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CpG oligodeoxynucleotides promote the host protective response against infection with *Cryptococcus neoformans* through induction of interferon-gamma production by CD4+ T cells

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Summary

In the present study, we elucidated the effect of synthetic CpG-containing oligodeoxynucleotides (ODN) on pulmonary and disseminated infection caused by *Cryptococcus neoformans*. CDF-1 mice were inoculated intratracheally with a highly virulent strain of this pathogen, which resulted in massive bacterial growth in the lung, dissemination to the brain and death. Administration of CpG-ODN promoted the clearance of *C. neoformans* in the lungs, decreased their dissemination to brain and prolonged the survival of infected mice. These effects correlated well with the enhanced production of interleukin (IL)-12 and interferon (IFN)-γ and attenuated secretion of IL-4 in bronchoalveolar lavage fluids (BALF) and promoted development of Th1 cells, as indicated by the increased production of IFN-γ by paratracheal lymph node cells upon restimulation with cryptococcal antigens. The IFN-γ synthesis in BALF was inhibited by depletion of CD8+ and CD4+ T cells on days 7 and 14 after infection, respectively, but not by depletion of NK and $\gamma\delta$ T cells. Consistent with these data, intracellular expression of IFN-γ was detected predominantly in CD8+ and CD4+ T cells in the lung on days 7 and 14, respectively. The protective effect of CpG-ODN, as shown by the prolonged survival, was completely and partially inhibited by depletion of CD4+ or CD8+ T cells, respectively, but not by depletion of other cells. Finally, TNF-α was markedly induced by CpG-ODN, and the protective effect of this agent was strongly inhibited by neutralizing anti-TNF-α MoAb. Our results indicate that CpG-ODN alters the Th1–Th2 cytokine balance and promotes host resistance against infection with *C. neoformans*.

Keywords: CpG-DNA, *Cryptococcus neoformans*, lung, Th1–Th2 balance

Introduction

Cryptococcus neoformans, a ubiquitous fungal pathogen, infects via an airborne route and causes a life-threatening infectious disease in the central nervous system in hosts with severely compromised immune responses, such as patients with acquired immunodeficiency syndrome [1]. Meningoencephalitis caused by this pathogen is often refractory to chemotherapy under these conditions, and development of a novel immune-based strategy is required. The host defence against *C. neoformans* is mediated largely by cellular immune responses [2], in which type-1 helper T (Th1) cells act as a critical population by producing interferon (IFN)-γ, while Th2 cells play a negative regulatory role [3]. Recent studies found that mice with a genetic disruption of Th1-related cytokines, such as IFN-γ, interleukin (IL)-12, IL-18 and tumour necrosis factor (TNF)-α, are highly susceptible to cryptococcal infection [4–8], while the infection was less severe in mice that did not synthesize Th2 cytokines, IL-4 and IL-10 [4,9]. Consistent with these observations, administration of IFN-γ, IL-12, IL-18 and TNF-α helps in the protection against infections caused by *C. neoformans* [10–13].

In earlier studies, it was found that purified deoxynucleotides (DNA) from *Mycobacterium bovis* bacille Calmette–Guérin (BCG) possessed immune stimulatory effects, including the activation of natural killer (NK) cells and

production of type-1 and type-2 IFN *in vitro* and the promotion of tumour regression *in vivo* [14–16]. Other investigators demonstrated that purified bacterial DNA induced B cell proliferation and immunoglobulin secretion, while vertebrate DNA did not [17]. Although the mechanisms of these effects had not been understood, Krieg and coworkers discovered that it was ascribed to an unmethylated CpG motif [18,19]. The oligo-DNA (ODN) containing this motif activate murine dendritic cells (DC) to produce IL-12 and expression of co-stimulatory molecules such as CD40, which results in the development of a pattern of Th1-like immune activation [20–22]. Indeed, *in vivo* injections of CpG-ODN induced systemic or local Th1-biased immune responses, including the synthesis of IL-12 and IFN-γ [23–25].

Based on the immune stimulatory activities, many investigations have addressed the therapeutic application of CpG-ODN in infections, malignancies and allergic diseases [19]. Administration of this agent was found to protect mice from infections by intracellular microbial pathogens, including *Listeria monocytogenes* [25], *Francisella tularensis* [26], *Leishmania major* [27,28] and *Plasmodium yoelii* [29]. In the present study, we examined the effect of CpG-ODN on the clinical course of infection caused by *C. neoformans* and the protective immune responses against this fungal pathogen. We show here that the beneficial effects of this treatment in protecting mice are related to the promotion of antigen-specific Th1-biased immune responses rather than the activation of innate immune lymphocytes, such as NK cells and γδ T cells.

Materials and methods

Mice

CDF-1 mice were purchased from Charles River Breeding Laboratories (Osaka, Japan) and used at 8–15 weeks of age. These mice were bred in a pathogen-free environment in the Laboratory Animal Center for Biomedical Science, University of the Ryukyus. All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of our university.

Microorganisms

A serotype A-encapsulated strain of *C. neoformans*, designated as YC-11, was recovered from a patient with pulmonary cryptococcosis. We established a mouse model of pulmonary cryptococcosis by directly instilling the yeast cells through the trachea, because in most cases this pathogen is acquired by inhalation. In this model, the infection was fatal with dissemination to the central nervous system [11]. The yeast cells were cultured on potato dextrose agar (PDA) plates for 2–3 days before use. Mice were anaesthetized by intraperitoneal injection of 70 mg/kg of pentobarbital

(Abbott Laboratory, North Chicago, IL, USA) and restrained on a small board. Live *C. neoformans* (1×10^5 cells) were inoculated in 50 µl per mouse by insertion of a 25-gauge blunt needle into and parallel to the trachea.

CpG- and CNT-ODN

All ODN were synthesized at Hokkaido System Science (Sapporo, Japan). The sequence of CpG-ODN was TCC ATG A<u>CG</u> TTC CTG A<u>CG</u> TT, and that of the control (CNT)-ODN was similar, except that the CpG motif (underlined) was replaced with GpC (TCC ATG A<u>GC</u> TTC CTG A<u>GC</u> TT). All ODN were phosphorothioated and purified by HPLC. The endotoxin content measured by *Limulus amoebocyte* lysate assay was less than 10 pg/ml.

Enumeration of viable *C. neoformans*

Mice were sacrificed 3 weeks after infection and lungs and brains were dissected carefully and excised, then homogenized separately in 10 and 2 ml of distilled water, respectively, by teasing with a stainless mesh at room temperature. The homogenates, diluted appropriately with distilled water, were inoculated at 100 µl on PDA plates, cultured for 2–3 days followed by counting the number of colonies.

Preparation of BALF

Mice were sacrificed on days 3, 7 and 14 after infection and samples of bronchoalveolar lavage fluid (BALF) were collected as described below. Briefly, after bleeding under anaesthesia with ether, the chest was opened and the trachea was cannulated with the outer sheath of 24G intravenous catheter/needle unit (BD Vascular Access, Sandy, UT, USA), followed by lavage of the lung twice with 0·5 ml of chilled normal saline.

In vitro stimulation of lymph node cells

Paratracheal lymph node (LN) cells were prepared from four mice on day 14 after infection with *C. neoformans* and cultured at 2×10^6/ml in flat-bottomed culture plates (Falcon no. 3047, Becton Dickinson, Franklin Lakes, NJ, USA) with various doses of viable organisms or purified protein derivatives (PPD: purchased from Nihon BCG Co., Tokyo, Japan) for 48 h. The culture supernatants were collected and kept at −70°C before use.

Measurement of cytokines

Murine IL-12p40, IFN-γ, IL-4 and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA) kits (BioSource International, Inc., Camarillo, CA, USA for IL-12p40; Endogen, Inc., Cambridge, MA, USA for IFN-γ and IL-4; R&D Systems, Inc., Minneapolis, MN, USA for TNF-α). The

detection limits of assays for IL-12p40, IFN-γ, IL-4 and TNF-α were 2, 15, 5 and 5·1 pg/ml, respectively.

