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Dendritic Cells Pulsed with Fungal RNA Induce Protective Immunity to *Candida albicans* in Hematopoietic Transplantation

Angela Bacci,* Claudia Montagnoli,* Katia Perruccio,† Silvia Bozza,* Roberta Gaziano,‡ Lucia Pitzurra,* Andrea Velardi,§ Cristiana Fe’ d’Ostiani,* Jim E. Cutler,§ and Luigina Romani*‡*

Immature myeloid dendritic cells (DC) phagocytose yeasts and hyphae of the fungus *Candida albicans* and induce different Th cell responses to the fungus. Ingestion of yeasts activates DC for production of IL-12 and Th1 priming, while ingestion of hyphae induces IL-4 production and Th2 priming. In vivo, generation of antifungal protective immunity is induced upon injection of DC ex vivo pulsed with *Candida* yeasts but not hyphae. In the present study we sought to determine the functional activity of DC transfected with yeast or hyphal RNA. It was found that DC, from either spleens or bone marrow, transfected with yeast, but not hyphal, RNA 1) express fungal mannoproteins on their surface; 2) undergo functional maturation, as revealed by the up-regulated expression of MHC class II Ags and costimulatory molecules; 3) produce IL-12 but no IL-4; 4) are capable of inducing Th1-dependent antifungal resistance when delivered s.c. in vivo in nontransplanted mice; and 5) provide protection against the fungus in allogeneic bone marrow-transplanted mice, by accelerating the functional recovery of *Candida*-specific IFN-γ-producing CD4+ donor lymphocytes. These results indicate the efficacy of DC pulsed with *Candida* yeasts or yeast RNA as fungal vaccines and point to the potential use of RNA-transfected DC as anti-infective vaccines in conditions that negate the use of attenuated microorganisms or in the case of poor availability of protective Ags. The *Journal of Immunology*, 2002, 168: 2904–2913.

In humans, *Candida albicans* is the most frequently isolated fungal pathogen (1). The delicate balance between the host and this otherwise harmless commensal fungus turn into a parasitic relationship, resulting in the development of severe infections. Invasive candidiasis is a significant cause of morbidity and mortality in differently immunocompromised patients, including those undergoing bone marrow transplantation (BMT). It is recognized that the nature and extent of the impairment of host defense influence the manifestation and severity of infection (1).

Clinical evidence and experimental data indicate that both the innate and the adaptive immune systems regulate resistance to *Candida* infections (3–5). In murine experimental models of infection, it has been demonstrated that Th cell reactivity plays a central role in regulating immune responses to the fungus, Th1 reactivity being responsible for resistance and Th2 reactivity being associated with susceptibility (6). The development of protective anticanidal Th1 responses requires the concerted actions of several cytokines, including IFN-γ and IL-12, in the relative absence of Th2 cytokines, such as IL-4 and IL-10, which inhibit development of Th1 responses (6).

However, the fungus is not a mere passive participant in the infectious process, and a hypothetical set of virulence factors has been attributed to it (7). Among these, the ability to reversibly switch between budding yeast to the filamentous growth form or hypha, all of which can be found in infected tissues (1), is thought to be important for virulence (8–10).

Recent evidence indicates that dendritic cells (DC) are uniquely capable of decoding the fungus-associated information required to elicit the qualitative nature of the adaptive immune response (11). DC finely discriminated between the two forms of *C. albicans* in terms of type of immune responses elicited. By the production of IL-12 and IL-4 in response to the nonviral and virulent forms of the fungus, DC were uniquely capable of Th1 priming and education in vitro and in vivo (11). This finding is particularly relevant in candidiasis, because the fungus behaves as a commensal as well as a true pathogen of skin and mucosal surfaces (1), known to be highly enriched for DC.

DC are uniquely specialized to initiate T cell immunity in vitro and in vivo (12). When delivered in vivo, DC charged with peptide Ags, tumor lysates, or viral vectors that encode the relevant Ags induce immune responses, which include protective and therapeutic immunity to tumors and pathogens in animals (11, 13–18) and clinical antitumor responses in humans (19–22).

