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Active Immunizations with Peptide-DC Vaccines and Passive Transfer with Antibodies Protect Neutropenic Mice against Disseminated Candidiasis

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Abstract

We previously report that peptide-pulsed dendritic cell (DC) vaccination, which targeting two peptides (Fba and Met6) expressed on the cell surface of *Candida albicans*, can induce high degree of protection against disseminated candidiasis in immunocompetent mice. Passive transfer of immune sera from the peptide immunized mice or peptide-related monoclonal antibodies demonstrated that protection was mediated by peptide-specific antibodies. In this study the efficacy of active and passive immunization against disseminated candidiasis was tested in mice with cyclophosphamide-induced neutropenia. Peptide-DC vaccines were given to mice prior to induction of neutropenia. We show active immunization with either Fba or Met6 peptide-DC vaccine significantly improved the survival and reduced the fungal burden of disseminated candidiasis in those immunocompromised mice. Importantly, we show that administration of two protective monoclonal antibodies also protect neutropenic mice against the disease, implying possibility of developing a successful passive immunotherapy strategy to treat the disease and protect against disseminated candidiasis. The results of this study are crucial as they address the fundamental questions as to whether the synthetic peptide vaccine induced immunity protects the host during a neutropenic episode. We anticipate that this peptide-vaccine study will serve as the foundation of future investigations into new peptide vaccines comprised of cell surface peptides from other medically important *Candida* species, as well as other fungi.

Keywords

peptide vaccine; antibody protection; candidiasis; neutropenia

INTRODUCTION

Disseminated candidiasis is the cause of more case fatalities than any other systemic mycosis (1-4), and it occurs most commonly in patients whose bodily defenses have been

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compromised by cancer, by major surgery, or by treatment with cytotoxic or immunosuppressive drugs (5,6). However, the most important predisposing factors are dysfunctional neutrophils or neutropenia (7-10). Unfortunately, mortality of disseminated candidiasis remains unacceptably high (11-13) in spite of current antifungal therapy (14). Therefore, more effective prophylactic and therapeutic strategies are needed.

Vaccination of high-risk groups is a particularly promising strategy to prevent invasive *Candida* infection (15). We first reported fully synthetic peptide vaccines that protect against disseminated candidiasis in immunocompetent mice (16-18). These peptide vaccines were selected from N-terminus of previously identified cell wall proteins that are expressed during pathogenesis of human disseminated candidiasis (19,20). We further demonstrated that antibodies specific for two peptide vaccines, Fba and Met6 (Fba, derived from fructose-bisphosphate aldolase and Met6, derived from 5 methyltetrahydropteroyltriglutamate homocysteine methyltransferase) are each protective (17,21). One major concern about a *Candida* vaccine is the belief that disseminated candidiasis occurs almost exclusively in immunocompromised patients, who may not be expected to respond immunologically to a vaccine. However, there is extensive literature confirming the immunogenicity and efficacy of vaccines even in patients with weakened immune systems—for example, those with neutropenia, active leukemia, HIV infections, or those receiving immunosuppressive corticosteroids (22-26). We also reported that protective antibodies specific for the β -(Man)₃, a carbohydrate epitope on *C. albicans* cell surface, enhanced resistance to disseminated candidiasis of both normal and neutropenic mice (27). Since neutropenia is one of the most common problems associated with disseminated candidiasis in human, we investigated whether the peptide vaccine induced immunity protects against disseminated candidiasis in neutropenic mice, as well as whether the protective peptide-specific MAbs protect neutropenic mice against the disease.

MATERIALS AND METHODS

Candida strains and culture conditions

C. albicans SC5314 (ATCC MYA-2876), were grown as stationary-phase yeast cells (24 h cells) in glucose-yeast extract-peptone (GYEP, 2% glucose, 1% peptone, 0.3% yeast extract) broth at 37°C, washed and suspended to the appropriate cell concentration (5×10^6 /ml, 1×10^6 /ml or 5×10^5 /ml) in Dulbecco's PBS (DPBS; Sigma), and used to infect mice intravenously (i.v.) as described (28,29). *C. albicans* strain SC5314 was also used for serum antibody absorption, immunofluorescence staining and flow cytometric analysis.