Preparation of pulmonary intraparenchymal leucocytes

Pulmonary intraparenchymal leucocytes were prepared as described previously [30]. Briefly, the chest of the mouse was opened, and the lung vascular bed was flushed by injecting 3 ml of chilled physiological saline into the right ventricle. The lungs were then excised and washed in physiological saline. The lungs, teased with a stainless steel mesh, were incubated in RPMI1640 (Gibco BRL: Grand Island, NY, USA) with 5% of fetal calf serum (FCS, Cansera: Rexdale, Ontario, Canada), 100 U/ml penicillin G, 100 μg/ml streptomycin, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 50 μM 2-mercaptoethanol, and 2 mM L-glutamine, containing 20 U/ml collagenase (Sigma Chemical Co., St Louis, MO, USA) and 1 μg/ml DNaseI (Sigma). After incubation for 60 min at 37°C with vigorous shaking, the tissue fragments and the majority of dead cells were removed by passing through the 50 μm-nylon mesh. After centrifugation, the cell pellet was resuspended in 4 ml of 40% (v/v) Percoll (Pharmacia, Uppsala, Sweden) and layered onto 4 ml of 80% (v/v) Percoll. After centrifugation at 600 *g* for 20 min at 15°C, the cells at the interface were collected, washed three times and counted with a haemocytometer. The obtained cells contained lymphocytes, macrophages and neutrophils.

Analysis of intracellular IFN-γ expression

The lung leucocytes were cultured at 1×10^6/ml with 5 ng/ml phorbol 12-myristate 13-acetate (PMA), 500 ng/ml ionomycin and 2 mM monencin (Sigma) in RPMI-1640 medium supplemented with 10% FCS for 4 h. The cells were washed three times in phosphate-buffered saline (PBS) containing 1% FCS and 0·1% sodium azide and then stained with phycoerythrin (PE)-conjugated anti-CD4 or -CD8 MoAb (clone GK1·5 or 53–6·7, respectively; BD Pharmingen, San Diego, CA, USA). After washing twice, the cells were incubated in the presence of cytofix/cytoperm (BD Biosciences, San Jose, CA, USA), washed twice in BD perm/wash solution and stained with fluorescein isothiocyanate (FITC)-conjugated anti-IFN-γ MoAb (clone XMG1·2, BD Pharmingen) or control IgG (clone R3-34, BD Pharmingen). The stained cells were analysed using an EPICS XL flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA). Data were collected from 15 000–20 000 individual cells using parameters of forward-scatter and side-scatter to set a gate on lymphocyte population.

Antibodies

Monoclonal anti-T cell receptor (TCR)-γδ (hamster IgG), -CD4, -CD8 and -TNF-α (rat IgG) antibodies were purified by using a protein G column kit (Kirkegaard & Perry Laboratory, Gaithersburg, MD, USA) from the culture superna-

tants of hybridomas (clone UC7–13D5, GK1·5, 53–6·72 and MP6-XT2·2–11, respectively; ATCC, Manassas, VA, USA). Asialo GM1 (ASGM1) antibody was purchased from Wako Pure Chemical Industries (Osaka, Japan). To delete NK, γδ T, CD4⁺ or CD8⁺ cells, mice were injected intraperitoneally with anti-ASGM1 antibody at 200 μg or -TCR-γδ, -CD4 or -CD8 MoAb at 400 μg on days –3, 0, + 3, + 7 and + 14 after infection. Rabbit IgG (Wako Pure Chemical Industries), hamster IgG (Organon Teknika Co., Durham, NC, USA) and rat IgG (ICN Pharmaceuticals, Inc., Aurora, OH, USA) were used as the control antibodies. We confirmed that treatment with each antibody greatly reduced the specific cell population in lung intraparenchymal leucocytes when lymphocyte populations were gated on the forward- and side-scatter profiles in a flow cytometric analysis: 22·5% to 1·1% in CD4⁺ T cells; 9·1% to 0·7% in CD8⁺ T cells; 1·6% to 0·2% in γδ T cells; and 18·2% to 0·9% in ASGM1⁺ cells. To block endogenously synthesized TNF-α, mice were injected intraperitoneally with MoAb at 400 μg on days −1, 0, + 3 and every 7 days post-infection. Rat IgG (ICN Pharmaceuticals, Inc.) was used as a control antibody. This MoAb completely neutralized the cytotoxic activity against L929 cells of 0·1 ng/ml recombinant murine TNF-α at 0·78 μg/ml.

Statistical analysis

Data were analysed using Statview II software (Abacus Concept, Inc., Berkeley, CA, USA) on a Macintosh computer. Data are expressed as mean ± standard deviation (s.d.). Differences between groups were examined for statistical significance using one-way analysis of variance (ANOVA) with a *post-hoc* analysis [Fisher's partial least squares difference (PLSD) test]. Survival data were analysed using the generalized Wilcoxon test. A *P*-value less than 0·05 was considered significant.

Results

Effect of CpG-ODN on the host defence to cryptococcal infection

Initially, we elucidated the effects of CpG-ODN treatment on the clinical course of *C. neoformans* infection. Mice received multiple doses of CpG- or CNT-ODN (10, 20 or 40 μg/mouse) on days −3, 0, 3, and every 7 days after infection. As shown in Fig. 1a, all the infected mice died within 5 weeks when they were treated with CNT-ODN irrespective of the dose. Administration of CpG-ODN at 20 μg, but not at 10 μg, significantly prolonged the survival time, although 80% of these mice died 14 weeks after infection. Such an effect also tended to occur when CpG-ODN was administered at 40 μg. Furthermore, we tested the effect of these treatments on the number of live microorganisms in the lung and brain 3 weeks after infection. As shown in Fig. 1b, administration of CpG-ODN at 20 or 40 μg significantly
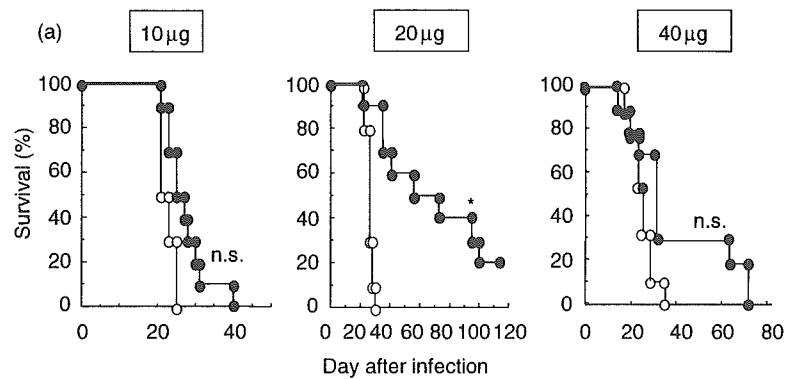
Fig. 1. Effect of CpG-oligodeoxynucleotides (ODN) on the clinical course of cryptococcal infection (a and b). Mice infected intratracheally with *Cryptococcus neoformans* were treated with 10, 20 or 40 μg/mouse of CpG- or CNT-ODN on days −3, 0, 3, 7 and every 7 days after infection. (a) The number of live mice was noted daily. Open circles, CNT-ODN (*n* = 10); closed circles, CpG-ODN (*n* = 10). (b) The numbers of live colonies in lung and brain were counted on day 21 post-infection. No live colonies were detected in the brain of one mouse treated with 20 μg CNT-ODN, which was not included when the mean value was calculated. Open bars, CNT-ODN; closed bars, CpG-ODN. Data are mean ± s.d. of six mice. (c) Mice were treated with 20 μg/mouse CpG- or CNT-ODN on the same schedule above (preventive) or on days 3, 7 and every 7 days after infection (therapeutic), and the number of live mice was noted daily. Open symbols, CNT-ODN; closed symbols, CpG-ODN; circles and solid lines, preventive; triangles and doted lines, therapeutic (*n* = 8 each). The experiments were repeated twice with similar results. *$P < 0.05$, compared with CNT-ODN.
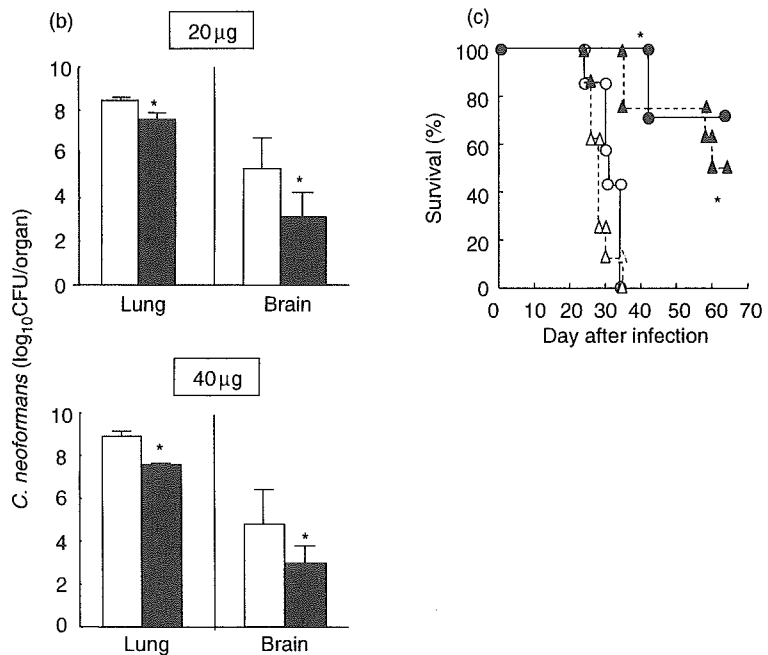
reduced the fungal burdens in both lung and brain, compared with the same dose of CNT-ODN. To examine the therapeutic effect, treatment with 20 μg CpG- or CNT-ODN per mouse was begun at 3 days after infection. As shown in Fig. 1c, the therapeutic treatment significantly prolonged the survival of infected mice as efficiently as did the preventive treatment. These results indicate that CpG-ODN protects mice against fatal and disseminated infection with *C. neoformans*.