Studies have shown that DC transfected with tumor RNA could also serve as potent cancer vaccines (23–25). DC transfected with chicken OVA RNA stimulate primary anti-OVA CTL responses in vitro. Moreover, vaccination of mice with OVA RNA-transfected DC has been used to treat OVA-positive tumor metastases to lung...
with focus to skin distance of 75 cm and 0.7 Gy/min dose rate. Unless
(Calco, Italy). Breeding pairs of homozygous IFN-
MHC class I Ag expression on cells from spleens.

cording to previous studies (27–29). Donor BM cells were prepared by differential agglutination with soybean

Candida

C. albicans

bacterial overgrowth, as described (11). Pulsing of DC with RNA was
performed in serum Opti-MEM medium (Life Technologies), as described (31). DC were washed twice in Opti-MEM and reseeded in Opti-MEM medium at 2–5 × 10^6 cells/ml and added to 15-ml polystyrene tubes (Falcon). The cationic lipid, N-[1-(2,3-dioleoyloxypropyl)N,N,N-trimethylammonium methylsulfate (DOTAP; Boehringer Mannheim, Mannheim, Germany) was used to deliver RNA into cells (33). RNA (25 μg in 250 μl Opti-MEM medium) and DOTAP (50 μg in 250 μl Opti-MEM medium) was mixed in 12 × 75-mm polystyrene tubes at room temperature for 20

Materials and Methods

Animals

Female, 8- to 10-wk-old, inbred C3H/HeJ and hybrid (BALB/c × DBA/2F1, CD2F1, mice were obtained from Charles River Breeding Laboratories (Calcio, Italy). Breeding pairs of homozygous IFN-γ-deficient (IFN-γ−/−) mice raised on BALB/c background (kindly provided by Dr. M. Kopf, Basel Institute for Immunology, Basel, Switzerland) were bred under specific pathogen-free conditions at the breeding facilities of the University of Perugia (Perugia, Italy). Mice of both sexes, 8–10 wk old, were used. Hybrid CD2F1, mice were used as wild-type mice. Bone marrow (BM)-transplanted mice were kept in small sterile cages (five animals in each cage) and fed with sterile food and water. Procedures involving animals and their care were conducted in conformity with national and international laws and policies.

Irradiation

C3H/HeJ mice were exposed to a single, lethal dose of 9 Gy from a gamma beam 150A, 60Co source (Clinac 600C Varian; Censuaco, Milan, Italy) with focus to skin distance of 75 cm and 0.7 Gy/min dose rate. Unless BM-transplanted, mice die within 14 days.

C. albicans strains and isolation of fungal RNA

The origin and characteristics of C. albicans highly virulent and live vaccine strains used in this study have already been described (11). Total RNA was isolated from actively growing yeasts and hyphae as described (30). Brieﬂy, cells were disrupted by repeated cycles of thawing and freezing on liquid nitrogen. Hot extraction buffer was added to the cells (a 1:1 mixture of phenol and 0.1 M LiCl. 100 mM Tris HCI (pH 8), 10 mM EDTA, and 1% SDS at 80°C) and a mixture (24:1, v/v) of chloroform and isoamyl

Preparation of T cell-depleted BM cells

Donor BM cells were prepared by differential agglutination with soybean agglutinin, as described (27). T cell-depleted soybean agglutinin-positive cells (containing <1% of contaminating T cells on FACS analysis) were injected at the concentration of 2.4 × 10^6/ml into recipient mice i.v. According to previous studies (27–29), >95% of the mice survived showing stable, donor-type hematopoietic chimerism, as revealed by donor-type MHC class I Ag expression on cells from spleens.

Purification, Candida pulsing, and culture of DC

DC were purified from spleens (SP-DC) by magnetic cell sorting with MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) conjugated to hamster anti-mouse CD11c mAbs (clone N-418), as described (11). Brieﬂy, after overnight plastic adherence to remove macrophages, 10^6 col-

