MYCOLOGY

Antifungal activity of interleukin-2-activated natural killer (NK1.1⁺) lymphocytes against *Candida albicans*

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Interleukin-2 (IL-2)-activated lymphocytes interact directly with, and inhibit, the growth of *Candida albicans* hyphae. *C. albicans*-stimulated natural killer (NK1.1⁺) lymphocytes were demonstrated to secrete a soluble product capable of directly affecting *C. albicans* yeast forms. Antibodies specific for interferon- γ completely eliminated the antifungal activity of the NK1.1⁺ lymphocyte product and diminished the antifungal activity of NK1.1⁺ lymphocytes against *C. albicans*. Antibodies specific for other cytokines had no such effect. These data demonstrate that *C. albicans*-stimulated NK1.1⁺ lymphocytes have antifungal activity against *C. albicans* yeast cells via the release of interferon- γ . This antifungal activity was demonstrable only against the yeast form of the fungus, with no effect on *C. albicans* hyphae.

Introduction

Natural killer (NK) cells have been shown to inhibit the growth of several fungi, including *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, *Coccidioides immitis* and *Histoplasma capsulatum* [1]. NK cells have no apparent capacity to inhibit the growth of *Candida albicans* [2–4]. NK cell-mediated inhibition of growth of fungi, other than *C. albicans*, is absolutely dependent on the direct contact of NK cells with the fungal surface. Even though NK cells do not inhibit the growth of *C. albicans*, NK cells as well as large granular lymphocytes (LGL), have been shown to interact with the surface of both *C. albicans* yeast cells and hyphae [3–6]. This interaction has been demonstrated by competitive inhibition of lymphocyte binding to *C. albicans* [3, 4], by direct measurement of adhesion of lymphocytes to fungal hyphae [2, 6] and by yeast cell stimulation of cytokine synthesis in LGL [5, 7].

NK cells and other lymphocyte populations can be activated by extended culture with interleukin-2 (IL-2) [8–10]. When C57B1/6 mouse lymphocytes are activated with IL-2, two lymphocyte populations are produced; a CD8⁺ NK 1.1⁺ pan-cytotoxic T lympho-

cyte population and a CD8⁺ NK 1.1⁺ activated NK cell population [9–11]. Both IL-2-activated CD8⁺ lymphocytes as well as IL-2-activated NK1.1⁺ lymphocytes bind *C. albicans* [9]. IL-2 activation produces lymphocytes with antifungal activity against the hyphal form of the fungus [3, 8]. The antifungal activity is produced by lymphocytes that express CD8 but do not express NK1.1 [9]. This CD8⁺ lymphocyte population not only affects the growth of *C. albicans* hyphae but is also cytotoxic for multiple tumour cell lines with distinct major histocompatibility complex (MHC) antigens. The functional activity of the lymphocyte population is consistent with that of lymphokine-activated killer (LAK) cells [9]. Under similar test conditions, IL-2-activated NK1.1⁺ lymphocytes had no antifungal activity against *C. albicans* hyphae.

This investigation aimed to determine whether IL-2-activated NK1.1⁺ lymphocytes were capable of exerting an antifungal activity against *C. albicans* yeast cells. In the C57B1/6 mouse, NK1.1 is a marker for both NK cells and cytokine-activated NK cells. NK cells, LGL and LAK cells can be induced to produce various cytokines, including interferon- γ (IFN- γ), IL-2, tumour necrosis factor (TNF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [12–14]. *C. albicans* yeast cells have been shown to induce the release of TNF [7] and GM-CSF [5] directly from such cells without the necessity for a priming signal.

Materials and methods

Mice

C57B1/6 female mice, aged 6–7 weeks, were obtained from Jackson Laboratory, Bar Harbor, ME, USA. Mice were 6–12 weeks of age when used in experiments.