Modulation of Th1–Th2 balance by CpG-ODN treatment in mice infected with *C. neoformans*

CpG-ODN directly activates DC, which results in preferential expression of Th1-related cytokines within a few hours [19]. Therefore, we next elucidated the effects of CpG-ODN treatment on the production of IL-12 and IFN-γ, as Th1-related cytokines, and IL-4, as a Th2 cytokine, in the lungs after infection with *C. neoformans*. For this purpose, the lev-

els of these cytokines in BALF were compared in mice treated with CpG- or CNT-ODN at 20 μg on days 7 and 14 post-infection. As shown in Fig. 2, IL-12 and IFN-γ were detected at a marginal level in the BALF of CNT-ODN-treated mice at both time points, and administration of CpG-ODN significantly enhanced the production of IL-12 on days 7 and 14 and of IFN-γ on day 14. In contrast, the levels of IL-4 in BALF were significantly lower in mice treated with CpG-ODN than those in CNT-ODN-treated mice on both days 7 and 14 post-infection. These results indicate that CpG-ODN modulates Th1–Th2 balance towards Th1-dominance at the primary site of cryptococcal infection.

Effect of CpG-ODN treatment on Th1 and Th2 cell development in mice infected with *C. neoformans*

To elucidate the effect of CpG-ODN on the development of fungus-specific Th1 and Th2 cells, on day 14 after cryptococcal infection paratracheal LN cells were prepared from
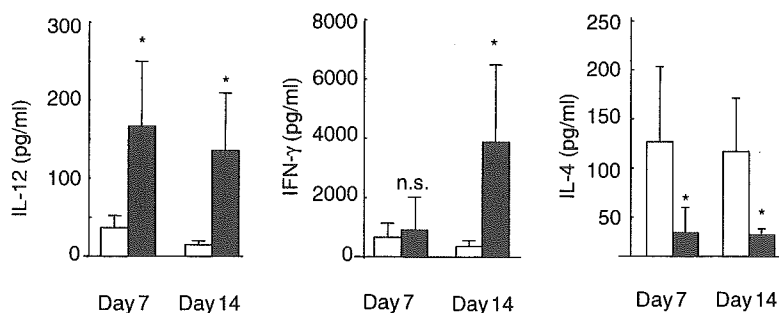
Fig. 2. Effect of CpG-oligodeoxynucleotides (ODN) on the production of cytokines in bronchoalveolar lavage fluids (BALF) after cryptococcal infection. Mice infected intratracheally with *Cryptococcus neoformans* were treated with 20 µg/mouse of CpG- or CNT-ODN on days −3, 0, 3, 7 and every 7 days after infection. The concentrations of interleukin (IL-12), interferon (IFN)-γ and IL-4 in BALF were measured on days 7 and 14 post-infection. Open bars, CNT-ODN; closed bars, CpG-ODN. Data are mean ± s.d. of six mice. The experiments were repeated twice with similar results. n.s., not significant; *$P < 0.05$.

mice treated with CpG- or CNT-ODN at 20 µg, and synthesis of IFN-γ and IL-4 by these cells upon restimulation with live microorganisms was measured. As shown in Fig. 3, unstimulated LN cells from infected and CNT-ODN-treated mice did not produce IFN-γ and only the highest amount of antigens (1×10^6 yeast cells/ml) induced the synthesis of this cytokine. In contrast, LN cells from infected and CpG-ODN-treated mice produced a considerable amount of IFN-γ even in the absence of cryptococcal antigens, and administration of live yeast cells promoted the synthesis of IFN-γ in a dose-dependent manner. The production upon restimulation with the highest dose of antigens was significantly higher in mice treated with CpG-ODN than in CNT-ODN-treated mice. The IFN-γ production was not detected when stimulated with PPD (data not shown). On the other hand, IL-4 synthesis by restimulated LN cells was almost undetectable in both CNT-ODN- and CpG-ODN-treated mice (data not shown). Thus, development of Th1 cells specific for *C. neoformans* was induced by administration of CpG-ODN.

## Lymphocyte subsets contribute to the protective response caused by CpG-ODN

To identify the lymphocyte subsets that contribute to the protective effect of CpG-ODN, we examined the effect of depletion of NK, γδ T, CD4+ T and CD8+ T cells on the survival of mice infected with *C. neoformans*. As shown in Fig. 4a and b, survival of CpG-ODN-treated mice was significantly longer than that of CNT-ODN-treated mice after cryptococcal infection, and the protective effect was not affected significantly by the depletion of either NK cells or γδ T cells. In contrast, the protective effect of CpG-ODN treatment was completely abrogated in CD4+ T cell-depleted mice (Fig. 4c). Administration of anti-CD8 MoAb partially, but significantly, suppressed the protective effect of CpG-ODN on the survival of infected mice, compared with control IgG

(Fig. 4d). These results indicate that CD4+ T cells, rather than NK, γδ T and CD8+ T cells, play a critical role in the CpG-ODN-induced host protection against lethal infection with *C. neoformans*.

Next, we elucidated the lymphocyte subsets responsible for the production of IFN-γ in CpG-ODN-treated mice after cryptococcal infection. For this purpose, the effect of the
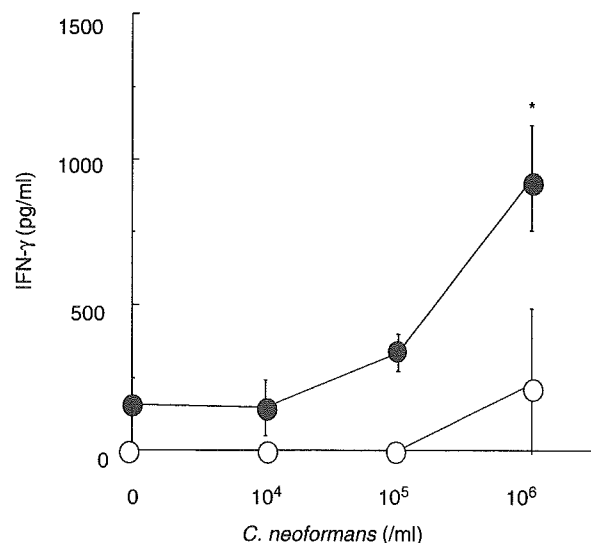
Fig. 3. Effect of CpG-oligodeoxynucleotides (ODN) on the development of Th1 cells after cryptococcal infection. Mice infected intratracheally with *Cryptococcus neoformans* were treated with 20 µg/mouse of CpG- or CNT-ODN on days −3, 0, 3, 7 and every 7 days after infection. The paratracheal lymph node (LN) cells obtained on day 14 post-infection were cultured with indicated doses of live yeast cells for 48 h, and the concentration of interferon (IFN)-γ in the culture supernatants was measured. Open circles, CNT-ODN; closed circles, CpG-ODN. Data are mean ± s.d. of triplicate cultures. The experiments were repeated twice with similar results. *$P < 0.05$.