Mice

BALB/c female mice (National Cancer Institute Animal Production Program, Frederick MD) 5 to 7 weeks old were used throughout. Mice were always maintained in our AAALAC—certified animal facility and all animal experiments were done in accordance with a protocol approved by the Institutional Animal Care and Use committee (IACUC) at LSU Health Sciences Center (LSUHSC).

Peptide vaccines

Two 14-mer peptides Fba and Met6 are derived from N-terminus of *C. albicans* cell wall proteins fructose-bisphosphate aldolase (Fba) and methyltetrahydropteroyltriglutamate (Met6). Fba peptide (YGKDVKDLFDYAE) and Met6 peptide (PRIGGQRELKKITE) were produced commercially (GenScript).

Protective MAbs

Hybridoma clones, which produce Fba peptide specific MAb IgM E2-9 (17) and Met6 peptide specific MAb IgG3 M2-4 (21) were generated from mice vaccinated with peptide Fba- or Met6-pulsed dendritic cell (DC) preparation as described previously (16). Briefly, BALB/c mice were immunized by injection of synthetic peptide pulsed DCs to stimulate the production of antibodies against peptide as described above. Ten days after the second booster, serum was taken from each animal to determine animals with the highest anti-peptide titers for subsequent sacrificing, removal of spleens and preparation of single cell suspensions. Hybridoma clones were established by the polyethylene glycol facilitation of fusion of spleen cells to an SP2/0-AG14 myeloma cell line by standard protocols. Hybridoma clones were screened by ELISA for production of specific anti-peptide antibody; only the highest titers and most rapidly growing clones were selected for subsequent cloning x3 or more by limiting dilution.

The hybridoma cell lines were initially grown in antibiotic-free RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum (Invitrogen) and 2 mM L-glutamine (Sigma) at 37°C and in the presence of 5% CO₂. For antibody production, the hybridoma clones were grown in antibiotic-free, BD cell MAb serum-free medium (but containing 1.1 mg bovine serum albumin/ml) in a CELLLine device (BD, Bedford, MA). MAb IgM E2-9 was purified and analyzed as described before (21). For the MAb IgG3 M2-4, the supernatant was collected and MAb was purified by affinity chromatography using a Protein A Sepharose 4FF column (GE Healthcare, USA). The isotype of MAb was determined with a Mouse Monoclonal Antibody Isotyping Kit (Pierce, USA).

Isolation and culture of dendritic cells (DCs) from mouse bone marrow

Dendritic cells (DCs) were generated from mouse bone marrow by a previously described method (16,30). Briefly, donor mice were euthanized by CO₂ asphyxiation, their long bones and tibias were aseptically removed, bone marrow was flushed from the bones by forcibly injecting several ml of RPMI-1640 and clumps were removed or dispersed by gentle pipetting through a sterile 70-mm cell strainer. Red blood cells were lysed (ACK lysing buffer, 0.15 M NH₄Cl, 1.0 mM KHCO₃, 0.1 mM EDTA) for 4 min and the remaining bone marrow cells were suspended in complete medium [CM, RPMI-1640 supplemented with 10% FBS (FBS), 2 mM L-glutamine, 1% of nonessential amino acids and 100 units/ml penicillin and 100 µg/ml streptomycin], adjusted to 2×10^5 cells per ml plated in 6-well plates at 5 ml per well and cultured for up to 9 days in the presence of 40 ng/ml of rmGM-CSF and rmIL-4 (R&D Systems) at 37°C, 5% CO₂. On days 4 and 7 of culture, the same amount of fresh GM-CSF and IL-4 was added to the wells.

