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Effect of Abrogation of Natural Killer Cell Activity on the Course of Candidiasis Induced by Intraperitoneal Administration and Gastrointestinal Candidiasis in Mice with Severe Combined Immunodeficiency

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Candida albicans **CFU per gram of tissue recovered from livers, spleens, and kidneys of 12 severe combined immunodeficiency (*scid*) and 12 BALB/c mice 5 days after intraperitoneal (i.p.) administration of 10^7 *C. albicans* cells were not significantly different. Nine *scid* mice given normal rabbit serum (NRS) as a control and eight *scid* mice given anti-asialo-GM1 (α-ASGM1) had *C. albicans* CFU per gram recovered from livers and spleens 1 week after i.p. administration of *C. albicans* that were not significantly different, despite virtual elimination of natural killer (NK) cell activity in mice treated with α-ASGM1. At 2 weeks after i.p. administration, despite significantly increased NK cell activity in eight infected NRS-treated *scid* mice and virtual elimination of NK cell activity by α-ASGM1 treatment of eight *scid* mice, *C. albicans* CFU per gram recovered from livers and kidneys were not significantly different. At 2 weeks after intragastric administration of 2×10^6 *C. albicans* cells, eight NRS- and eight α-ASGM1-treated *scid* mice had identical proportions colonized with *C. albicans* and similar *C. albicans* CFU per gram recovered from feces. There was no evidence of hematogenous dissemination in either group. Similar results were seen 1 week after intragastric administration of 10^7 *C. albicans* cells. We conclude that NK cell activity is increased by i.p. administration of *C. albicans* in *scid* mice, but nonetheless, abrogation of NK cell activity is not associated with enhanced susceptibility to candidiasis induced by i.p. administration and also is not associated with enhanced susceptibility to gastrointestinal colonization or hematogenous dissemination after intragastric administration of *C. albicans*.**

Mice with severe combined immunodeficiency (*scid*) have been used to analyze host defense mechanisms involved in *Candida albicans* infections (15, 19, 22). C.B-17 mice with the *scid* mutation have a defect in the recombinase system that is essential for the gene rearrangement required for production of immunoglobulins and T-cell receptors; as a consequence, *scid* mice lack mature B and T cells (6). *scid* mice are not more susceptible to candidiasis induced by intravenous (i.v.) administration than immunologically normal BALB/c mice are (15, 19). However, a greater proportion of *scid* than BALB/c mice develop persistent gastrointestinal (GI) colonization after receiving a single oral challenge with *C. albicans* (22). Despite more frequent and higher levels of *C. albicans* GI colonization, *scid* mice do not consistently develop hematogenously disseminated candidiasis arising from this GI colonization (15, 22). Thus, host defense systems, other than those mediated by B and T cells, can provide the host with normal clearance of candidiasis induced by i.v. administration and protect the host from hematogenous dissemination of *C. albicans* from foci of GI colonization. Cells that are candidates for this role include natural killer (NK) cells, cells of monocyte and macrophage lineage, and polymorphonuclear leukocytes (12).

The NK cell system is intact in *scid* mice (17). We hypothesized that NK cell function is an important component of host defense against candidiasis induced by i.v. administration and hematogenously disseminated candidiasis arising from GI tract colonization in the *scid* mouse model for several reasons. First, intraperitoneal (i.p.) injection of merthiolate-inactivated whole *C. albicans* or specific cell wall components activates NK cell activity and induces lymphokine-activated killer (LAK) cell activity in the peritoneal cavity in a murine model of *C. albicans* infection (20, 25, 26). Also, although neither human nor murine NK cells appear to be directly involved as natural killers of *C. albicans* (10, 27, 30), the presence of LAK cells with in vitro inhibitory activity against *C. albicans* can be demonstrated after prolonged incubation in vitro with interleukin-2 (3, 4). Third, gnotobiotic *scid/scid beige/beige* mice develop disseminated *C. albicans* infection after oral challenge, whereas gnotobiotic *scid* mice or beige mice do not (7); the abnormal NK cell function in these mice may be responsible for this enhanced susceptibility to hematogenous dissemination of GI candidiasis. Fourth, human NK and LAK cells stimulated in vitro by *C. albicans* produce tumor necrosis factor, gamma interferon, and granulocyte-macrophage colony-stimulating factor, all of which enhance phagocytic cell function against *C. albicans* (5, 10, 11, 28). Finally, studies with *scid* mice demonstrate a critical role for NK cell production of gamma interferon in host defense during *Listeria monocytogenes* infection (2, 29). Therefore, we hypothesized that *C. albicans* infection induces NK cell activation and production of lymphokines that modulate other cells to become more effective in phagocytosis and killing of the organism.