Acting as nonadherent cells and fresh medium containing GM-CSF and IL-4 was added. On day 6 of culture, DC were isolated by transferring nonadherent cells to new culture plates and incubating at 37°C for at least 2 h. Consistent with previous reports (31, 32), >90% SP-DC (at ~1% yield recovery) routinely express high levels of CD11c integrin, while BM-DC (at ~10% of the BM population) uniformly showed low level of CD11c staining. More than 90% of BM-DC did not react with the 2.43 anti-CD8α mAb, while 25–30% of SP-DC expressed CD8α (data not shown). For pulsing, 2 × 10^6 DC were exposed to live yeasts or hyphae (at DC:yeast ratio of 1:1 and 1:10, respectively) for 2 h before addition of 2.5 μg/ml amphotericin B (Sigma-Aldrich) to prevent Candida overgrowth, as described (11). Pulsing of DC with RNA was performed in serum Opti-MEM medium (Life Technologies), as described (31). DC were washed twice in Opti-MEM and reseeded in Opti-MEM medium at 2–5 × 10^6 cells/ml and added to 15-ml polystyrene tubes (Falcon). The cationic lipid, N-[1-(2,3-dioleoyloxypropyl)N,N,N-trimethylammonium methylsulfate (DOTAP; Boehringer Mannheim, Mannheim, Germany) was used to deliver RNA into cells (33). RNA (25 μg in 250 μl Opti-MEM medium) and DOTAP (50 μg in 250 μl Opti-MEM medium) was mixed in 12 × 75-mm polystyrene tubes at room temperature for 20

Adaptive immunization, fungal challenge, and assessment of protection

DC (3–5 × 10^3/each injection) were injected either s.c. in 20 μl of PBS or i.v. in 0.5 ml of PBS following different protocols, as described in Results. In BM-transplanted recipients, DC were administered s.c. twice, 1 and 7 days after BMT. For infection, a week after the last DC administration, mice were i.v. infected with 10^7 C. albicans cells of the live vaccine or the virulent strain in 0.5 ml of PBS as described (11). Infection with the virulent strain was done 14 days after the primary infection with the live vaccine strain. Resistance to infection was assessed by quantifying the number of CFU (mean ± SE) per organ and production of Th1 and Th2 cytokines in culture supernatants of Ag-stimulated splenocytes (40) splenocytes stimulated with 10^3 heat-inactivated C. albicans cells for 48 h, and by enumerating the IFN-γ-producing cells, by ELISPOT assay, as described (11). Total and differential white blood cell counts were done by hemocytometry and by staining blood smears from transplanted mice with May-Grunwald Giemsa reagents (Sigma-Aldrich) before analysis.

Flow cytometry

For DC analysis, cell surface phenotype was assessed with the following mAb reagents: FITC-conjugated mAb anti-CD11c (N418), anti-CD8α (2.43), anti-IAα (34–5–3), anti-CD80 (1G10), and anti-CD86 (GL1) (all from BD PharMingen, San Diego, CA). For cytokine analysis, splenocytes were reacted with FITC-conjugated goat anti-mouse IgA plus IgG plus IgM (H and L) (Euroimmun, Germany) was used to deliver RNA into cells (33). RNA (25 μg in 250 μl Opti-MEM medium) and DOTAP (50 μg in 250 μl Opti-MEM medium) was mixed in 12 × 75-mm polystyrene tubes at room temperature for 20

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CD4 (L3T4, clone GK1.5), anti-CD8α (Ly-2, clone 53-6.7), and anti-SE6NK (clone SE6). Staining with PE-conjugated anti-CD44 (Pgp-1, clone 5E6). Staining with PE-conjugated anti-CD4 (37). Briefly, freshly isolated splenocytes or purified CD4+ T cells were cultured (1 × 10^5–1 × 10^6 cells/well) in complete medium (RPMI 1640 with 10% FCS, 50 mM 2-ME, and 50 μg/ml gentamicin sulfate) for 18 h in 96-well plates previously coated with rat anti-murine α4-IAA-2 mAb. Biotinylated AN-18.17.24 mAb was used as the detecting reagent, avidin-biotinylated peroxidase complex and 3,3′-diaminobenzidine (Life Technologies, Gaithersburg, MD) was used as the enzyme, and 5-bromo-4-chloro-3-indolyl phosphate-<i>p</i>-tolydilute salt (Life Technologies, Gaithersburg, MD) was used as the substrate. Results were expressed as the mean of IFN-γ-producing cells (± SE) per 10⁶ cells, calculated using replicates of serial 2-fold dilutions of cells.

