the C57BL/Ks strain; amyloid is induced more slowly and to a lesser extent by viable candida in C3H and AKR mice, and by both viable *Saccharomyces cerevisiae* and heat-killed *C. albicans* in C57BL/Ks mice.

During studies of immunological responses of mice to *Candida albicans* infection, we have found that yeast infections induce a fulminating amyloidosis in a majority of mice of a susceptible strain, and a more slowly developing amyloidosis in mice of less susceptible strains. The experimental model being used to study candidiasis is the mouse-thigh lesion, originally described by O'Grady (11) and recently developed in our laboratories (12). The production of amyloid is often associated with chronic infectious diseases or prolonged antigenic challenge with substances such as casein (5, 7). There is also considerable evidence linking abnormalities of T-cell function with amyloidosis (4, 6). Therefore, it was of particular interest to investigate the relationship between fungus infections and amyloid production.

**MATERIALS AND METHODS**

**Mice.** Inbred mice of the following strains were used: C57BL/Ks (H-2d, histocompatibility group), C3H (H-2k), and AKR (H-2a). Mice of both sexes, aged 3 to 12 months, were used.

**Fungi.** *Candida albicans* and *Saccharomyces cerevisiae* from stock collections were used. Organisms to be injected were grown for 24 to 48 h in Sabouraud glucose broth at 37°C, centrifuged from the broth, washed once in saline, counted, and suspended in diluent to achieve a concentration of 2.5 × 10⁶ yeast cells per ml.

**Thigh lesion.** The induction of thigh lesions with *C. albicans* has been described (12). Briefly, 5 × 10⁴ viable *C. albicans* cells are injected intramuscularly into the thigh. The size of the thigh lesion that ensues serves as an index of the course of the infection. The lesions peak in size generally during the second week and gradually decrease to normal size over a period of about 4 to 6 weeks after the initiation of infection. Histologically, the lesion has the characteristics of an abscess.

The thigh model was developed in C57BL/Ks mice and was subsequently found to follow the same course in inbred mice of other strains, including C3H and AKR (unpublished data).

**Histopathology.** Mice were killed and autopsied at various intervals, as described in the text. Tissues were fixed in 10% formalin, cut in 6- to 7-μm sections, and stained with either hematoxylin and eosin, periodic acid-Schiff, or Congo red. Congo red-stained sections were examined with polarized light to demonstrate dichroism of amyloid.

**RESULTS**

**Induction of amyloidosis by candida infection.** Profound changes occurred in the architecture of the spleen and other organs, beginning early during candida infection. The amyloid appears initially in the perifollicular region and progressively replaces the red pulp of the spleen. The identification of the amorphous, eosinophilic deposits as amyloid was confirmed by its dichroic Congo red staining. Control, noninfected mice of this strain have been consistently free of amyloid, whereas a majority of the candida-infected mice developed amyloidosis (Fig. 1). After the first 2 weeks of infection, 20 out of 23 or 87% of the mice examined showed evidence of amyloidosis. Figure 2 indicates the extent of amyloid deposition in the spleen, which is the organ most markedly affected. Amyloid is also demonstrable in the liver, adrenal, and kidney.

The experiments described in Fig. 1 showed conclusively that *C. albicans* can induce amyloidosis in mice. Additional experiments were performed to determine the relative importance of different strains of mice, and of differences in the fungi, in the induction of amyloidosis.

**Relative susceptibility of various mouse strains to fungus-induced amyloidosis.** Considerable variation in susceptibility to amyloid induction occurred among the mouse strains tested (Table 1). The C57BL/Ks mice were extremely susceptible, C3H mice much less so.
and AKR mice failed to develop amyloidosis in the small number of animals tested. The difference between the incidence of amyloid induced in C57BL/Ks mice and in the other two strains of animals examined more than 2 weeks after injection of viable candida is significant by the \( \chi^2 \) test (\( P < 0.01 \)).