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In this investigation, we more directly examine the role of NK cells and their products in host defense in *C. albicans* infections. To do this, we further compromised *scid* mice by abrogation of NK cell activity with anti-asialo-GM1 (α-ASGM1), a polyclonal antiserum that causes NK cell lysis, in the presence of complement (16). Previous studies demonstrated abrogation of murine NK cell activity in vivo by administration of this antibody (13), including studies with *scid* mice (15, 18). We compared the course of candidiasis induced by i.p. administration of *C. albicans* in *scid* mice with and without NK cell abrogation and studied GI candidiasis and the hematogenous dissemination of *C. albicans* from GI foci under these conditions.

(These data were presented in part in abstract form at the 30th Annual Meeting of the Infectious Diseases Society of America, 1992 [12a].)

MATERIALS AND METHODS

Organism. *C. albicans* type A strain B311 (no. 32354; American Type Culture Collection, Rockville, Md.) was maintained in frozen Sabouraud glucose broth (Difco) stock cultures. Sabouraud glucose broth was inoculated from these stock cultures, and after overnight incubation on an orbital shaker at 37°C, yeast-phase organisms were harvested by centrifugation. The yeast cells were washed three times in sterile phosphate-buffered saline (PBS), a viable cell count was made (by using trypan blue exclusion) in a hemacytometer, and appropriate dilutions for administration to mice were prepared in PBS. Final suspensions were quantitatively cultured in Sabouraud glucose agar (Difco) to confirm concentration.

Mice. Male and female mice, 6 to 10 weeks of age, were used in all experiments. BALB/c mice were obtained from the Oklahoma Medical Research Foundation (Oklahoma City, Okla.) and were housed in ordinary mouse cages. C.B-17 *scid* mice, derived from the colony at the Oklahoma Medical Research Foundation (19), were bred at the Oklahoma City Department of Veterans Affairs Medical Center and were housed in microisolator cages. Cages, bedding, food, and water for the *scid* mice were sterilized before use. All mice were shown not to be colonized with *C. albicans* by negative stool cultures before experimental manipulation. The phenotypic purity of the *scid* mice was confirmed by the absence of immunoglobulin M, as determined by radial immunodiffusion (22), in serum obtained at necropsy.

i.p.-induced *C. albicans* infection in BALB/c and *scid* mice. Preliminary experiments administering escalating i.p. concentrations, beginning with 10^5 *C. albicans* cells, established that i.p. administration of 10^7 *C. albicans* cells resulted in a quantifiable but not immediately lethal disseminated infection. Therefore, this dose was chosen for further studies. Groups of 12 BALB/c and 12 *scid* mice were given 10^7 *C. albicans* cells i.p. and were necropsied on the fifth day after *C. albicans* administration. Livers, spleens, and kidneys were harvested under sterile conditions, diluted in PBS, and homogenized in a stomacher-type homogenizer (Tekmar, Cincinnati, Ohio), and then the samples were quantitatively cultured for *C. albicans* by a standard pour plate technique in Sabouraud glucose agar containing 25 mg of gentamicin sulfate and 50 mg of chloramphenicol sodium succinate per liter. After 48 h of incubation at 37°C, colonies were enumerated, and the number of *C. albicans* CFU per gram of tissue was determined. Because of necessary dilutions, the minimum detectable CFU per gram was 10; negative cultures were assigned 10 CFU/g for quantitative analyses.