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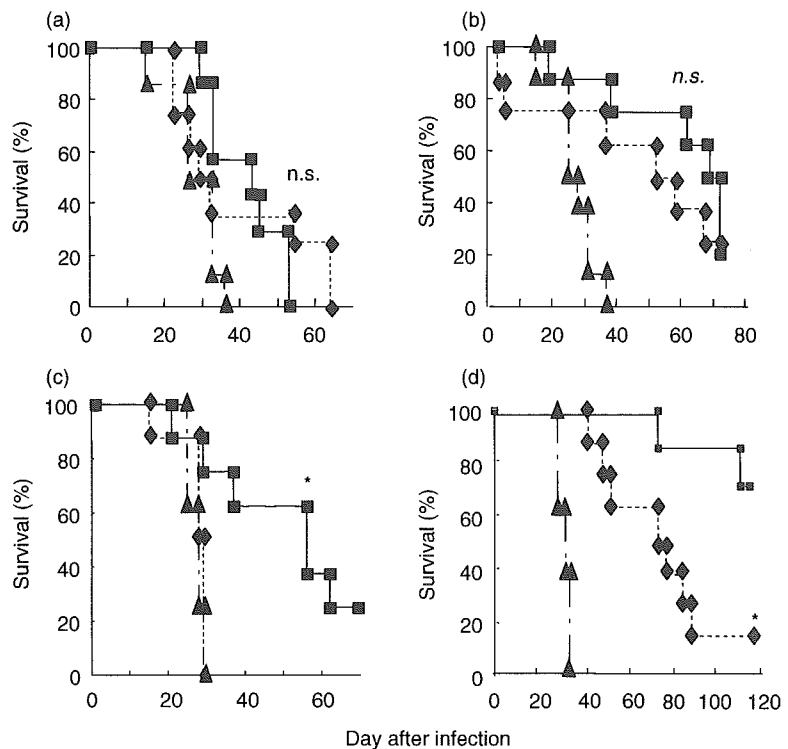
Fig. 4. Lymphocyte subsets responsible for CpG-oligodeoxynucleotides (ODN)-induced host protection. Mice infected intratracheally with *Cryptococcus neoformans* were treated with 20 μg/mouse of CpG- or CNT-ODN on days −3, 0, 3, 7 and every 7 days after infection. The CpG-ODN-treated mice received antibodies against Asialo GM1 (ASGM1) (a), T cell receptor (TCR)-γδ (b), CD4 (c) or CD8 (d) or respective control IgG. The number of live mice was noted daily. Each group consists of seven to eight mice. The experiments were repeated twice (a, b and c) and four times (d) with similar results. Triangles, CNT-ODN; squares, CpG-ODN + control IgG; diamonds, CpG-ODN + specific antibody. n.s., not significant; *P < 0·05, compared between control IgG and specific antibody.

depletion of NK, γδ T, CD4⁺ T and CD8⁺ T cells was examined on days 3, 7 and 14 post-infection. As shown in Table 1, on day 3 the BALF levels of IFN-γ in infected and CpG-ODN-treated mice were not reduced significantly by depletion of any lymphocyte subsets. On day 7, IFN-γ production was inhibited significantly in CD8⁺ T cell-depleted mice, but not in mice depleted of other lymphocyte subsets, compared with control IgG-treated mice. In contrast, depletion of CD4⁺ T cells strongly inhibited the production of IFN-γ in infected and CpG-ODN-treated mice on day 14 post-infection, whereas no influence was noted for depletion of other lymphocyte subsets.

Induction of intracellular IFN-γ expression in CD4⁺ T and CD8⁺ T cells in lung by CpG-ODN
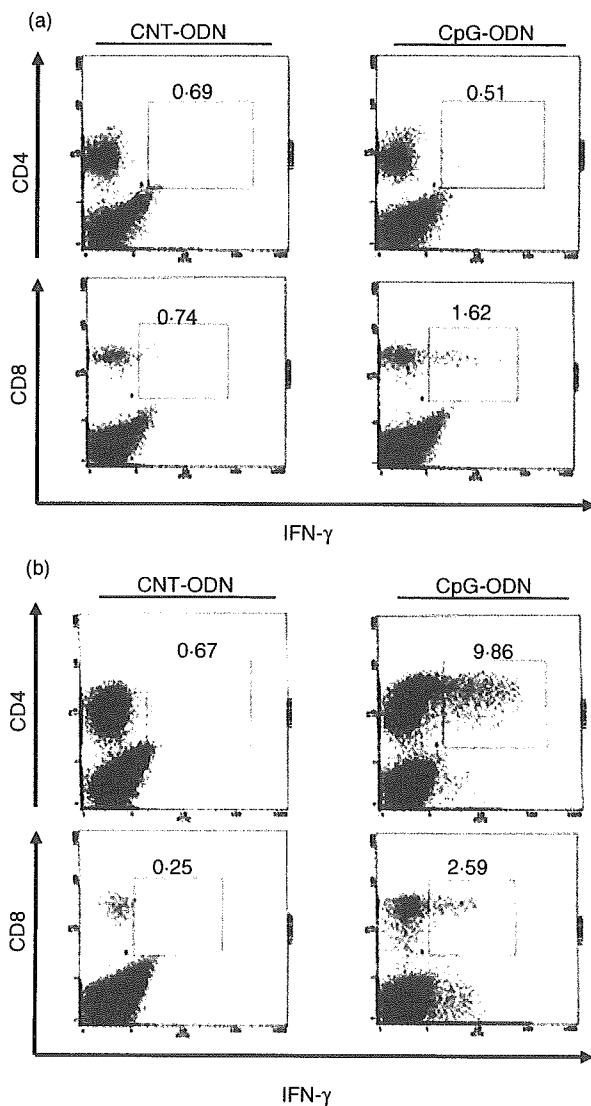
In further experiments, we examined the expression of intracellular IFN-γ in CD4⁺ T and CD8⁺ T cells in the lung on days 7 and 14 after infection with *C. neoformans*. As shown in Fig. 5a, intracellular IFN-γ was detected at a small level in CD4⁺ T and CD8⁺ T cells from CNT-ODN-treated mice on day 7 post-infection. Administration of CpG-ODN did not alter the expression of this cytokine in CD4⁺ T cells compared with CNT-ODN-treated mice. In contrast, such expression was considerably augmented in CD8⁺ T cells although the magnitude was not pronounced (0·74% and 1·62% in CNT- and CpG-ODN, respectively). On day 14 post-infection, IFN-γ synthesis was not detected in CD4⁺ T

and CD8⁺ T cells from CNT-ODN-treated mice, and the expression of this cytokine was induced in both T cell subsets by treatment with CpG-ODN. Interestingly, CpG-ODN induction of IFN-γ synthesis was detected at a higher level in CD4⁺ T cells than in CD8⁺ T cells (9·86% versus 2·59%) (Fig. 5b). These results indicate that CpG-ODN treatment stimulates the synthesis of IFN-γ predominantly in CD4⁺ T cells rather than in CD8⁺ T cells.

Table 1. Effect of lymphocyte subset depletion on IFN-γ production.[a]

	IFN-γ (pg/ml) in BALF		
	Day 3	Day 7	Day 14
Rabbit IgG	1176 ± 114	9714 ± 1167	6053 ± 3167
aASGM1 antibody	1622 ± 229[b]	4557 ± 6284[c]	5654 ± 1617[c]
Hamster IgG	705 ± 417	7451 ± 4842	11371 ± 816
α γδ T antibody	1296 ± 232[c]	8636 ± 2785[c]	11238 ± 3432[c]
Rat IgG	1141 ± 478	3680 ± 2018	4820 ± 2071
αCD4 antibody	1451 ± 143[c]	2317 ± 1850[c]	573 ± 134[b]
Rat IgG	1296 ± 232	3680 ± 2018	5527 ± 3906
αCD8 antibody	1249 ± 101[c]	1083 ± 226[b]	5761 ± 1566[c]

[a]Infected and CpG-oligodeoxynucleotides (ODN)-treated mice received control IgG or specific antibody, and the concentrations of interferon (IFN)-γ in bronchoalveolar lavage fluids (BALF) (pg/ml) were measured at each time point. The values are the mean ± s.d. of four to five mice. The experiments were repeated twice with similar results. [b]P < 0·05, compared to control IgG-treated mice. [c]Not significant, compared to control IgG-treated mice.

(a)

(b)

whereas CpG-ODN markedly induced such levels. In the next experiments, we examined the effect of neutralizing anti-TNF-α MoAb on the host protective responses to cryptococcal infection caused by CpG-ODN. As shown in Fig. 7a, CpG-ODN treatment significantly extended the survival of infected mice compared with CNT-ODN-treated mice, and this protective effect was abrogated almost completely by administration of anti-TNF-α MoAb. In addition, anti-TNF-α MoAb significantly suppressed CpG-ODN-stimulated IFN-γ production in the infected lungs (Fig. 7b).