Active Immunizations with peptide pulsed dendritic cells

A time course of the protocols for active immunization, the induction of neutropenia, and survival studies after *C. albicans* infection is summarized in Fig. 1. All active vaccinations were conducted as previously described (16,17,21). DCs were pulsed *in vitro* with Fba peptide or Met6 peptide as described before with minor modification by totally removing CFA for last booster (16). Briefly, DCs in culture were pulsed with the peptide antigen (1 μ M) on day 6. On day 7, PGE₂ (10⁻⁷M) was added along with LPS (2 μ g/ml, Sigma) for 24h to induce DC maturation. On day 9, antigen-pulsed DCs were washed extensively and 5×10^5 in 200 μ l DPBS were given by intraperitoneal (i.p.) route as the priming dose to mice. The mice were boosted i.p. at day 14 and day 28 with fresh antigen-pulsed DCs without adjuvant.

To test the efficacy of the vaccine in immunocompromised mice, vaccinated mice were induced neutropenia by intraperitoneal injection of 200 mg/kg of cyclophosphamide (CY; Sigma-Aldrich) on day -3 followed by another 4 doses (150 mg/kg) every 10 days on days 10, 20, 30 and 40 relative to infection (Fig. 1).

Induction of neutropenia

Before challenge, BLAB/c mice were made neutropenic by intraperitoneal receipt of a 200 mg/kg dose of cyclophosphamide (CY; Sigma-Aldrich) at day -3. The i.p. injection (150 mg/kg) is repeated every 10 days after infection (i.e., at days 10, 20, 30, and 40 post-infection) in order to maintain low neutrophil counts for the entire experimental procedure. The experiment was set up to test this regimen, which has been shown to render mice neutropenic (The absolute neutrophil count is <500 cells/mm³) within 3–4 day of the first cyclophosphamide injection, and neutropenia lasts until the termination of the experiments (day 50). To evaluate leukocytopenia in these mice, blood samples were collected from tail veins 3 days after each CY or saline injection ($n = 10$ in each group). Total leukocyte and differential cell counts were determined on a hemacytometer and by Wright-Giemsa staining. Body weights of the mice were also measured and compared for 14 days after the first CY or saline injection ($n = 10$ in each group).

Fungal challenge dose and assessment of protection

Due to defective fungal host defense and impaired survival in neutropenic mice, *C. albicans* challenge dose need to be modified to obtain the similar survival curves as shown in immunocompetent mice. Neutropenic mice and age- and sex-matched normal control mice ($n = 5$ in each group) were injected i.v. with different doses of live *C. albicans* yeast cells (5×10^4 , 1×10^5 , and 5×10^6 in 0.1 ml DPBS). After two independent experiments, 5×10^4 was determined as lethal challenge dose for neutropenic mice in all the following survival studies. Two weeks after the second boost on day 0, immune and control neutropenic mice were infected i.v. with a lethal dose of live *C. albicans* yeast cells (5×10^4 in 0.1 ml of DPBS) prepared as described above and as before (16). Passively immunized mice (below) also received the same challenge doses. Protection was evaluated by monitoring animal survival for 50 days. The mice were monitored for the development of a moribund state, defined as being listless, disinterested in food or water, and nonreactive to finger probing. At the time that a mouse was deemed moribund, it was sacrificed and their kidneys were

homogenized in DPBS and plated onto a nutrient agar to determine colony forming units (CFUs).