In vivo depletion of NK cells. To abrogate NK cell activity, rabbit polyclonal α-ASGM1, (Wako Chemicals, Richmond, Va.) was used. In preliminary experiments, we determined that i.p. administration of 20 μl (0.328 mg) of α-ASGM1 every third day resulted in persistent abrogation of NK cell activity in *scid* mice. This dose was therefore chosen for all subsequent experiments. As a control, 0.328 mg of normal rabbit serum (NRS [Cappel Division, Organon Teknika, West Chester, Pa.]) was administered with the same schedule.

Assay of NK cell activity. NK cell activity was measured by a standard 18-h chromium release assay with YAC-1 target cells and nylon wool nonadherent splenocytes from two mice in the same treatment group as the effector cells at an effector/target ratio of 50:1 (14). Eight *scid* mice had spleens harvested for determination of NK cell activity in *scid* mice in the absence of *C. albicans* administration.

i.p.-induced *C. albicans* infection after NK cell depletion. Groups of nine control and eight experimental *scid* mice were given i.p. injections of NRS and α-ASGM1, respectively, beginning 1 day before i.p. administration of 10^7 *C. albicans* cells. Mice were killed on the seventh day after *C. albicans* administration, and *C. albicans* CFU per gram in liver and kidney tissues were quantified. Spleens were harvested for assay of NK cell activity.

In a second group of experiments, two groups of eight *scid* mice were treated with NRS or α-ASGM1 as described above, were administered *C. albicans* i.p. as described above, and were killed on the 14th day after *C. albicans* administration. Studies at necropsy were performed as described above.

GI candidiasis after NK cell depletion. Two groups of eight *scid* mice each were given NRS or α-ASGM1 i.p., as previously described. One day after the initial injection, each mouse received intragastric administration of 2×10^6 *C. albicans* cells by a metal feeding tube. Stool samples were collected from the mice every other day and were quantitatively cultured for *C. albicans* by a pour plate technique (22). Because of necessary dilutions, the minimum detectable *C. albicans* CFU per gram of feces was 10^2; negative cultures were assigned 10^2 CFU/g for quantitative analyses. At 14 days after intragastric *C. albicans* administration, the mice were killed; spleens were harvested for assay of NK cell activity, and livers and kidneys were harvested for culture of *C. albicans*.

In a final series of experiments, mice received intragastric administration of *C. albicans* as before, but a fivefold higher concentration (10^7 *C. albicans* cells) was used and the mice were killed on the seventh day after intragastric administration of *C. albicans*. In this experiment, 13 mice received α-ASGM1 and 14 mice received control NRS treatment in the same dose and schedule as previously employed. Liver and kidney samples were again quantitatively cultured for *C. albicans*.

Data analysis. Statistical analyses were performed with an Apple (Cupertino, Calif.) Macintosh computer with SYSTAT software (SYSTAT Inc., Evanston, Ill.). For hypothesis testing, two-tailed tests were used, and $P < 0.05$ was considered significant.

RESULTS

i.p.-induced *C. albicans* infection in BALB/c and *scid* mice. All mice survived i.p. administration of 10^7 *C. albicans* cells. All mice in both groups had *C. albicans* recovered from at least one of the three cultured organs. Mean ± standard