**Amyloid induction by Saccharomyces cerevisiae and by killed fungi.** The results shown in Table 1 indicate that another fungus, *S. cerevisiae*, can also induce amyloidosis in susceptible mice. Viable saccharomyces yeast cells were injected into the thigh muscle, in the same manner as described for candida; the resulting thigh lesions followed an analogous self-limiting course. The incidence of amyloidosis in saccharomyces-injected C57BL/Ks mice was five out of nine (56\%). Viable saccharomyces did not induce amyloid production in any of the 11 C3H mice examined at intervals varying from 2 to 43 days. *Candida albicans* killed by heating at 60 C for 1 h and injected as described above for

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**Fig. 1.** Occurrence of amyloidosis in C57BL/Ks mice, either normal or infected with Candida albicans.

**Fig. 2.** Sections of spleen from C57BL/Ks mice. (A) Normal spleen. (B) Spleen 2 days after initiation of infection with Candida albicans. The light areas are filled with amyloid, as shown by Congo red staining.
viable candida induced amyloid production in one out of six C3H mice and in three out of ten C57BL/Ks mice.

**Influence of abscess formation on amyloid induction.** To determine whether abscess formation per se contributed to amyloid induction, sterile abscesses were induced by the injection of 0.2 ml of turpentine into the thigh muscle of C57BL/Ks mice. The turpentine-induced lesions followed a course similar to the lesions induced by fungi. Examination of mice bearing turpentine abscesses at various time intervals showed no evidence of amyloid in 10 of 11 mice; however, amyloid was demonstrable in one mouse examined 1 month after the injection of turpentine (Table 1).

**DISCUSSION**

These experiments show that the injection of either viable or nonviable yeast cells of two different genera readily induced amyloidosis in mice of a susceptible strain. Both *C. albicans* and *S. cerevisiae* were effective in inducing amyloid production. Viable yeast cells were more active in this respect than killed cells; however, this may merely reflect the final number of fungal cells in the tissues, since the viable yeasts proliferated during the first week of infection.

It is clear that different strains of inbred mice vary in their susceptibility to fungus-induced amyloidosis. Differences in the susceptibility of various mouse strains to casein-induced amyloidosis have often been reported (10).

Two other reports of amyloidosis in fungus-infected mice are of interest. Mankowski (9) suggested that mice experimentally infected with *C. albicans* develop amyloidosis; however, data concerning incidence were not presented, and it was noted that some of the control, noninfected mice were also amyloidotic. Another brief report (8) deals with the examination of two mice that had extensive dermatitis caused by *Trichophyton mentagrophytes* and that were found to be amyloidotic. Neither of these reports present any data concerning the kinetics of amyloid production.

The rapidity with which amyloid can be produced following candida infection is indicated in Fig. 1 and 2. Extensive amyloidosis was noted in five of the twenty-three C57BL/Ks mice examined during the first 2 weeks of infection. Such rapid deposition suggests that preformed precursors of amyloid may be available in noninfected mice.

Although amyloid has been studied for more than a century, much remains to be learned about its synthesis and its significance. Two kinds of amyloid have been described: one contains fragments of immunoglobulin light chains and is formed during various disease states, such as multiple myeloma; the other contains a unique protein, called amyloid A protein, and is commonly formed during chronic infections and prolonged antigenic stimuli (1, 5). It is not known whether amyloid occurs normally in small amounts and is deposited in large, detectable quantities only under certain conditions, or alternatively, whether the protein is only produced under abnormal circumstances. The A-protein-containing amyloid has been found in several species, including birds and mammals, and the amino acid composition and sequences are remarkably similar among diverse species (2). This suggests that the protein has an important function in order to have had its amino acid sequences conserved throughout evolution.

There is evidence that amyloidosis accompanies aging; in one study, virtually all human...
beings over 55 who were examined showed some amyloid deposition (13). Amyloid is deposited in some but not all organs during aging, and the amounts deposited are small (14).

Of particular interest are recent studies showing a correlation between T-cell depletion and amyloid production (3, 4, 6). Experiments are under way to assess the effects of the infection and amyloid production on T-lymphocyte populations in candida-infected mice.

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LITERATURE CITED