Passive transfer of MAbs by intraperitoneal (i.p.) route

The preventive effect of MAbs M2-4 and E2-9 in neutropenic mice was examined by passive transfer experiments. A time course of the protocols for passive immunization and survival studies after *C. albicans* challenge is summarized in Fig. 1. The MAb IgM E2-9 was appropriately diluted in DPBS (0.2µg/µl) to give a 100,000 ELISA titer against Fba peptide coated on the plate, and the MAb IgG3 M2-4 was appropriately diluted in DPBS (0.5µg/µl) to give a 100,000 ELISA titer against Met6 peptide coated on the plate. For testing, mice received 0.5 ml of MAb M2-4 or MAb E2-9 intraperitoneally. Control mice received 0.5 ml of the DPBS diluents. For each condition, neutropenic BALB/c mice were given 0.5 ml of test MAb, or control materials intraperitoneally, followed 4 h later by 0.1 ml intravenously of a suspension containing 5×10^5 yeast cells per milliliter of DPBS. Mice were divided into groups with five mice each and three independent experiments were carried out. After challenge, some groups of mice were given the same dose of antibodies (MAb E2-9 or MAb M2-4) as above or control materials on every other day post-challenge for two weeks as we described before (21). All mice were sacrificed on day 50.

Statistical Analysis

Survival times were statistically evaluated by Kaplan–Meier (GraphPad Prism, version 6), and statistical significance was subsequently calculated for each preset time point of analysis. A *P* value < 0.05 was considered to be statistically significant.

RESULTS

Induction of neutropenia in mice

Vaccinated BALB/c Mice were rendered severe neutropenic (polymorphonuclear leukocyte count, <500/mm³) 3 days prior to the beginning of the challenge study (Day 0) with 200 mg/kg of body weight intraperitoneal (i.p.) cyclophosphamide (CY, Sigma). Significant decreases in the numbers of total leucocytes and neutrophils were observed in the CY-treated mice compared with those in the control mice. The effects of CY treatment were monitored every 2-3 days during the entire experimental procedure. The total neutrophil counts were reduced (<500 cells/mm³) within 3 day of the first CY injection, and the severe neutropenia was maintained until the termination of the experiments at day 50 (Fig. 2). Monocyte counts didn't change significantly in cyclophosphamide-treated mice as compared to normal control group (immunocompetent mice) without CY treatment (Data not shown).

Vaccination with peptide vaccines significantly improved survival and decreased fungal burden in immunocompromised mice challenged intravenously with *C. albicans*

Neutropenic mice and age- and sex-matched normal control mice (BALB/c mice) were injected i.v. with different doses (5×10^4 , 1×10^5 , and 5×10^5) of *C. albicans* to determine the challenge dose for neutropenic mice. CY treated neutropenic mice had dose-dependent, significantly accelerated and enhanced mortality as compared with normal littermate

controls (data not shown). To obtain similar survival curves in neutropenic animals as in immunocompetent mice, one-tenth of challenge dose for normal animals, which is 5×10^4 is required.

It is known that a significant fraction of immunocompromised patients do respond to a variety of vaccines (15,22,31). Having demonstrated protective efficacy in immunocompetent mice (15,17,21), we next evaluated the potential for the Fba and Met6 peptide vaccines to induce protective antibodies that protect neutropenic mice against disseminated candidiasis. Here, we used the same antigen-pulsed DC-based immunization strategy favoring production of antibodies as described before (16,30). After the first booster, immune sera from mice immunized with Fba or Met6 peptide showed that significantly increased antibody responses ($p < 0.001$, data not shown) to each peptide over background sera obtained from mice that received DPBS or DCs only. Following the first and second booster immunization, an isotype switch from IgM to IgG of both Fba and Met6 specific antibodies were observed in the sera from immunized mice (data not shown), which suggested induction of immune memory responses. Vaccinated BALB/c Mice were rendered severe neutropenic (polymorphonuclear leukocyte count, $<500/\text{mm}^3$) 3 days prior to the beginning of the challenge study (Day 0) as described in Fig. 1. Each peptide vaccine was able to induced good protection in neutropenic mice as evidenced by significantly improved survival ($p < 0.01$) (Fig. 3A) and significantly reduced fungal burden (colony forming units, CFUs) in kidney ($P < 0.001$) (Fig. 3B), a target organ in disseminated candidiasis, in mice challenged with the fungus.