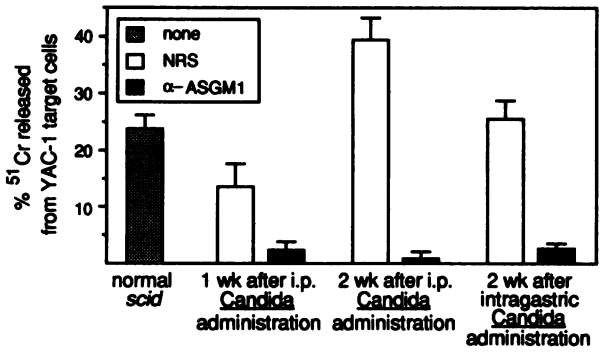

FIG. 1. NK cell activity of nylon wool nonadherent splenocytes, as determined by percentage ^{51}Cr release by lysis of YAC-1 target cells. The first bar represents NK cell activity of eight normal *scid* mice. Comparisons of control *scid* mice treated with 0.328 mg of NRS every third day with *scid* mice treated with 0.328 mg of rabbit polyclonal α-ASGM1 every third day are then shown for three different experiments: (i) 1 week after i.p. administration of 10^7 *C. albicans* cells (six assays of splenocytes from two mice of two groups of NRS-treated mice and two groups of α-ASGM1-treated mice), (ii) 2 weeks after i.p. administration of 10^7 *C. albicans* cells (four assays of splenocytes from two mice of two groups of NRS-treated mice and two groups of α-ASGM1-treated mice), and (iii) 2 weeks after intragastric administration of 2×10^6 *C. albicans* cells (four assays of splenocytes from two mice of two groups of NRS-treated mice and two groups of α-ASGM1-treated mice). In the latter three experiments, differences between NRS- and α-ASGM1-treated mice were significant ($P < 0.05$).

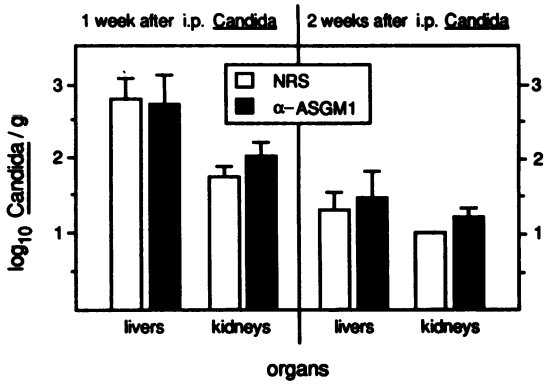

FIG. 2. Mean (+SEM) $\log_{10}$ *C. albicans* CFU per gram recovered from liver and kidney tissue of nine *scid* mice treated with NRS and eight *scid* mice treated with α-ASGM1 1 week after i.p. administration of 10^7 *C. albicans* cells are shown in the left panel. The difference between treatment groups was not significant ($P = 0.72$). The right panel represents mean (+SEM) $\log_{10}$ *C. albicans* CFU per gram recovered from liver and kidney tissue of eight *scid* mice treated with NRS and eight *scid* mice treated with α-ASGM1 2 weeks after i.p. administration of 10^7 *C. albicans* cells. The difference between groups at this interval was also not significant ($P = 0.37$).

error of the mean (SEM) recoveries of *C. albicans* from livers of BALB/c and *scid* mice were 3.21 ± 0.29 and 4.20 ± 0.48, respectively; from spleens, mean ± SEM recoveries were 4.04 ± 0.35 and 4.47 ± 0.4; and from kidneys, mean ± SEM recoveries were 2.41 ± 0.27 and 2.88 ± 0.28. One-way analysis of variance, with a repeated measures design for the recovery from the various organs of the same mouse, demonstrated that the difference in recovery of *C. albicans* from BALB/c and *scid* mice was not significant ($P = 0.103$).

i.p.-induced *C. albicans* infection after NK cell depletion. We then sought to determine whether abrogation of NK cell activity in *scid* mice would alter their susceptibility to i.p. administration of *C. albicans*. The efficacy of abrogation of splenic NK cell activity by treatment with α-ASGM1 is shown in Fig. 1. Splenic NK cell activity, as determined by the percentage of lysis of YAC-1 target cells by nylon wool nonadherent splenocytes, was 13.67% ± 3.98% (mean ± SEM of four measurements) in NRS-treated mice and 2.32% ± 1.36% in α-ASGM1-treated mice studied 1 week after i.p. administration of *C. albicans* ($P = 0.036$ by Student's *t* test). The NRS-treated mice had higher splenic NK cell activity (39.33% ± 3.93%) at 2 weeks after i.p. administration of *C. albicans* than at 1 week after i.p. administration of *C. albicans*, and this was again significantly higher than that of α-ASGM1-treated mice (1.0% ± 1.0%) at this interval ($P = 0.001$ by Student's *t* test). Furthermore, a one-way analysis of variance test ($P = 0.003$), followed by Tukey's multiple comparisons test, performed on data from the four groups of mice in Fig. 1 not treated with α-ASGM1 revealed that NK cell activity in the mice studied 2 weeks after i.p. *C. albicans* administration was significantly higher than the NK activities of normal *scid* mice and mice studied 1 week after i.p. *C. albicans* administration ($P < 0.05$).