Discussion

The present study shows that CpG-ODN treatment protected mice against infection with *C. neoformans* by promoting local clearance of this fungal pathogen and preventing its dissemination to the central nervous system. This beneficial effect was associated with alteration in the Th1–Th2 cytokine balance toward a Th1-dominant condition. In our earlier studies [31], aggravation of cryptococcal infection was associated with a Th2-biased cytokine balance in which Th1-related cytokines, IL-12, IL-18 and IFN-γ, were hardly detected in the infected lung tissues, compared with overproduction of Th2 cytokines, IL-4 and IL-10. Administration of recombinant IL-12 results in strong Th1-like immune responses and reduced mortality by this infection [11]. Similar data were obtained when CpG-ODN was administered in these mice. The synthesis of IL-12 and IFN-γ was induced strongly in the infected lungs by this treatment, whereas

Fig. 5. CpG-oligodeoxynucleotides (ODN) stimulates intracellular interferon (IFN)-γ expression. Mice infected intratracheally with *Cryptococcus neoformans* were treated with 20 μg/mouse of CpG- or CNT-ODN on days −3, 0, 3, 7 and every 7 days after infection. The lung leucocytes prepared on days 7 (a) and 14 (b) post-infection were stained with fluoroscein isothyocyanate (FITC)-anti-IFN-γ MoAb and phycoerythrin (PE)-anti-CD4 or -CD8 MoAb and analysed by flow cytometry. Each number indicates the proportion of each subset. The experiments were repeated twice with similar results.
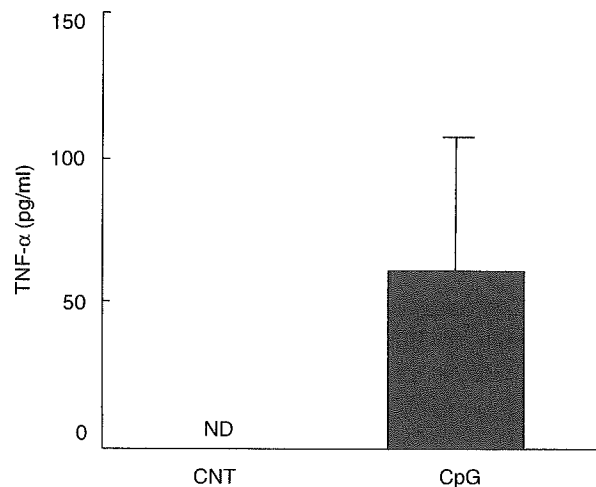
Involvement of TNF-α in the protective effect of CpG-ODN

Finally, we elucidated the contribution of TNF-α to CpG-ODN-induced host protection against a lethal infection with *C. neoformans*. For this purpose, we examined the effect of CpG-ODN on the synthesis of TNF-α in the lung. As shown in Fig. 6, TNF-α levels were under detection limit in the BALF of CNT-ODN-treated mice on day 14 post-infection,

Fig. 6. Effect of CpG-oligodeoxynucleotides (ODN) on the production of tumour necrosis factor (TNF)-α in bronchoalveolar lavage fluids (BALF) after cryptococcal infection. Mice infected intratracheally with *Cryptococcus neoformans* were treated with 20 μg/mouse of CpG- or CNT-ODN on days −3, 0, 3, 7 and every 7 days after infection. The concentrations of TNF-α in BALF were measured on day 14 post-infection. Data are mean ± s.d. of five mice. The experiments were repeated twice with similar results. n.d., not detected.
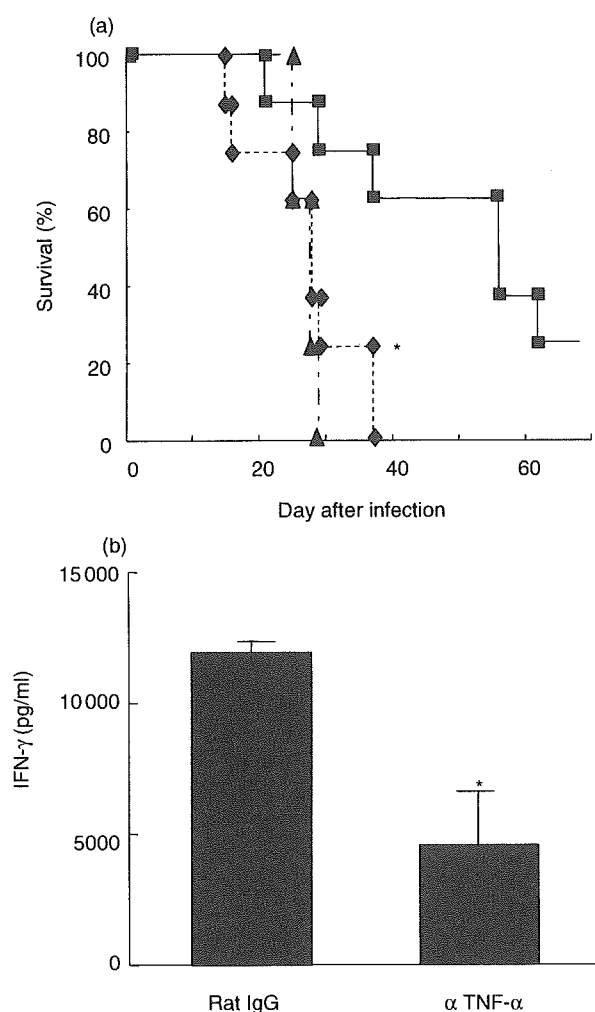
Fig. 7. Effect of anti-tumour necrosis factor (TNF)-α MoAb on the host protective responses stimulated by CpG-oligodeoxynucleotides (ODN). Mice infected intratracheally with *Cryptococcus neoformans* were treated with 20 μg/mouse of CpG- or CNT-ODN on days –3, 0, 3, 7 and every 7 days after infection. The CpG-ODN-treated mice received anti-TNF-α MoAb or control rat IgG. (a) The number of live mice was noted daily. Each group consists of eight mice. Triangles, CNT-ODN; squares, CpG-ODN + control IgG; diamonds, CpG-ODN + specific antibody. *$P < 0.05$, compared between control IgG and anti-TNF-α MoAb. (b) The concentrations of IFN-γ in bronchoalveolar lavage fluids (BALF) were measured on day 14 post-infection. Data are mean ± s.d. of five mice. The experiments were repeated twice with similar results. *$P < 0.05$, compared with control IgG and anti-TNF-α MoAb.

IL-4 production was significantly inhibited. CpG-ODN is well known to polarize the immune response towards Th1 over Th2 by promoting IL-12 synthesis and expression of co-stimulatory molecules by DC [20–22]. Similar observations are shown also in previous reports addressing the effect of CpG-ODN in other infectious diseases [25–29].

Bacterial DNA was found to activate NK cell production of IFN-γ [14–16]. The major source of initial IFN-γ produc-

tion in CpG-ODN-stimulated mouse spleen cells is supposed to be NK cells [32]. Recently, Iho and colleagues reported that CpG-ODN directly activate human NK cells [33]. In our study, by contrast, the role of NK cells in CpG-ODN-induced IFN-γ synthesis as well as host protection against cryptococcal infection could not be confirmed from the experiments in which this lymphocyte subset was depleted. In addition, we did not obtain any evidences indicating the contribution of γδ T cells in these processes, although there is no report that described the involvement of this particular lymphocyte subset. We also found no difference in the protective effect of CpG-ODN between control C57BL/6 mice and Jα18 gene-disrupted mice [mice lacking invariant natural killer T (iNKT) cells] (unpublished data). These observations demonstrated that the contribution of innate immune lymphocytes to the CpG-ODN effects on cryptococcal infection was limited, although we could not exclude the possibility that these subsets were directly or indirectly activated at an earlier phase by this treatment. In fact, there are many investigations indicating the contribution of NK cells to elimination of this fungal pathogen in a direct or indirect manner [34–37]. In other studies, we reported the roles of iNKT cells and γδ T cells in the host defence to cryptococcal infection [38,39] and the contribution of NK cells and γδ T cells to IFN-γ synthesis in the lung caused by combined treatment with IL-12 and IL-18 [40]. Further studies are necessary to define the relationship to the present observations.

Generally, CpG-ODN does not have a direct stimulatory effect on resting T cells [19,41–43], although direct activation of purified human T cells by this agent has been reported recently [33]. CpG-ODN-induced T cell activation would be conducted in an indirect way by antigen-presentation and expression of cytokines and co-stimulatory molecules by DC, as reviewed by Krieg [19]. This notion is thoroughly consistent with our unpublished data showing complete abrogation of CpG-ODN induction of host defence to cryptococcal infection by anti-CD11c MoAb that depletes DC. Previous studies reported the contribution of both CD4+ and CD8+ T cells to IFN-γ production induced by CpG-ODN treatment of different animal models, although their contribution varied from one model to another [44,45]. In our hands, using intracellular cytokine analysis we found that the major source of IFN-γ production was CD8+ T cells rather than CD4+ T cells in lung on day 7 after infection, although the overall proportion of intracellular IFN-γ+ CD8+ T cells was not very high (2·6%). In contrast, on day 14 CD4+ T cells became the major IFN-γ-producing cells instead of CD8+ T cells, as shown by intracellular cytokine production ratio in each subset (CD8+ IFN-γ+ 2·59% versus CD4+ IFN-γ+ 9·86%). These findings were consistent with the results showing that IFN-γ synthesis detected at a protein level in BALF was significantly inhibited in CD8+ T cell-depleted, but not CD4+ T cell-depleted mice on day 7 and vice versa on day 14 after infection with *C. neoformans*. These

findings indicate that CD8$^+$ T cells are the major source of IFN-γ production stimulated by CpG-ODN, although not large production, at an earlier phase and CD4$^+$ T cells are the main IFN-γ producer at a later phase of infection. In further experiments, the protective effects of CpG-ODN treatment against fatal infection were abrogated completely by depletion of CD4$^+$ T cells and only partially affected by depletion of CD8$^+$ T cells. Thus, in our model, the development of *C. neoformans*-specific Th1 cells, rather than type-1 cytotoxic T cells (Tc1), contributes more profoundly to the induction of host protective immune responses caused by CpG-ODN.