Passive immunization with MAbs E2-9 and M2-4 enhanced resistance of neutropenic mice to disseminated candidiasis

Since some immunocompromised patients might not respond to an active vaccine strategy, in this study we evaluated the possibility of using passive MAb immunotherapy targeting Fba and Met6 peptides in neutropenic mice. The preventive effect of MAbs M2-4 and E2-9 against disseminated candidiasis in neutropenic mice was examined by passive transfer experiments. To verify that the protection elicited by antibodies was indeed due to anti-Fba and anti-Met peptide antibodies, MAbs were absorbed with *C. albicans* yeast cells prior to testing for their protective activity against hematogenously disseminated candidiasis, with ELISA assays indicated the anti-peptide MAbs were successfully eliminated (data not shown). Mice received either MAb M2-4 or MAb E2-9 (one dose or multiple doses during 14 day period) all had significantly prolonged survival as compared to control animals that received DPBS or absorbed MAbs (Fig. 4, A & C); consistently, the group received treatment of either protective MAb had significantly reduced CFUs in kidneys compared with control mice (Fig 4, B & D). When each MAb was given every other day for two weeks, MAb M2-4 was able to increase protection from 20% to 40%, and E2-9, from 20% to 60%, with similar improved efficacy as we observed in immunocompetent mice (21).

Two different challenge doses, 1×10^5 and 5×10^5 were also tested in comparison with protective efficacy of protective MAbs. With both challenge doses are lethal for neutropenic mice, the survival of the neutropenic mice passively immunized with either MAb (E2-9 or

M2-4) was significantly improved (data not shown). Consistently, results also show the adsorbed MAbs didn't protect mice from disseminated candidiasis.

DISCUSSION

We conducted this study to investigate the usefulness of peptide vaccine based strategy to prevent lethal disseminated candidiasis during neutropenia. In a previous study, we reported fully synthetic peptide and glycopeptide vaccines protect against disseminated candidiasis in immunocompetent mice (16-18). We defined six T-cell peptides found in *C. albicans* cell wall proteins and conjugated each peptide to the fungal cell wall β -1,2-trisaccharide [β -(Man)₃] by novel tether linker to create glycopeptide conjugates (16). Although the six peptides were originally designed as the carriers for the protective glycan epitope, we further demonstrated that antibodies specific for two peptide carrier, peptides Fba and Met6, are each protective as well (17,21). Having demonstrated protective efficacy in immunocompetent mice, the potential for peptide vaccine and protective MAbs to induce immunity and protect neutropenic mice against disseminated candidiasis was evaluated in this study.

We have developed a neutropenic murine model of *C. albicans* to study vaccine efficacy, which emulate the most common host risk factor leading to susceptibility to disseminated candidiasis in human. We extended observations made by others on the effects of CY in mice (31-33). This widely used method was further modified by us, and resulted in neutropenia 3 days after administration of the drug in mice. Importantly, prolonged neutropenia can be maintained for 50 days by giving each animal a 150mg/kg body weight i.p. dose of CY every 10 days. As noted in Fig. 2, the total neutrophil counts remained below 500/ml during entire experiments, thus fulfilling the criterion for protracted neutropenia/leucopenia (34). This neutropenic mouse model is considered appropriate model for mimicking patients who develop the deficiency, including neutropenia, as a result of chemotherapy against various malignancies.

We found that rendering mice immunocompromised by CY made them hyper-susceptible to disseminated candidiasis, mimicking the enhanced susceptibility of neutropenic patients to the disease (27). In this intravenous murine model of *C. albicans* systemic infection, neutrophil depletion notably increases fungal burden and mortality, which is also reported by the others (35-37). However, to obtain survival curves in neutropenic animals similar to that seen in immunocompetent animals required a yeast cell challenge dose of one-tenth that used for normal animals. Although neutropenic mice were more susceptible to a lower dose of *C. albicans* than were normal mice, vaccination prior to rendering the mice immunocompromised provided significant protection evidenced by prolonged survival and greatly reduced CFU in kidneys of vaccinated neutropenic mice as compared to controls. We showed that immunity induced by active immunization with each of peptides (Fba and Met6) was protective in the neutropenic mice against the disease.