RAPD-PCR

Fungal RNA was isolated as described above. SP-DC transfected with yeast or fungal RNA were subjected to RNA extraction by the guanidium thiocyanate-phenol-chloroform procedure, as previously described (11). Before cDNA synthesis, all RNA samples were treated with RNase-free DNase I (Life Technologies). cDNA was synthesized from 1 μg of total RNA according to the manufacturer’s directions (Superscript Moloney murine leukemia virus reverse transcriptase; Life Technologies). cDNAs were amplified with random primers derived from the M13 phage core sequence (5′-GAGGTTGCGTTCT-3′), known to amplify hypervariable interrepeat DNA sequences from Candida strains (38). Amplifications were performed in volumes of 25 μl containing 10 ng of cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each dATP, 2′-dCTP, dTTP, and dGTP, 3 mM magnesium acetate, 10 ng of primer, and 1.5 U of AmpliTaq DNA polymerase. The PCR was performed in a PerkinElmer thermal cycler (model 480; PerkinElmer, Wellesley, MA) with an initial denaturation of 97°C for 3 min, followed by 40 cycles of 20 s at 93°C, 60 s at 50°C, and 20 s at 72°C, and a final cycle of 5 min at 72°C. Amplification products were separated by electrophoresis in 1.5% agarose gels in 1× Tris-acetate-EDTA buffer for 12 h at 2 V/cm. A 100-bp ladder (New England Biolabs, Cebio, Milan, Italy) was run in parallel for approximate PCR product band sizing. Amplification products were analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical analysis

Student’s t test was used to determine significance of values among experimental groups (significance was defined as p < 0.05). In vivo groups consisted of six to eight animals. The data reported were pooled from three to five experiments.

Results

Maturation and cytokine production by DC upon exposure to Candida yeasts or transfected with yeast or hyphal RNA

To assess whether DC undergo functional maturation upon exposure to viable yeasts or RNA from yeasts or hyphae, the surface expression of MHC class II Ags and of CD80 and CD86 costimulatory molecules was assessed in SP-DC and BM-DC exposed to either type of stimuli. The results showed that the expression of MHC II, CD80, and CD86 was evaluated both as the percentage of positive cells and as median fluorescence intensity (MFI).
Effect of different treatments on the ability of yeast RNA to induce maturation of DC

FIGURE 2. Effect of different treatments on the ability of yeast RNA to induce DC maturation. Splenic DC were transfected yeast RNA for 24 h at 37°C before the assessment of CD80 (A) and CD86 (B) costimulatory Ag expression by immunofluorescence analysis, as detailed in Fig. 1. Before transfection, some RNAs were treated with 100 U/ml RNase A, 100 U/ml DNase I, or 400 μg/ml proteinase K for 30 min at 37°C. The enzymes were inactivated by incubating samples at 65°C for 15 min. MFI of nontransfected DC was 12 for CD80 and 10 for CD86. *, p < 0.05, RNA-treated vs untreated (none) RNA.

In contrast, transfecting with hyphal RNA did not result in up-regulated expression of costimulatory molecules (Fig. 1), a finding suggesting that maturation of DC is a specific effect of yeast RNA and not a consequence of a nonspecific effect of the transfection method itself. The inability of hyphal RNA to sensitize DC was not due to a defective entry of RNA into the cells, as both yeast and hyphal RNA could be detected inside the cells by random amplified polymorphic DNA (RAPD) analysis (Fig. 3). An 867-bp band that was similar in size to that amplified in fungal cDNAs (Fig. 3) or in genomic DNAs from Candida strains (38) was detected in DC transfected with either yeast or hyphal RNA. As higher amounts of hyphal RNA still failed to up-regulate costimulatory molecule expression on DC (data not shown), all together, these data suggest that the induction of DC maturation is a property of yeast RNA.