Fungal cultures

C. albicans ATCC 58716 was obtained from Dr T. Hashimoto, Loyola University Medical Center, Maywood, IL, USA, and used throughout this investigation. The cultures were stored at 25°C on Sabouraud's Dextrose Agar (SDA) (Becton Dickinson, Cockeysville, MD, USA). The cells were cultured overnight at 37°C on SDA, collected as isolated colonies and washed once in Hanks's Balanced Salts Solution (HBSS). *C. albicans* hyphal forms were obtained by incubation at 37°C in RPMI 1640. Clinical isolates of *C. albicans* were obtained from the Clinical Laboratories, Loyola University Medical Center.

Recombinant molecules, antibodies and the detection of IFN- γ

Recombinant human IL-2 was obtained from Hoffmann LaRoche, Nutley, NJ, USA. Recombinant mouse IFN- γ , recombinant mouse TNF and recombinant mouse GM-CSF were obtained from Genzyme, Cambridge, MA, USA. The effects of IFN- γ were tested as described in the text. The effects of the other cytokines were evaluated with maximal assessment of IL-2 at 1500 units/assay, TNF at 1500 units/assay and GM-CSF at 100 units/assay. Murine IFN- γ was detected with ELISA kits obtained from Genzyme. Monoclonal hamster anti-mouse IFN- γ , monoclonal hamster anti-mouse TNF and monoclonal rat anti-mouse GM-CSF antibodies (MAbs) were obtained from Genzyme and assessed for effects at concentrations of up to 0.5 μ g/assay. These MAbs were all IgG.

IL-2 activation of splenocytes

Spleens from untreated mice were removed aseptically. Single cell suspensions were prepared by dissociating the spleen through a 60-gauge wire mesh with the hub of a syringe. Spleen cells were washed once in HBSS and dispensed in culture medium containing 5×10^{-5} M 2-mercaptoethanol (2-ME) at a concentration of 2.5×10^6 cells/ml with IL-2 1500 U/ml in Falcon Multiwell plates (Becton Dickinson). This quantity of IL-2 has been shown previously to be optimal for the induction of antifungal activity in IL-2-activated lymphocytes [8]. Non-adherent cells were harvested after incubation at 37°C, overlaid on to Lymphocyte Separation Medium (Litton Bionetics, Kensington, MD, USA) and centrifuged at 1000 g for 20 min. The cells at the interface were washed twice with HBSS before assessment of growth inhibitory activity. These splenocytes were >99% lymphocytes, as judged by Wright-Giemsa staining.

Stimulation of IL-2-activated lymphocytes with *C. albicans*

IL-2-activated lymphocytes were prepared as described above and 1×10^6 cells were placed into Falcon multiwell plates containing 1 ml of RPMI 1640 and either 1×10^5 *C. albicans* yeast or the hyphal forms prepared from 1×10^5 *C. albicans* yeasts as described above. After stimulation with *C. albicans* for 1–4 h at 37°C in air with CO₂ 5%, the supernates of the co-cultures of lymphocytes with *C. albicans* were collected, centrifuged at 1000 g, filtered through a 0.45- μ m filter (Gelman Sciences, Ann Arbor, MI, USA) and immediately assayed for effects on *C. albicans*. These conditions were found to be optimal for the production of antifungal supernates. Larger numbers of *C. albicans* or longer periods of incubation did not increase the antifungal activity of the culture supernates.