TNF-α is known to play a critical role in the host defence to intracellular microorganisms [46]. Mice with a genetic disruption of this cytokine are highly susceptible to infection with *C. neoformans* [8]. In a series of studies by Huffnagle and colleagues [47,48], TNF-α was shown to function at an early phase by initiating the accumulation of inflammatory leucocytes and development of Th1-based immune responses to *C. neoformans*. Here, we demonstrated that TNF-α was an important cytokine in the CpG-ODN induction of IFN-γ synthesis and host protection against this infectious pathogen. Although the cellular source remains to be elucidated, DC or macrophages may be directly involved in TNF-α production caused by CpG-ODN, as reported in earlier investigations [20,49].

In conclusion, we demonstrated in the present study that CpG-ODN protects mice against infection with *C. neoformans* by promoting Th1-mediated immune responses. Recently, clinical trials using CpG-ODN in cancer therapy, treatment of allergic diseases and in vaccination against infectious diseases has been reported [50,51], suggesting that this treatment can be conducted without any serious adverse effects. Thus, CpG-ODN could be a candidate therapeutic agent against refractory cryptococcal infection in patients with compromised immune responses. However, our data identifying CD4$^+$ T cells as a major subset of IFN-γ production may limit the usefulness of this agent, because the compromised immune response associated with cryptococcosis patients is a severe reduction in this T cell subset [1]. Further investigations will be necessary to make the CpG-ODN treatment useful in such clinical settings.

Acknowledgements

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58 抗真菌薬

種類と適応

* 従来，わが国で市販されている全身投与可能な抗真菌薬は，①世界で初めて抗真菌活性を有する薬剤として開発されたグリセオフルビン，②ポリエン系薬剤（ナイスタチン，アムホテリシンB），③ピリミジン系薬剤（フルシトシン），④アゾール系薬剤（ミコナゾール，フルコナゾール，イトラコナゾール），の4系統7薬剤のみであったが，昨年キャンディン系薬剤（ミカファンギン）が市販され，5系統8薬剤となった。
* これらの薬物にはそれぞれ有効な適応菌種があり，それ以外の菌種には効果はないか，あっても軽微である。また，その薬物動態により適応菌種が制限される薬物もある。

1）グリセオフルビン
* 経口薬のみで，深在性真菌症に適応はなく，皮膚白癬症のみである。

2）ポリエン系薬剤
* アスペルギルスを含む糸状菌にも有効な抗真菌活性を有するが，両薬剤とも経口投与では吸収が悪く，消化管以外の深在性真菌症に効果は期待できない。ナイスタチンは副作用が強いため，注射用製剤はない。
* アムホテリシンBは，糸状菌を含めて極めて良好な抗真菌活性を有する。注射用製剤は有効性が高いものの，その副作用が問題となる。

3）ピリミジン系薬剤
* フルシトシンは，耐性化しやすいため単剤では用いず，アムホテシリンBやフルコナゾールと併用する。

4）アゾール系薬剤
* ミコナゾールやフルコナゾールは糸状菌には抗菌活性は弱く，糸状菌に適応があるのはイトラコナゾールのみである。フルコナゾールは耐性化の問題があり，イトラコナゾールは吸収が不安定で，髄膜炎は適応外である。
* ホスフルコナゾールはフルコナゾールのプロドラッグであり，抗真菌スペクトルや活性はフルコナゾールと同じである。

5）キャンディン系薬剤
* ミカファンギンは，アスペルギルス属やフルコナゾール低感受性カンジダ属に強い抗真菌活性を有し，かつ安全性は極めて高い。ただし，作用機序の理由からクリプトコックスやトリコスポロンには抗真菌活性を有していない。

使用方法と注意事項

* 皮膚白癬などの浅在性真菌症に対して一般的には外用薬（「14 皮膚科用薬」129ページ参照）で十分であるが，皮膚の角化が強い場合や，爪白癬など難治性の場合には内服薬（グリセオフルビン，イトラコナゾール）の適応となる。なお，爪白癬に対してはイトラコナゾールのパルス療法（400mg/日の7日間連続投与を1クールとして，4週毎に合計3クール）が保険適応となった。

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＊深在性真菌症に対して治療薬の中心となっている薬剤は，アムホテリシンBである。
しかし，この薬剤は，抗真菌活性は高いものの低カリウム血症や腎機能障害が用量
存的にほぼ必発であり，使用に際しては注意深い観察が必要である。

＊フルシトシンやアゾール系薬剤は，副作用は少なく使用しやすいため，軽症〜中
にはこれらの薬剤が選択される。その使用に際しては，フルシトシンは骨髄抑制に
意が必要で，アゾール系薬剤は他薬剤との相互作用が問題となる。

＊リファンピシン，フェニトイン，カルバマゼピン，フェノバルビタールの併用でア
ール系薬剤の代謝が亢進し，アゾール系薬剤の血中濃度が低下する。

＊イトラコナゾールの吸収は胃内のpHに左右され，それが上昇するような状態（胃
H_2受容体拮抗薬，PPI，スクラルファート）では極端に低下する。

＊アゾール系薬剤との併用で血中濃度が上昇する薬物としては，シクロスポリン，
ロリムス，フェニトイン，スルフォニールウレア，トリアゾラム，ミタゾラム，
フェナジン，ワルファリン，抗HIV薬等があり，併用により腎障害，中毒症状，
糖，睡眠効果遷延，QT延長，出血傾向等を引き起こす。

＊ミカファンギンは副作用も少なく，使用しやすい薬剤であるが，重症の症例では
量またはアムホテリシンBとの併用が選択される。

〔河野

剤名・規格単位	適応	用法・用量	備考（副作用・禁忌等）

◯グリセオフルビン *griseofulvin*

剤名・規格単位	適応	用法・用量	備考（副作用・禁忌等）
グセルビンFP Guservin FP （中外） 錠 125mg ¥11.20 ポンシルFP Poncyl （武田） 錠 125mg	皮膚糸状菌による白癬・黄癬・渦状癬	250〜500mg／日，1〜数回に分服 初回：1,000mgを投与し効果が現れれば上記まで減量 小児：125〜250mg／日，1〜数回に分服，375〜500mg／日まで増量可	副中毒性表皮壊死症，剥脱性皮膚炎，SLE様症状，精神錯乱，末梢神経炎，ポルフィリン症，肝機能障害，黄疸，過敏症状，血管神経浮腫，光線過敏症，発疹，紅斑，蕁麻疹，色素沈着，顆粒球減少，白血球増多，食欲不振，悪心，嘔吐，下痢，心窩部痛，味覚異常，舌痛，口渇，めまい，頭痛，頭重，眠気，不眠，耳鳴，抑うつ状態，視力障害，蛋白尿，倦怠感，発熱，関節痛，女性化乳房
動 t1/2：22hr，V：105L／70kg，腎外消失：1.0			禁ポルフィリン症，肝障害，本剤の成分に対し過敏症歴，妊婦又は妊娠の可能性，授乳婦