The recoveries of *C. albicans* from the livers and kidneys of *scid* mice with NRS or α-ASGM1 treatment and i.p. administration of *C. albicans* are shown in Fig. 2. At 1 week after i.p. administration of *C. albicans*, cultures of livers and kidneys of all mice yielded *C. albicans*, except for the cultures of tissues from the kidneys of one NRS-treated mouse. These cultures yielded somewhat lower CFU per gram than did cultures of the *scid* mice at 5 days after i.p. administration of *C. albicans* in the previous experiment. Recoveries of *C. albicans* from livers and kidneys of the NRS- and α-ASGM1-treated mice were similar ($P = 0.72$; analysis of variance with a repeated measures design).

The recoveries of *C. albicans* from the livers and kidneys of control- and α-ASGM1-treated *scid* mice at 2 weeks after i.p. administration are also shown in Fig. 2. *C. albicans* CFU per gram recovered at 2 weeks were lower than at 1 week after i.p. administration. In fact, many cultures were negative, suggesting that the infection had been eradicated at this postinoculation interval. Cultures of the liver tissue were positive in three of eight mice in the NRS control group and four of eight mice in the α-ASGM1 group. Cultures of kidneys were positive in two of eight mice in the NRS group and three of eight mice in the α-ASGM1 group. When the negative cultures were assigned a value of 1.0 $\log_{10}$ CFU/g, and the quantitative data were analyzed by analysis of variance (with a repeated measures design for the two organs), the difference in recoveries of *C. albicans* from the NRS- and α-ASGM1-treated mice was not significant ($P = 0.37$).

GI candidiasis after NK cell depletion. Next, we sought to determine if abrogation of NK cell activity would alter the susceptibility of *scid* mice to GI colonization with *C. albicans* and/or facilitate hematogenous dissemination of *C. albicans* from foci of GI colonization. To assess susceptibility to GI candidiasis, fecal specimens from the eight *scid* mice treated with α-ASGM1 and eight NRS control-treated mice were cultured for *C. albicans* every other day for 2 weeks after intragastric administration of 2×10^6 *C. albicans* cells. The NK cell activity of nylon wool nonadherent splenocytes from the mice in this experiment is shown in

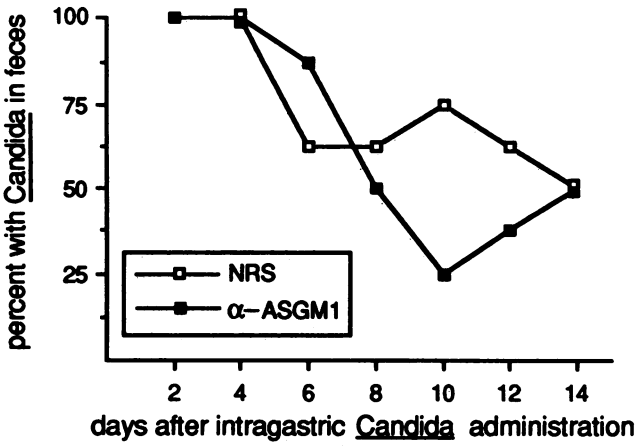

FIG. 3. Proportions of eight *scid* mice receiving treatment with NRS and eight *scid* mice receiving treatment with α-ASGM1 that had positive fecal cultures for *C. albicans* at intervals after intragastric administration of 2×10^6 *C. albicans* cells.