Complement-mediated depletion analysis

Antibodies used to deplete cell populations were MAb anti-CD8 (clone AD4; mouse IgM) from Accurate Chemical and Scientific Corp. (Westbury, NY, USA) and MAb anti-NK 1.1 (clone PK136; mouse IgG) obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Viable lymphoid cells were obtained with lymphocyte separation medium as above and washed twice with HBSS. Lymphocyte populations were depleted of those cells bearing specific antibodies by incubating 1×10^7 cells with saturating levels of antibody for 1 h at 4°C. The cells were washed three times with HBSS to remove excess antibody and then 100 μ l of Low Tox M rabbit complement (Accurate Chemical and Scientific Corp.) were added to the cell pellet. After incubation for 1 h at 37°C, cells were washed once with HBSS, separated by use of lymphocyte separation medium as above and washed twice with HBSS before assessment of their growth inhibitory activity. The effectiveness of the depletion was assessed by direct immunofluorescence and the number of cells recovered from treated populations was compared with that from untreated populations. IL-2-activated lymphocytes depleted by treatment with anti-CD8 and complement showed background levels (5.3 SD 3.3) of CD8⁺ cells as judged by direct microscopic immunofluorescence. Anti-NK1.1- and complement-treated IL-2-activated lymphocytes showed background levels (4.6 SD 4.1) of NK1.1⁺ cells as judged by direct microscopic immunofluorescence. Cells were re-counted after complement depletion and resuspended to the indicated cell concentration before measurement of antifungal activity.

Inhibition growth of *C. albicans*

The antifungal activity of lymphocytes for *C. albicans* was determined as described previously [11]. Briefly, fungal cells used for experimentation were collected from isolated, overnight SDA colonies and washed

once in HBSS. Yeasts were resuspended to 2×10^5 /ml in RPMI 1640 and 1×10^4 yeasts were added to individual wells of 96-well, flat-bottomed plates (Corning 25861, Corning, NY, USA). *C. albicans* hyphal forms were obtained by incubation at 37°C in air with CO₂ 5% for 2 h. Lymphocytes were added to give varying effector to target ratios. After incubation for 2 h at 37°C in air with CO₂ 5%, effector lymphocytes were lysed and removed by washing with water in a PHD cell harvester (Cambridge Technology, Cambridge, MA, USA). Then 0.05 ml of RPMI 1640 containing 1 μ Ci of [³H] uridine (ICN Radiochemicals, Irvine, CA, USA) was added to individual wells. After incubation for 1 h at 37°C, in air with CO₂ 5%, the [³H] uridine was removed by washing and 25 U of lyticase (Sigma) in 50 μ l of HBSS was added to individual wells for 0.5 h at 25°C. Cells were then harvested with a PHD cell harvester and incorporated radioactivity was determined with a Beckman LS5801 β liquid scintillation counter. Growth inhibition of *C. albicans* = {(DPM *C. albicans* control) – DPM (effector and *C. albicans* – effector control)/DPM *C. albicans* control} $\times$ 100. All cultures were prepared at least in triplicate and the mean inhibition values were determined. The effects of lymphocytes on the yeast form of *C. albicans* were determined as described above except that lymphocytes were added to assay wells containing *C. albicans* yeast cells. *C. albicans* yeasts cells were placed in flat-bottomed plates in HBSS 15 min before the addition of lymphocytes to facilitate the association of the yeasts with the plastic surface of the assay wells. After 2 h, the lymphocytes

were removed. All further procedures were as described above for hyphae. Yeast germination was scored microscopically.

Statistical analysis

The results are expressed as means and SD. The data were analysed by one-way ANOVA and further analysed by Student Newman-Kvels test for multiple comparisons between groups; $p < 0.05$ was considered significant.

Results

Effect of C. albicans-stimulated culture supernates on the growth of C. albicans yeast and hyphae

A previous study showed that conditioned media from IL-2-activated lymphocytes (unstimulated culture supernates) had no antifungal effect against the hyphal form of *C. albicans* [3]. This study assessed whether culture supernate of *C. albicans*-stimulated IL-2-activated lymphocytes could affect the fungus. Lymphocytes cultured for 7 days with IL-2 were stimulated with either the yeast or hyphal forms of *C. albicans*. These stimulated culture supernates were then assessed for their capacity to inhibit the growth of the hyphal form of *C. albicans* (Fig. 1). No effect of yeast or hyphae-stimulated culture supernates was seen against the hyphal form of *C. albicans*. When these stimulated culture supernates were assessed for their capacity to