We have already shown that myeloid DC produce opposite sets of Th cytokines upon exposure to yeasts (IL-12 p70 and IL-6) or hyphae (IL-4) (11). In this study we found that both types of DC produce IL-12 p70 and IL-6, but not IL-4 or IL-10, upon exposure to either yeasts or yeast RNA (Table I). Therefore, these data suggest that both live yeasts and yeast RNA are effective stimuli capable of inducing functional maturation and cytokine production by myeloid DC.
that the fungal growth was significantly decreased in mice who had kidneys was recorded a week after the infection. The results show yeasts or yeast RNA. Splenic DC from CD2F1 mice were pulsed with Th1 (IFN-γ) had been observed with yeast-pulsed DC (11), the antifungal re-
cant inhibition transfected DC s.c., twice, also resulted in a signifi-
cano effect was observed when DC were given 14 days before by the
before challenge (Fig. 5). However, consistent with previous data
received either type of yeast-pulsed DC administered s.c. 14 days
infected i.v. with virulent \textit{C. albicans} and fungal growth in the
sificantly reduced in organs that are sites of fungal disease also signifi-
fungal Ag expression by immunofluorescence analysis with the IgM B6.1
controls with the temporal occurrence of Th2 and Th1 cell responses, with
Th2 reactivity accounting for susceptibility to the infection in the
early engraftment period (26). To assess whether the infusion of \textit{Candida}-pulsed DC would accelerate Th1 cell recovery and anti-
fungal resistance in transplanted mice, recipients mice received at
1 and 7 days post-BMT donor DC in vitro pulsed with yeasts or yeast RNA. One week after the last DC administration, mice were i.v. infected with the \textit{Candida} live vaccine strain and 14 days later they were infected with the virulent strain. Mice were monitored for resistance to infection with virulent cells and for parameter of Th1-mediated antifungal resistance, such as the frequency of IFN-γ-producing CD4+ T splenocytes. The results (Fig. 7) showed that the infusion of yeast-pulsed or RNA-transfected DC greatly accelerated the recovery of antifungal resistance, as compared with control mice not receiving DC. A reduced fungal burden was observed in the kidneys and was comparable to that seen in donor-vaccinated mice upon reinfection. Interestingly, the fungal growth was also significantly reduced in organs that are sites of fungal disease and pathology in BMT, such as the gastrointestinal tract, the liver, and the lungs (26). The infusion of yeast-pulsed or RNA-transfected DC also increased resistance toward the primary infection with virulent \textit{Candida} cells (data not shown). Total and differential counts of blood leukocytes indicated that the absolute number of circulating lymphocytes significantly increased in DC-infused and transplanted mice and was similar to that of donor-vaccinated mice, as shown in Fig. 8 for mice infused with RNA-transfected DC. The number of monocytes and neutrophils did not show significant variations among groups (data not shown). Cytotoxicometric analysis of spleen cells revealed that the numbers of CD4+, CD8+, and NK cells were significantly higher in mice who had

\textbf{Immunization with yeast RNA-transfected DC induces Th1-mediated antifungal resistance}

DC pulsed with \textit{Candida} yeasts, but not hyphae, were capable of inducing Th1 priming and antifungal resistance when adoptively transferred into naive recipients (11). To assess whether DC transfected with yeast RNA also induce antifungal resistance in vivo, SP-DC or BM-DC were exposed to yeasts or to RNA from yeasts or hyphae in vitro and injected in vivo into naive recipients following different experimental protocols, which include different routes and timing of DC administration. Mice were subsequently infected i.v. with virulent \textit{C. albicans} and fungal growth in the kidneys was recorded a week after the infection. The results show that the fungal growth was significantly decreased in mice who had received either type of yeast-pulsed DC administered s.c. 14 days before challenge (Fig. 5). However, consistent with previous data (11), the fungal growth was even more restrained when DC were administered twice, 14 and 7 days before the infection. In contrast, no effect was observed when DC were given 14 days before by the i.v. route. Results with yeast or hyphal RNA-transfected DC showed that the adoptive transfer of yeast, but not hyphal, RNA-transfected DC s.c., twice, also resulted in a significant inhibition of fungal growth (Fig. 5). To understand whether, similar to what had been observed with yeast-pulsed DC (11), the antifungal resis-
tance correlates with the priming of Th1 cells, the production of Th1 (IFN-γ) and Th2 (IL-4 and IL-10) cytokines was evaluated in the spleens of mice who had received multiple s.c. injections of RNA-transfected SP- or BM-DC. The results (Fig. 6) show that production of IFN-γ was higher and that of IL-4/IL-10 was lower in mice who had received multiple s.c. injections of yeast RNA-transfected DC as compared with either mice who had received DC transfected with hyphal RNA or control mice not receiving