◯アムホテリシンB *amphotericinB(AMPH-B)* 動（腐図）禁（注用）

剤名・規格単位	適応	用法・用量	備考（副作用・禁忌等）
ファンギゾン Fungizone （ブリストル） 錠 100mg ¥49.60 図 10% 注用 50mg ¥1099	内服：消化管におけるカンジダ異常増殖 注用：アスペルギルス，カンジダ，ムコール，クリプトコッカス，ブラストマイセス，ヒストプラズマ，コクシジオイデス，ホルモデンドラム，ヒアロホーラ，ホルミシチウム等による深在性感染症	内服：100mg×2〜4回／日 小児（図）：50〜100mg×2〜4回／日 点滴静注：0.25mg／kgから開始し，0.5mg／kg／日まで漸増 最高1mg／kg／日，又は1.5mg／kg×1回／2日 気管内注入：初回1mg／日又は5〜10mg／日から漸増し，10〜20mg×1回／2日 胸膜内注入：1mg／日から漸増し，5〜20mg×1〜3回／週 髄腔内注入：0.25〜1mg×1回／日，採取髄液量を超えない液量で漸増法により隔日又は3日ごと 膀胱内注入：15〜20mg×1〜2回／日 皮内注：0.5〜2mg／10〜30日 吸入：2.5〜5mg／mL×2〜5回／日，1〜2ヵ月継続	副内服：皮膚粘膜眼症候群，中毒性表皮壊死症，悪心，嘔吐，食欲不振，腹痛，下痢，口内炎，心窩部痛，発熱，腎障害，肝障害，蕁麻疹，血管浮腫 注用：心停止，心不全，不整脈（心室細動等），急性肝不全，腎障害，皮膚粘膜眼症候群，中毒性表皮壊死症，アナフィラキシー様反応，無顆粒球症，肺水腫，頭痛，倦怠感，食欲不振，悪心，嘔吐，筋肉痛，関節痛，過敏症，貧血，顆粒球減少症，血圧降下，血圧上昇，斑点状丘疹性皮疹，肝障害，複視，末梢性神経障害，めまい，痙攣，低K血症，BUN・クレアチニン上昇 禁本剤の成分に対し過敏症歴，授乳婦 併禁注用：白血球輸注
ポイント▶注用毒性が非常に強いので深在性の重篤疾患にのみ適用．注射速度はできるだけ遅くする．⊕での溶解不可．動CL：1.8L／hr（70kg），t1/2：360hr，V：280L／70kg，腎外消失：0.95			

◯ナイスタチン *nystatin (NYS)*

剤名・規格単位	適応	用法・用量	備考（副作用・禁忌等）
ナイスタチン錠 nystatin （明治） 50万単位	有効菌種：カンジダ 消化管カンジダ症	50万単位×3回／日	副皮膚粘膜眼症候群，発疹，瘙痒感，悪心・嘔吐，食欲不振，下痢

58 抗真菌薬

薬剤名・規格単位	適　応	用法・用量	備考（副作用・禁忌等）
フルシトシン *flucytosine (5-FC)* ⊛			
アンコチル Ancotil （共和） 錠500mg ¥314.70	有効菌種：クリプトコッカス，カンジダ，アスペルギルス，ヒアロホーラ，ホンセカエア 真菌血症，真菌性髄膜炎，真菌性呼吸器感染症，黒色真菌症	25～50mg／kg×4回／日	⊛テガフール・ギメラシル・オテラシルカリウム配合剤との併用にて，重篤な血液障害等のおそれがあるので併用不可. ⊛汎血球減少，腎不全，無顆粒球症，貧血，白血球・顆粒球・血小板減少，腎障害，肝障害，食欲不振，嘔吐，腹痛，下痢，頭痛，しびれ感，視力低下，幻覚，難聴，傾眠，不随意運動，痙攣，発疹，光線過敏症，血清K・血清Ca・血清P値等の低下
㊟ココール（錠500 mg）.	尿路真菌症，消化管真菌症	12.5～25mg／kg×4回／日	㊝本剤の成分に対し過敏症歴，妊婦又は妊娠の可能性，授乳婦 ㊟㊝テガフール・ギメラシル・オテラシルカリウム配合剤投与中及び投与中止後7日以内
動F：84%，CL：0.8L/hr(70g)，t₁/₂：4.2hr，V：55L/70kg，腎外消失：0.03			
ミコナゾール *miconazole (MCZ)* ⊛（-Fのみ）			
フロリード-F Florid・F （持田） 注200mg(20mL) ¥2481 点滴静注用：0.267 %(75・150mL) フロリードゲル Florid （持田） 経口用ゲル：2% ¥122.20 g	有効菌種：クリプトコッカス，カンジダ，アスペルギルス，コクシジオイデス 注：真菌血症，肺真菌症，消化管真菌症，尿路真菌症，真菌髄膜炎 ゲル：口腔・食道カンジダ症治療	注 点滴静注：初回200mg以後200～400mg×1～3回／日，30～60分かける 髄腔内注入：5～20mg×1回／1～7日 ゲル：200～400mg(10～20g)4回に分けて使用(食後，就寝前) 口腔カンジダ症：口腔内にまんべんなく塗布，病巣が広範囲の場合は口腔内にできるだけ長く含んだ後，嚥下 食道カンジダ症：口腔内に含んだ後，少量ずつ嚥下	⊛・F：ショック，アナフィラキシー症状，肝機能障害，黄疸，急性腎不全，QT延長，心室性不整脈，汎血球・血小板減少，搔痒感，発疹，悪寒，発熱，悪心，嘔吐，食欲不振，下痢，頭重感，倦怠感，腎障害，静脈血圧低下，期外収縮，血管痛，振戦 ゲル：嘔気・嘔吐，食欲不振，口渇，腹鳴，AST・ALTの上昇，発疹，口腔内疼痛等 ㊝本剤の成分に対し過敏症歴，妊婦又は妊娠の可能性，授乳婦 ㊟㊝シサプリド，ピモジド，トリアゾラム，テルフェナジン，キニジン，シンバスタチン，アゼルニジピン，酒石酸エルゴタミン，メシル酸ジヒドロエルゴタミン
			ポイント▶薬物代謝酵素CYP3A阻害. 動F(経口)：27%，CL：46L/hr(70kg)，t₁/₂：23hr，V：1400L/70kg，腎外消失：1.0

剤名・規格単位	適　　応	用法・用量	備考（副作用・禁忌等）
）フルコナゾール　fluconazole			
ジフルカン Diflucan （ファイザー） 図 50mg 　100mg 　　　￥1310.60 静注：50mg 　　　（50mL） 　　　100mg 　　　（50mL） 　　　200mg 　　　（100mL）	有効菌種：カンジダ属，クリプトコッカス属及びアスペルギルス属 真菌血症，呼吸器・消化管・尿路真菌症，真菌髄膜炎	カンジダ症：50〜100mg×1回／日 クリプトコッカス症，アスペルギルス症：50〜200mg×1回・日内服，静注 重症・難治性：400mg×1回・日まで増量可	重ショック，皮膚粘膜眼症候群，中毒性表皮壊死症，血液障害，急性腎不全，肝障害，意識障害，痙攣，高カリウム血症，心室頻拍，QT延長，不整脈，間質性肺炎，偽膜性大腸炎，発疹，発熱，嘔気，しゃっくり，食欲不振，下痢，腹部不快感，頭痛，倦怠感，手指のこわばり，腎障害，浮腫，乏尿，熱感，好酸球増多，高コレステロール血症，高血糖，注射部位血管痛（注） 禁本剤に対し過敏症歴，妊婦又は妊娠の可能性，授乳婦 併禁トリアゾラム，シサプリド，テルフェナジン
類フルコナメルク，ミコシスト（図 50・100mg静注液0.1% 50mL・0.2% 50mL・0.2% 100mL），フラノス（静注：0.1% 50mL・0.2% 50mL・0.2% 100mL），アルナゾール（静注キット100mg 50mL・200mg 100mL）．			ポイント▶アムホテリシンB注用との混合で白濁，重F：90%，t₁₂：30hr，V：56L 70kg，腎外消失：0.3
）ホスフルコナゾール　fosfluconazole			
プロジフ Prodif （ファイザー） 注 100mg(1.25mL) 　　　￥5860.0 　200mg(2.5mL) 　　　￥11160.0 　400mg(5mL) 　　　￥21251.0 新03年12月	カンジダ属及びクリプトコッカス属による下記感染症：真菌血症，呼吸器真菌症，真菌腹膜炎，消化管真菌症，尿路真菌症，真菌髄膜炎	カンジダ症：50〜100mg×1回・日，静注 クリプトコッカス症：50〜200mg×1回・日，静注 重症・難治性：400mg×1回・日まで増量可，どちらの場合も初日，2日目は倍量を投与	重ショック，皮膚粘膜眼症候群，中毒性表皮壊死症，無顆粒球症・汎血球減少症・血小板減少等血液障害，急性腎不全，肝障害，意識障害，痙攣，高K血症，心室頻拍，QT延長，不整脈，間質性肺炎，偽膜性大腸炎，ALT・ビリルビン増加・黄疸等肝機能障害，脱毛，発疹，剥脱性皮膚炎，しゃっくり・腹部不快感・消化不良等消化器障害，頭痛，手指のこわばり，浮動性めまい，BUN・クレアチニン増加，乏尿，高コレステロール血症，高TG血症，高血糖，好酸球増加，好中球減少，貧血，高血圧，静脈炎，心雑音，鼻炎，鼻出血，関節痛，筋痛，背部痛，熱感，味覚倒錯，発熱 禁本剤の成分又はフルコナゾールに対して過敏症歴，妊婦又は妊娠の可能性，授乳婦 併禁トリアゾラム，シサプリド，テルフェナジン
		ポイント▶フルコナゾールのプロドラッグ．	