Fig. 1. Inhibition of *C. albicans* growth by culture supernates (various volumes, μ l) of IL-2-activated lymphocytes stimulated with *C. albicans*; 1×10^6 IL-2-activated lymphocytes were stimulated with either 1×10^5 *C. albicans* yeasts or 1×10^5 *C. albicans* hyphae and the effect of the culture supernates on *C. albicans* yeast or hyphae forms was judged by *C. albicans* incorporation of [³H] uridine. Yeast-stimulated IL-2-activated lymphocyte culture supernates were assessed for effect on yeasts (○) and hyphae (□). Hyphae-stimulated IL-2-activated lymphocyte culture supernate were assessed on yeasts (●) and hyphae (■). Data are expressed as mean percentage growth inhibition and SD for three individual experiments.

affect the yeast form of the fungus, an antifungal effect was obtained with yeast-stimulated but not with hyphae-stimulated culture supernates. To determine whether the antifungal effect of the yeast-stimulated culture supernates was limited to *C. albicans* strain ATCC 58716, seven separate clinical isolates of *C. albicans* were evaluated. Each clinical isolate was similarly responsive to yeast-stimulated culture supernates as was *C. albicans* strain ATCC 58716 (data not shown). Media conditioned by either yeast or hyphae (without mammalian cells) had no effect on the growth of either yeast or hyphae. Media conditioned by IL-2-activated lymphocytes (without either yeast cells or hyphae) had no effect upon the growth of either yeasts or hyphae (data not shown).

Effect of C. albicans yeast-stimulated culture supernates on the germination of C. albicans yeasts

Microscopic observation of *C. albicans* treated with culture supernates, from co-culture of IL-2-activated lymphocytes with *C. albicans*, showed these to be retarded in their capacity to germinate (Fig. 2). The retardation in germination of these yeasts was apparent from 30 to 90 min after the addition of the stimulated culture supernate. By 120 min all had germinated. In contrast, no effect on germination was observed when yeasts were treated with supernates from co-culture of IL-2-activated lymphocytes with *C. albicans* hyphae. Media conditioned by either yeast or hyphae (without mammalian cells) had no effect on yeast germination. Media conditioned by IL-2-activated lymphocytes

(without either yeasts or hyphae) had no effect upon germination.

Cytokine effects on the germination of C. albicans yeasts

C. albicans has been shown previously to induce lymphocytes to release TNF and GM-CSF [5, 7]. Those cytokines and IFN- γ were evaluated for their capacity to inhibit yeast germ-tube formation (Fig. 3). IFN- γ directly inhibited germination. Antibodies reactive with IFN- γ (0.1 μ g/culture well) abolished the effect of the cytokine, whereas antibodies reactive with either TNF or GM-CSF had no effect on IFN- γ inhibition of yeast germ-tube formation at concentrations as high as 0.5 μ g of antibody/yeast culture (data not shown). TNF and IL-2 (1500 units/yeast culture) and GM-CSF (100 units/yeast culture) had no effect upon germ-tube formation when added directly to yeasts.

Effect of anti-IFN- γ on the antifungal activity of IL-2-activated lymphocytes

Extended culture of C57B1/6 splenocytes with high dose IL-2 produced two LGL populations, one which was CD8⁺ and another which was NK1.1⁺ [10]. These cell populations were enriched by use of specific antibodies and complement and their capacity to inhibit the growth of *C. albicans* yeast cells was determined (Fig. 4). Both CD8⁺ and NK1.1⁺ cell populations had the capacity to inhibit the growth of *C. albicans* yeast as judged by the incorporation of [³H] uridine into the fungus. The addition of anti-IFN- γ to

Fig. 2. The effect of *C. albicans*-stimulated culture supernates of IL-2-activated lymphocytes on germ-tube formation by *C. albicans* yeasts; 1×10^6 IL-2 activated lymphocytes were stimulated with either 1×10^5 *C. albicans* yeast (○) or 1×10^5 hyphae (●) and the effect of the culture supernates on *C. albicans* yeast determined by microscopic observation of germ-tube formation by the yeasts. Culture supernates from yeasts cultured alone (□) or hyphae cultured alone (■), and culture supernates from IL-2-activated lymphocytes cultured alone (△) were also evaluated. Data are expressed as mean percentage germination of yeasts into germ-tubes and SD for three individual experiments.