\textbf{DC vaccination in murine candidiasis}

In a mouse model of allogeneic T-depleted BMT, we have recently shown that susceptibility or resistance to candidiasis correlates with the temporal occurrence of Th2 and Th1 cell responses, with Th2 reactivity accounting for susceptibility to the infection in the early engraftment period (26). To assess whether the infusion of \textit{Candida}-pulsed DC would accelerate Th1 cell recovery and anti-
fungal resistance in transplanted mice, recipients mice received at 1 and 7 days post-BMT donor DC in vitro pulsed with yeasts or yeast RNA. One week after the last DC administration, mice were i.v. infected with the \textit{Candida} live vaccine strain and 14 days later they were infected with the virulent strain. Mice were monitored for resistance to infection with virulent cells and for parameter of Th1-mediated antifungal resistance, such as the frequency of IFN-γ-producing CD4+ T splenocytes. The results (Fig. 7) showed that the infusion of yeast-pulsed or RNA-transfected DC greatly accelerated the recovery of antifungal resistance, as compared with control mice not receiving DC. A reduced fungal burden was observed in the kidneys and was comparable to that seen in donor-vaccinated mice upon reinfection. Interestingly, the fungal growth was also significantly reduced in organs that are sites of fungal disease and pathology in BMT, such as the gastrointestinal tract, the liver, and the lungs (26). The infusion of yeast-pulsed or RNA-transfected DC also increased resistance toward the primary infection with virulent \textit{Candida} cells (data not shown). Total and differential counts of blood leukocytes indicated that the absolute number of circulating lymphocytes significantly increased in DC-infused and transplanted mice and was similar to that of donor-vaccinated mice, as shown in Fig. 8 for mice infused with RNA-transfected DC. The number of monocytes and neutrophils did not show significant variations among groups (data not shown). Cytotoxicometric analysis of spleen cells revealed that the numbers of CD4+, CD8+, and NK cells were significantly higher in mice who had

\textbf{Yeast RNA-transfected DC induce antifungal resistance in allogeneic T-depleted BM-transplanted mice}

DC. Together, these data suggest that yeast RNA-transfected DC, similar to yeast-pulsed DC, are capable of inducing Th1 priming and antifungal resistance in vivo, and that the priming capacity is strictly dependent on mode of DC delivery in vivo.
Lethally irradiated C3H/HeJ mice were transplanted with C. albicans and IL-2 production in hematopoietic BM recipients upon adoptive transfer. Yeasts (A) were lower than those of vaccinated donor mice (Fig. 9). Upon Ag stimulation in vitro, donor-vaccinated mice (Fig. 9a) compared with recipients not receiving DC and was similar to that of mice receiving either yeast-pulsed or RNA-transfected DC as compared with recipients not receiving DC and was similar to that of donor-vaccinated mice (Fig. 9a). Upon Ag stimulation in vitro, splenocytes from DC-infused mice produced levels of IL-4 that were lower than those of mice not receiving DC and were similar to those of vaccinated donor mice (Fig. 9b). Interestingly, however, levels of IL-2 were also lower and those of IL-10 higher in DC-infused mice, as compared with not only untreated recipients but also donor-vaccinated mice. Therefore, the activation of CD4+ T lymphocytes producing IFN-γ occurs together with IL-10 but not IL-2 production in hematopoietic BM recipients upon adoptive transfer of Candida-pulsed DC. Experiments in which RNA-transfected DC as compared with mice not receiving DC and were similar to those of donor-vaccinated mice. Interestingly, CD4+ T cells also stained positive for the CD44 and CD25 activation surface markers (Fig. 8).