薬剤名・規格単位	適　応	用法・用量	備考(副作用・禁忌等)
⟩イトラコナゾール　*itraconazole*			
イトリゾール Itrizole (ヤンセンファーマ) 囲50mg ¥614.50	有効菌種：皮膚糸状菌(トリコフィトン属，ミクロスポルム属，エピデルモフィトン属)，カンジダ属，マラセチア属，アスペルギルス属，クリプトコッカス属，スポロトリックス属，ホンセカエア属		㊿うっ血性心不全，肺水腫，肝障害(黄疸，AST・ALT・LDH・γ-GTP・-P・総蛋白・総コレステロール・血ビリルビン・LAPの上昇等)，皮膚粘膜眼症候群，腹痛，嘔気，便秘，下痢，嘔吐，消化不良，食欲不振，鼓腸放屁，発疹，光線性過敏性反応，瘙痒，めまい，頭痛，肩こり，倦怠感，不眠等，BUNの上昇，尿蛋白及び尿糖の陽性等，白血球減少，血小板減少，好酸増多，浮腫，トリグリセライド・血尿酸・血清Kの上昇，低K血症，発熱，味覚倒錯，耳鳴，心室性期外収縮，房室ブロック
	内臓真菌症(真菌血症，呼吸器真菌症，消化器真菌症，尿路真菌症，真菌髄膜炎)，深在性皮膚真菌症(スポロトリコーシス，クロモミコーシス)	100〜200mg×1回/日，食直後	
	表在性皮膚真菌症(白癬，カンジダ症，癜風，マラセチア毛包炎)(爪白癬以外)	50〜100mg×1回/日，食直後 爪カンジダ症及びカンジダ性爪囲爪炎：100mg×1回/日，食直後 最大：1日200mg	㊗本剤の成分に対し過敏症歴，重篤な肝疾患又はその既往歴，妊娠又は妊娠の可能性，授乳婦
㊹イトラート，イコナゾン，イトラコン(囲50mg).	爪白癬	200mg×2回/日，食直後，1週間経口投与しその後3週間休薬．これを1サイクルとし，3サイクル繰り返す(パルス療法)	(肝禁)シサプリド，トリアゾラム，ピジド，キニジン，シンバスタチン，ゼルニジピン，エルゴタミン，ジヒドロエルゴタミン，バルデナフィル ポイント▶薬物代謝酵素CYP3A阻害．㊿F：40%，t₁₂：30hr
⟩ミカファンギンナトリウム　*micafungin sodium* ⬤			
ファンガード Funguard (藤沢) 点滴用50mg ¥7387 75mg ¥10680 ポイント▶光によって徐々に分解する．又，他剤と配合すると直後に濁りが生じたり，力価低下がみられる場合がある．	アスペルギルス属及びカンジダ属による真菌血症，呼吸器真菌症，消化管真菌症	アスペルギルス症：50〜150mg×1回/日，点滴静注，〜300mg/日まで カンジダ症：50mg×1回/日，点滴静注，〜300mg/日まで ＊いずれも㊹，⑦，又は補液に溶解し，75mg以下では30分以上，75mg以上では1時間以上かけて点滴	㊿好中球減少，血小板減少，溶血性貧血，ショック，アナフィラキシー様症状，肝機能障害・黄疸，急性腎不全，K上昇・低下，好酸球増多，発疹，高血圧，動悸，下痢，軟便，BUN・クレアチニン上昇，クレアチニンクリアランス低下，静脈炎，関節炎，血管痛 ㊗本剤の成分に対し過敏症歴，授乳婦

薬剤名・規格単位	適　　応	用法・用量	備考(副作用・禁忌等)
◯ 塩酸テルビナフィン　*terbinafine hydrochloride*			
ラミシール Lamisil 　(ノバルティス) 錠 125mg 　　¥293.50 錠 1%(10g) 　　¥56.70 g ク 1%(10g) 　　¥56.70 g 液 1%(10g) 　　¥85.20 g	錠：深在性皮膚真菌症(白癬性肉芽腫, スポロトリコーシス, クロモミコーシス), 表在性皮膚真菌性, 白癬(爪白癬, 手・足白癬, 生毛部白癬, 頭部白癬, ケルスス禿瘡, 白癬性毛瘡, 生毛部急性深在性白癬, 硬毛部急性深在性白癬), 爪カンジダ症 軟ク：白癬(足白癬, 体部白癬, 股部白癬), 皮膚カンジダ症(指間びらん症, 間擦疹, 乳児寄生菌性紅斑), 癜風	125mg×1回/日(外用抗真菌薬では治療困難な患者に限る) 1日1回患部に塗布	警(錠のみ)重篤な肝障害及び汎血球減少, 無顆粒球症, 血小板減少から死亡例の報告あり. 投与前, 投与中には肝機能検査, 血液検査を行う. 副錠：**重篤な肝障害, 汎血球減少, 無顆粒球症, 血小板減少, 皮膚粘膜眼症候群, 中毒性表皮壊死症, 横紋筋融解症, 過敏症状, 肝障害, 白血球減少, 消化器障害, 頭痛, めまい, ふらつき, 頻尿, BUN上昇, 味覚異常** 軟ク：接触皮膚炎, 紅斑, 発赤, 瘙痒感, 刺激感等 禁本剤の成分に対し過敏症歴, 錠のみ：(重篤な肝障害, 血液障害, 授乳婦)
◯ クロトリマゾール　*clotrimazole*			
エンペシド Empecid 　(バイエル) ト 10mg	HIV感染症における口腔カンジダ症(軽症, 中等症)	10mg×5回/日	副AST・ALT上昇, 嘔気, 嘔吐, 口内乾燥, 口腔疼痛, 口内灼熱感, 瘙痒 禁本剤の成分に対し過敏症歴, 授乳婦

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抗真菌薬

3

感染症

感染症の治療の動向

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感染症医療の現状

感染症における最大のトピックは，重症急性呼吸器症候群（severe acute respiratory syndrome：SARS）の流行である．この感染症は瞬く間に世界に拡大し，グローバル化時代の新興感染症の特徴を示すと同時に，感染危機管理体制の脆さをも露呈した．世界保健機構（WHO）によると，SARSは8,000人以上の感染者と900人以上の死者を計上し，以後終息に向かったとされている．その後，SARSの病原ウイルスであるSARSコロナウイルス（SARS‐CoV）が同定され，全塩基配列が報告された．日本国内では奇跡的に患者が発生しなかったものの，再流行やわが国での発生が懸念されている．2003年12月にLAMP（loop‐mediated isothermal amplification）法と呼ばれる新しい遺伝子法によるSARSコロナウイルスの検出が保険適用された．糞便と鼻腔咽頭拭い液が対象となるが，糞便のほうが検出率が高い．治療に関しては，抗ウイルス薬やインターフェロンの有効性の報告はあるものの，十分とはいえない．現在世界中で最も注目されているSARS対策の1つがワクチン開発である．現時点では，SARS Co‐Vの構造蛋白からのペプチドを使用して，ワクチン開発に利用しようと研究が行われているが，臨床応用にはまだ時間がかかると思われる．

また，鳥インフルエンザの世界規模の発生は国際的に大きな問題を投げかけている．本ウイルスは変異を繰り返してヒトへの感染力を増すことがおそれられている．この感染症によるパンデミーが発生すれば，数十万～数百万人の犠牲者が危惧される．既にヒト‐ヒト感染の擬似例も報告されるなど，世界規模の抑制対策が求められている．

2. 感染症法改正と新しいガイドライン

2003年11月5日には感染症法が4年ぶりに改定された．主なポイントは，SARSおよび痘そう（天然痘）が新たに1類感染症に追加されたことと，従来の4類感染症のうち，消毒，動物の輸入禁止等の措置が必要なものを新2類，それ以外を新5類に分類した点である．

感染症領域のガイドラインとして，日本呼吸器学会は2003年に気道感染症のガイドラインである「成人気道感染症診療の基本的考え方」を刊行した．また，各科領域における真菌感染症に対しても「深在性真菌症の診断・治療ガイドライン」が2003年に刊行された．

3. 最近（1年間）保険適用となった診断法ならびに治療法

EIAキットによるレジオネラ尿中抗原測定，LAMP（loop‐mediated isothermal amplification）法を用いたSARSコロナウイルスの検出，マクロライド誘導体のケトライド系抗菌薬であるケテック（テリスロマイシン），キャンディン系抗真菌薬であるファンガード（ミカファンギン），新規アゾール系抗真菌薬の開発であるプロジフ（フォスフルコナゾール）．