Fig. 3. Effects of cytokines on *C. albicans* germ-tube formation. *C. albicans* yeasts were incubated with 10 (●), 5 (▲) and 0.5 units of IFN- γ (▼) and with 1500 units of TNF (■), 100 units of GM-CSF (□) or 1500 units of IL-2 (○) or were untreated (▽). *C. albicans* yeasts were also incubated with a combination of 10 units of IFN- γ and 0.1 μ g of anti-IFN- γ (△). Final volumes were 200 μ l. Data are expressed as mean percentage germination of yeasts into germ-tubes and SD for three individual experiments.

Fig. 4. Growth inhibition of *C. albicans* yeast cells by IL-2-activated lymphocytes after in-vitro antibody and complement depletions. IL-2-activated cells were treated with complement plus anti-CD8 (NK1.1⁺ lymphocytes) (□), or anti-NK 1.1 (CD8⁺ lymphocytes) (○). Anti-IFN- γ (0.1 μ g/assay) was added separately to *C. albicans* yeasts cultured with IL-2-activated lymphocytes treated with complement plus anti-CD8 (NK1.1⁺ lymphocytes) (■) or anti-NK 1.1 (CD8⁺ lymphocytes) (●). Inhibition of *C. albicans* growth was assessed by the incorporation of [³H] uridine. Data are expressed as mean percentage inhibition and SD of three independent experiments.

the NK1.1⁺ cell population significantly ($p < 0.05$) reduced the antifungal effect of the lymphocytes at effector: target ratios of 50:1 and 25:1. In contrast, the addition of anti-IFN- γ to the CD8⁺ cell population had no effect on the capacity of the lymphocytes to inhibit the growth of the yeast form of the fungus. No effect was observed when antibodies to either TNF or GM-

CSF were added to either cell population (data not shown).

IFN- γ production by IL-2-activated lymphocytes

CD8⁺ and NK1.1⁺ cell populations were prepared by antibody and complement depletion and assessed for

their capacity to produce IFN- γ when stimulated with *C. albicans* yeast cells. As shown in Fig. 5, the NK1.1⁺ cell population released IFN- γ when stimulated with *C. albicans* but the CD8⁺ cell population did not. When the CD8⁺ cell population was assayed by ELISA for cell-associated IFN- γ , none was detected (data not shown).

Discussion

To date, investigations in this laboratory have focused on the effects of IL-2-activated lymphocytes upon the hyphae of *C. albicans*. They have shown that extended culture of mouse lymph node cells, mouse splenocytes or human peripheral blood mononuclear cells with high concentrations of IL-2 results in a population of lymphocytes capable of inhibiting the growth of *C. albicans* hyphae *in vitro* [3, 8, 11]. In the mouse, the investigations have focused upon the C57B1/6 strain

because it has been demonstrated to be the most resistant to challenge with *C. albicans* [15]. In this murine system, studies have shown that IL-2-activated CD8⁺ lymphocytes mediate an antifungal effect against hyphae but NK1.1⁺ lymphocytes do not [9]. Even cytokine-activated NK1.1⁺ lymphocytes had no effect against hyphae. This is consistent with recent observations with human IL-2-activated NK cells that failed to demonstrate an antifungal effect against *C. albicans* [2]. Those IL-2-activated lymphocytes were MHC unrestricted in their cytotoxic activity for tumour cell lines, but the cell population contained only 1.5% T cells. The inability of that cell population to inhibit the growth of *C. albicans* hyphae *in vitro* is most probably due to absence of CD8⁺ T lymphocytes [9]. This IL-2-activated cell population was produced with culture conditions different from the methods employed in the present study and in our previous investigations [3, 8, 9]. The difference in culture conditions may directly relate to the observed differences in antifungal effect upon *C. albicans* hyphae. An absolute requirement for the generation of antifungal effector lymphocytes is the extended culture period with high dose IL-2 [3, 8, 9]. It is clear that all LAK cells do not mediate an antifungal effect, particularly if they are activated NK cells [2]. The production of an antifungal effect against *C. albicans* hyphae requires IL-2-activated CD8⁺ lymphocytes in the murine system.