On assaying the pattern of cytokine production, we found that the frequency of IFN-γ-producing cells was increased in both freshly isolated T splenocytes and purified CD44+ T cells from mice receiving either yeast-pulsed or RNA-transfected DC as compared with recipients not receiving DC and was similar to that of donor-vaccinated mice (Fig. 9a). Upon Ag stimulation in vitro, splenocytes from DC-infused mice produced levels of IL-4 that were lower than those of mice not receiving DC and were similar to those of vaccinated donor mice (Fig. 9b). Interestingly, however, levels of IL-2 were also lower and those of IL-10 higher in DC-infused mice, as compared with not only untreated recipients but also donor-vaccinated mice. Therefore, the activation of CD4+ T lymphocytes producing IFN-γ occurs together with IL-10 but not IL-2 production in hematopoietic BM recipients upon adoptive transfer of Candida-pulsed DC. Experiments in which RNA-transfected DC were infused into recipient mice who had received T-depleted BM cells from IFN-γ−/− mice demonstrated that the efficacy of RNA-transfected DC was mediated by the occurrence of donor IFN-γ-producing cells. Fig. 10 shows that the fungal growth in these mice was comparable to that observed in mice not receiving DC and significantly higher than that of mice transplanted with T-depleted BM cells from IFN-γ-sufficient mice. As a result, similar to what we observed in mice not receiving DC, all the mice succumbed to the infection as opposed to the long-term survival, with no signs of diseases, of mice who had received T-depleted BM cells from IFN-γ−/− mice.

**Discussion**

The present study shows that, consistent with previous results obtained with myeloid DC pulsed with Candida yeasts (11), DC transfected with yeast RNA are fully competent to induce Th1 priming upon adoptive transfer in vivo. Induction of antitumor immunity with tumor RNA-transfected DC represents a recently described approach for DC-based vaccines (25). In this study we show that this approach could be used to induce antimicrobial immunity as well.

It has been shown that DC transfected with MUC1 RNA expressed MUC1 tumor Ag along with costimulatory and adhesion molecules (24), a finding suggesting that newly synthesized molecules are potential targets for CTL recognition of RNA-transfected cells (24). We found in this study that DC from both BM and spleen underwent functional maturation in vitro upon pulsing with either live yeasts or yeast RNA, but not with hyphal RNA, as revealed by the up-regulated expression of MHC class II Ags and costimulatory molecules. No variation in CD8α expression was observed upon pulsing with either type of stimulus (data not shown), a finding suggesting that, in our system, myeloid DC do not convert into lymphoid DC upon activation, as suggested (40). Moreover, similar to yeast-pulsed DC, DC transfected with yeast but not hyphal RNA produced IL-12 p70 along with IL-6. As the ability of yeast-pulsed DC to induce Th1 priming in vivo was lost in conditions of IL-12 deficiency (11), these data suggest that DC transfected with yeast and not hyphal RNA would be fully competent to induce Th1 activation.

For this to occur, a prerequisite would be the expression of fungal Ags capable of inducing protective immunity on the surface of DC upon transfection with yeast RNA. This appeared to be the case as DC transfected with yeast but not hyphal RNA positively reacted with polyclonal Abs specifically reacting with Candida cells (36) and, partially, with mAb binding to a specific component of the acid-labile portion of the phosphomannan complex, which is known to be endowed with the ability to induce protective immunity in experimental candidiasis (39).
Pathways and mechanisms of fungal RNA and Ags processing by DC are largely unknown at the moment and remain to be explored.

Upon adoptive transfer in vivo, yeast RNA-transfected DC were capable of inducing the activation of Candida-specific Th1 cells, as revealed by the predominant production of IFN-γ over that of...
IL-4/IL-10. Moreover, the IL-12/β2R, a marker of CD4+ Th1 cells in candidiasis (5), was expressed on CD4+ T lymphocytes (data not shown). Associated with the occurrence of Candida-specific Th1 reactivity was the induction of a state of antifungal resistance, as revealed by the significant decrease of fungal growth in the target organs, such as the kidneys and the brain (data not shown).

Previous work in vivo in mice and in humans has suggested that, in addition to the maturation state (41), the mode of delivery of DC is important in eliciting T cell responses (42–45). DC given i.v. localized in the reticuloendothelial system and lung, while those given s.c. preferentially homed to the T cell areas of the draining lymph nodes (42). In particular, induction of IFN-γ production, but not IL-4, was seen only with DC injected intradermally or intralymphatically but not i.v. (43). We found a similar result, as Th1 cell activation was observed after s.c. but not i.v. administration of DC. Although multiple injections of DC could lead to clonal exhaustion of the responding T cell population (43), the optimal protection against C. albicans was observed after two s.c. injections of Candida-pulsed DC. Labeling studies will clarify whether DC loaded with Candida home to different organs upon administration by different routes and whether the extent to which the ability of DC to migrate to relevant lymphoid organs will regulate the type and magnitude of the antifungal immune response.