Fig. 1. YAC-1 target cell lysis was 25.47% ± 5.94% in the NRS group (not significantly different from that in the normal *scid* mice) and 2.65% ± 0.89% in the α-ASGM1 group ($P = 0.001$ by Student's t test). The proportions of these mice from whom stool cultures at the various intervals were positive for *C. albicans* are shown in Fig. 3. At the end of the experiment, an identical 50% of both NRS- and α-ASGM1-treated *scid* mice had fecal specimens positive for *C. albicans*. Figure 4A presents the results of quantitative stool cultures from these mice. The colonization magnitudes were very similar in the NRS- and α-ASGM1-treated *scid* mice ($P = 0.44$; analysis of variance with repeated measures design). *C. albicans* was not cultured from the liver and kidney tissue of any of the mice in either group.

To confirm these results, we next administered an intragastric inoculum of 10^7 *C. albicans* cells to groups of NRS- and α-ASGM1-treated *scid* mice. There were three deaths in this experiment: one NRS-treated mouse and two α-ASGM1-treated mice. At 6 days after intragastric administration of *C. albicans*, 10 of 12 surviving NRS-treated mice and all 12 α-ASGM1-treated mice had fecal cultures positive for *C. albicans* ($P = 0.48$; Fisher's test). Results of quantitative fecal cultures are shown in Fig. 4B. Again, no significant difference between groups was demonstrated ($P = 0.77$; analysis of variance with a repeated measures design).

There was not consistent evidence of dissemination of *C. albicans* from the GI tract. However, one NRS-treated mouse had <10 *C. albicans* CFU/g of liver and kidney, while similar numbers of *C. albicans* CFU per gram were isolated from the liver of one α-ASGM1-treated mouse and the kidneys of another α-ASGM1-treated mouse. Overall, 10 of 13 NRS-treated *scid* mice in this experiment survived to necropsy and had no evidence of hematogenously disseminated candidiasis, and 10 of 14 α-ASGM1-treated *scid* mice survived to necropsy and had no evidence of hematogenously disseminated candidiasis ($P = 1.0$; Fisher's test).

DISCUSSION

Our primary purpose in further studying i.p. administration of *C. albicans* was to begin to dissect the mechanisms by which *scid* mice are able to contain this infection as well as BALB/c mice do (15, 19). We found that i.p. injection of 10^7 *C. albicans* cells reproducibly resulted in a quantifiable infection and that *scid* and BALB/c mice were identically susceptible. This inoculum is 100 to 1,000-fold higher than that used in studies in which *C. albicans* has been administered i.v. to *scid* mice (15, 19), and this inoculum resulted in recoveries of *C. albicans* from livers, spleens, and kidneys that were roughly equivalent at 5 days after i.p. administration to those seen at 7 days after i.v. administration.

Normal clearance of candidiasis induced by i.p. administration should not be taken as evidence that B- and T-cell mechanisms are of no importance in eradicating this infection, particularly in hosts that have prior exposure to *C. albicans*. $CD4^+$ and $CD8^+$ T-cell mechanisms have been shown to be important in animal models of candidiasis induced by i.p. or i.v. administration (8, 9, 23, 24). Rather, the *scid* model affords an opportunity to investigate additional host defense mechanisms that are sufficient in *scid* mice to eliminate candidiasis induced by i.p. or i.v. administration and may be important in other hosts.

We found that abrogation of NK cell activity did not enhance susceptibility of *scid* mice to candidiasis induced by i.p. administration. We and others had formulated hypotheses that NK cell activity would be important in the *scid* mouse model of candidiasis induced by i.p. or i.v. administration (7, 19, 21) on the basis of evidence of activation of