Fig. 5. Release of IFN- γ by IL-2-activated lymphocytes after stimulation with *C. albicans* yeasts. IL-2-activated cells were treated with complement plus anti-CD8 (NK1.1⁺ lymphocytes), or anti-NK 1.1 (CD8⁺ lymphocytes), or complement (C) alone and cultured with 1×10^5 *C. albicans* yeasts for 1, 2 or 4 h. Released IFN- γ was assessed by ELISA. No IFN- γ was detected in IL-2-activated lymphocyte culture supernates before stimulation with *C. albicans* yeasts. Data are expressed as units of released IFN- γ /ml of culture supernate and are the means and SD of three independent experiments.

The assay used to measure antifungal effects against *C. albicans* in the present study is one which has been shown to be sensitive, rapid and reproducible [11]. It compares well with other assays of antifungal activity and in this laboratory is the most sensitive and reproducible of the described assays. This may be important with regard to the capacity of IL-2-activated NK1.1⁺ lymphocytes to inhibit the yeast-to-hyphal transition by the production of IFN- γ . The effect of IFN- γ upon the yeast-to-hyphal transition is only a retardation in the ability of the yeast to enter a germ-tube stage. This effect of IFN- γ was maximal during the first 90 min with all yeast cells germinated by 120 min. The use of radioactive precursor incorporation assays that extend for significantly longer periods of time would not necessarily detect this effect on the growth of the micro-organism. Arancia *et al.* showed human IL-2-activated NK cells to have no effect on the yeast form of *C. albicans* [2]. This may be a consequence of the more lengthy assessment of [³H] glucose incorporation into the fungus. In that study, [³H] glucose incorporation into *C. albicans* was permitted to occur for 3 h after incubation for 4 h with IL-2-activated NK cells. In the assay reported here, [³H] uridine incorporation for 1 h after incubation for 2 h with IL-2-activated lymphocytes was studied. This difference in the period of incubation and assessment may result in the fungus incorporating indistinguishable levels of radioisotope. Culture conditions or culture media other than those described have not been investigated in this laboratory and the

results may be different with other culture conditions or media. The observed effects are apparent only within a very early period of incubation. Human LGL are stated to produce IFN- γ when stimulated with *C. albicans* [10]. Therefore it is probable that the differences in results of the present study and those described by Arancia *et al.* are a consequence of the methods of measurement of antifungal activity [2].

IFN- γ has been shown previously to inhibit germ-tube formation by *C. albicans* [16]. LGL stimulated with *C. albicans* have been reported to produce this cytokine [17]. More recently, IFN- γ has been shown to be released by human lymphocytes directly stimulated with *C. albicans* [18]. In this investigation the release of IFN- γ was induced quickly in IL-2-activated NK1.1⁺ cells after stimulation with the yeast form of the fungus. Previously, TNF has been shown to be maximally released by *C. albicans*-stimulated NK cells after culture for 24 h, with a modest release of the cytokine as early as 2 h following *C. albicans* stimulation [7]. GM-CSF has been shown to be maximally released by *C. albicans*-stimulated LGL after 7 days in culture, with detection of the cytokine as early as 1 day after *C. albicans* stimulation [5]. Those investigations employed *C. albicans* yeast cells for the stimulation of cytokine release by NK cells or LGL. The present study demonstrated that IL-2-activated NK1.1⁺ lymphocytes were rapidly induced to release IFN- γ when stimulated by *C. albicans* yeasts but not by hyphae. There is abundant evidence that NK cells or LGL can bind to both the yeast and the hyphal form of *C. albicans* [2–4] but the precise means by which the fungus induces the release of IFN- γ from NK1.1⁺ lymphocytes is unknown. The differential release of IFN- γ by NK1.1⁺ cells after stimulation with yeast but not after stimulation with hyphae does not appear to be a consequence of differential binding of lymphocytes to these two distinct forms of the fungus [3–6]. The lymphocyte surface molecules that engage the fungus and lead to the release of IFN- γ by IL-2-activated NK1.1⁺ lymphocytes are under investigation.