An interesting observation of the present study was that the infusion of Candida-pulsed DC accelerated the recovery of functional antifungal Th1 responses in a murine model of T cell-depleted allogeneic BMT. Patients receiving T cell-depleted BMT are unable to develop Ag-specific T cell responses soon after transplant (46). It has been demonstrated that T cell depletion of allogeneic BMT is associated with a slow recovery of CD4+ and CD8+ T cells (46). However, functional recovery of the T cell system after T cell-depleted allogeneic BMT has been demonstrated (47, 48), and both donor and recipient DC may contribute to the reconstitution of the T cell repertoire in transplantation through distinct pathways of Ag presentation (49). Interestingly, it has been reported that T cell reactivity to Candida readily recovered after T cell-depleted allogeneic BMT, and the fact that recovery was related to the absence of antifungal prophylactic measures indicates that antifungal reactivity of residual T cells could be maintained through continuous exposure to the fungus (50).

We have recently demonstrated that an unbalanced production of Th1 and Th2 cytokines was responsible for the susceptibility to candidiasis observed in our BMT model. However, readressing the balance between Th1 and Th2 subsets, as by treatment with Th2 cytokine antagonists, accelerated the recovery of Th1-mediated antifungal resistance (26). In this study we show that recovery of functional NK and T cells producing IFN-γ could also be accelerated by the infusion of Candida-pulsed DC. This, along with the decreased IL-4 production, translated in the occurrence of a state of antifungal resistance as revealed by the impaired fungal growth in different target organs, including those most frequently associated with fungal disease and pathology in BMT settings, such as the gut, liver, and lungs. Therefore, although conflicting data exist as to whether donor DC are “friends” or “foes” in transplantation (49), our findings suggest that DC may contribute to the educational program of T cells in BMT during reconstitution, in line with the recent observation of the immunization capacity of tumor-pulsed (51) or Aspergillus-pulsed (18) DC in BM-transplanted mice and humans.

The observation that the occurrence of a protective Th1 reactivity coexisted with the detection of significant levels of IL-10 is intriguing. It is known that high levels of IL-10 are associated with tolerance to HLA-mismatched BM stem cells (52) and IL-10 is required for the induction of regulatory T cells mediating tolerance to alloantigens in vivo (52–55). Different regulatory T cells have been reported and they are known to differentiate upon the encounter with immature DC (53, 55). However, fully competent DC were also found to be inducers of T cell anergy when presenting a self-epitope with altered peptide ligand features (56). Whether IL-10 produced in BM-transplanted mice infused with Candida-pulsed DC may serve to support the growth of regulatory T cells preventing donor Th1 alloreactivity remains a working hypothesis, although it may help to explain the long-term, disease-free survival of the mice. In terms of interference with the development and activity of antifungal Th1 reactivity, it is well established that the effect of IL-10 is dependent upon the dose of the cytokine. At high doses, IL-10 had a negative effect on Th1-mediated antifungal resistance (57), but at low doses IL-10 was required for optimal induction and maintenance of antifungal Th1 immune response (58).

A recent study showed that yeast cells could act as an efficient vaccine and elicited protective cell-mediated immunity by virtue of their ability to induce DC maturation, IL-12 production, and efficient priming for MHC class I- or II-restricted Ag-specific T cell responses (59). We show in this work that not only the yeasts but yeast RNA could be an efficient trigger of DC maturation, IL-12 production, and induction of protective Ag-specific responses to Candida. Interestingly, inactivated yeasts failed to induce DC maturation in vitro, and DC pulsed with inactivated yeasts failed to promote Th1 immunity upon adoptive transfer in
vivo (data not shown). Therefore, these data may account for the long-standing observation of the inability of inactivated Candida to induce memory anticalendal protective immune responses.

It has been shown that human DC phagocytose and process C. albicans (60, 61) as well as other fungi (18, 62), and ex vivo-generated DC pulsed with Aspergillus conidia restored antifungal immunity in BM-transplanted patients (18). This work suggests that DC could act as effective vaccines against fungal infections and that RNA-transfected DC could be of vaccinating potential in conditions that negate the use of attenuated microorganisms, such as immunosuppression, or in the case of poor availability of protective Ags.

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References

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