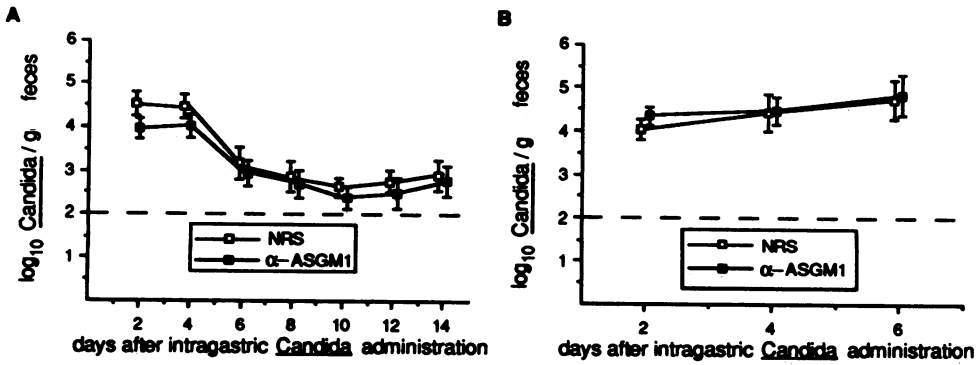

FIG. 4. Mean (±SEM) $\log_{10}$ *C. albicans* CFU per gram of feces at various intervals after intragastric administration of 2×10^6 *C. albicans* cells in two groups of eight *scid* mice treated with either control NRS or α-ASGM1 (0.328 mg i.p. every third day, beginning the day before intragastric administration of *C. albicans*). The difference between groups was not significant ($P = 0.44$). (B) Mean (±SEM) $\log_{10}$ *C. albicans* CFU per gram of feces at various intervals after intragastric administration of 10^7 *C. albicans* cells and treatment with NRS (14 *scid* mice) or α-ASGM1 (13 *scid* mice) as described above. The difference between groups was not significant ($P = 0.77$).

NK and LAK activity by *C. albicans* (20, 25, 26). Our data (Fig. 1) demonstrate increased splenic NK cell activity in NRS control-treated mice 2 weeks after i.p. administration of *C. albicans*, confirming this induction of NK cell activity. Nonetheless, abrogation of this activity was not associated with impairment of clearance of *C. albicans* from livers and kidneys. It has previously been reported that recovery of *C. albicans* 24 h after i.v. administration in *scid* mice treated with α-ASGM1 is not significantly different from recovery in control *scid* mice (15). Our data here extend that observation to an interval after infection at which induction of NK cell activity was evident. Thus, this NK cell activity is not demonstrated to be important in vivo in host defense against candidiasis induced by i.p. administration.

LAK cells or LAK-like cells with inhibitory activity against *C. albicans* have been generated from an NK cell-enriched population of splenocytes after several days of incubation with interleukin-2 (3, 4). A role for such cells in host defense has not been established. It is not clear that such cells or their precursors express α-ASGM1 and thus were abolished in our model. It is also unknown whether such cells have the same functional characteristics in *scid* mice that they do in C57BL/6 mice, which are intrinsically more resistant to candidiasis induced by i.v. or i.p. administration than BALB/c mice.

The mechanisms whereby *scid* mice are made more susceptible to GI colonization than immunologically normal mice are unknown (22). B and/or T cells and their products must play a role in preventing colonization. It was conceivable therefore that NK cells and their products also might serve a role in limiting GI colonization with *C. albicans*. However, abrogation of NK cell activity—at least NK cell activity measured in the spleen—did not enhance GI colonization in this study.

Hematogenous dissemination of candidiasis from foci of GI colonization does not consistently occur in *scid* mice (15, 22), suggesting that NK cells and their products might serve a role in host defense against this form of infection. Hematogenous dissemination does occur in gnotobiotic *scid/scid beige/beige* mice (7). Although initially attributed to the NK cell defect in beige mice, this now seems more likely to be related to their phagocytic cell defect (1). Hematogenous dissemination also occurs in gnotobiotic *scid* mice treated with poly(I · C), apparently because of macrophage inhibition by stimulation of alpha/beta interferon production (15). In the present study, we found no consistent evidence of hematogenous dissemination of *C. albicans* from the GI tracts of NK cell-depleted *scid* mice.

Thus, we conclude that host defense systems remaining intact in NK cell-depleted *scid* mice are capable, in the absence of functional B, T, and NK cells, of providing elimination of candidiasis induced by i.p. administration and prevent hematogenously disseminated candidiasis from arising from foci of GI colonization. The effectors of clearance of *C. albicans*, cells of monocyte and macrophage lineage and polymorphonuclear leukocytes (as reviewed in reference 12), are ultimately responsible for this. The roles of other host defense systems in supporting and modulating these effectors and their relative roles in effecting the clearance of *C. albicans* remain to be elucidated.

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