IL-2-activated CD8⁺ lymphocytes were also capable of an antifungal effect against the yeast form of *C. albicans*. However, this antifungal effect was not a consequence of the production of IFN- γ as the cytokine was not detected in culture supernates after stimulation with the fungus. Likewise, antibodies specific for murine IFN- γ did not obviate the antifungal effect of the IL-2-activated CD8⁺ cells. These results, coupled with previous observations at this laboratory, demonstrate that IL-2-activated CD8⁺ lymphocytes are capable of an antifungal effect against both the hyphal and the yeast forms of the fungus [3, 8, 9]. The mechanism by which CD8⁺ lymphocytes produce their antifungal effect is unknown, but may relate to the process of *C. albicans* hyphae-induced release of cytoplasmic granule content [19].

It is well known that the C57B1/6 mouse strain produces high levels of IL-2 and IFN- γ when challenged with *C. albicans* [20]. The production of these cytokines in high quantity has been directly related to the capacity of this strain of mouse to resist challenge with the micro-organism [20]. *C. albicans* cells, as well as cell wall components have been shown to activate lymphocytes *in vivo* [21, 22]. Such activated lymphocytes may then produce IFN- γ . The production of IFN- γ at sites of yeast localisation may lead to host protection by retarding germination into the hyphal form of the fungus. The transition from the yeast to the hyphal form is known to be associated with the invasion of host tissue by this micro-organism [23]. The ability of *C. albicans* to form hyphae or pseudo-hyphae has been associated directly with the virulence of this fungus [24]. In addition, IFN- γ has been shown to protect endothelial cells from *C. albicans*-induced damage [25]. Hence, both IL-2-activated NK1.1⁺ and CD8⁺ T lymphocytes can exert antifungal effects against *C. albicans*. These results, combined with investigations by others showing lymphocyte-mediated antifungal effects against the yeast-like micro-organism *Cryptococcus neoformans* [26–28] suggest that such lymphocytes may play a role in protection of individuals from other opportunist infections as well. Reports of lymphocyte populations conferring enhanced protection against opportunist microbial infections confirm this suggestion [29, 30]. The lymphocytes described here may contribute to protection from opportunist microbial pathogens.

The data presented in this study show that IL-2-activation of mouse splenocytes results in a population of NK1.1⁺ lymphocytes capable of affecting the yeast stage of *C. albicans in vitro*. Whether this phenomenon functions *in vivo*, as a form of host defence, has yet to be established. However, *C. albicans* can induce the production of IFN- γ and IL-2 *in vivo* and these cytokines can act in synergy with other cytokines to induce IFN- γ synthesis by NK cells [31]. It is possible that the elaboration of cytokines and the concomitant induction of activated lymphocytes may indeed serve as a line of defence against *C. albicans*. The results of the present study demonstrate that IL-2-activated NK1.1⁺ lymphocytes, by the production of IFN- γ , can exert an antifungal effect against the yeast stage of *C. albicans*. This suggests a possible role for the cytokine and for IL-2-activated NK 1.1⁺ lymphocytes in the immune response to this fungus. Even though mice depleted of NK1.1⁺ lymphocytes show no obvious changes in qualitative or quantitative expression of T lymphocyte response, the local production of IFN- γ by NK1.1⁺ lymphocytes could postpone tissue invasion by the hyphal form of the fungus [32]. Hence a delay in this form of filamentous growth would be expected to be advantageous to the mammalian host. The results of this study coupled with previous observations show that both IL-2-activated NK1.1⁺

and CD8⁺ lymphocytes can produce antifungal effects against *C. albicans*.

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