We have previously shown that the use of either peptide-specific MAb (E2-9 or M2-4) is promising as a single prophylactic component (17,21). Here we did show that passive vaccination with MAbs, either administered as a single dose or given every other day for

two weeks, resulted in marked improvement in survival and significant reductions in fungal burden during otherwise rapidly fatal hematogenously disseminated candidiasis in neutropenic mice. When MAb M2-4 or E2-9 was administered as single dose, (0.5 ml 4h before i.v. infection of *C. albicans*), either MAb was able to provide 20% protection of recipient animals. However, when each MAb was given every other day for two weeks, MAb M2-4 was able to increase protection from 20% to 40%, and E2-9 from 20% to 60%. This latter finding demonstrates that the efficacy of immunoprophylaxis is augmented when MAbs E2-9 and M2-4 were used for longer time. Of interest are the kidney fungal burden results from individual neutropenic mice received MAb treatments, demonstrating that approximately all the mice had kidney fungal burdens less than 4 log CFU/g, which is most critical predictor of the disease outcome. Consistently, passive vaccination with protective MAbs also resulted in significant improvements in time to death and overall survival. Furthermore, for all of the passive transfer experiments, passive protection was prevented by removal of the protective MAbs through absorption with *C. albicans* yeast cells before transfer, which provided strong additional evidence for the protection being due to the MAbs.

Our study showed that both vaccination prior to onset of neutropenia and administration of protective MAbs at the time of infection reduced the susceptibility of neutropenic mice to disseminated candidiasis, which based on better survival and lower fungal burdens in these animals. Since neutropenic patients have enhanced susceptibility to disseminated candidiasis, our results are encouraging because they show peptide vaccines and protective MAbs can offer enhanced protection even in immunocompromised individuals. Such patients, who have a high risk of developing disseminated candidiasis, would be an obvious target population for instituting preventive measures through either active immunization to induce protective immunity or direct administration of the protective antibody by passive transfer. The mechanism by which MAbs E2-9 and M2-4 protect against disseminated candidiasis is not known. Previous studies from our laboratory (38) and others (39-41) have indicated the importance of macrophages in host defense against disseminated candidiasis. Therefore, the protective antibodies may enhance anti-*Candida* activities of macrophages in neutropenic animals, thus conferring protection against lethal challenge of *C. albicans*. The possibility that MAbs E2-9 and M2-4 enhance macrophage activity against the fungus is under investigation. We hypothesized that immunotherapy targeting these two cell surface peptides would have the dual benefit of the immune system recognizing the fungus and enhancing phagocyte killing of *C. albicans*. Most recently, we designed a novel double chimeric peptide vaccine (Fba-Met6) that was able to synergistically enhance the level of protection in addition to avoiding immune evasion (21). We will continue to test the efficacy of this chimeric peptide vaccine in neutropenic mice in future study.

In summary, the Fba and Met6 peptide vaccines are the promising candidates for prevention of increasingly common and highly lethal disseminated candidiasis. Both peptide vaccines and related specific MAbs are efficacious in both immunocompetent and neutropenic mice. This paradigm of vaccine-based immunity during neutropenia may potentially serve as a framework for new preventive and therapeutic strategies against infectious diseases in the immunocompromised host.

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Fig. 1. The experimental protocols of active and passive immunization, the induction of neutropenia, and survival studies

For active immunization, mice were immunized by peptide-pulsed DC-based vaccine strategy as we described before (14,15). To induce neutropenia, vaccinated mice received 200 mg/kg dose of CY by i.p. on day -3. On day 0, mice were intravenously infected with 5×10^4 viable *C. albicans* yeast cells in 0.1 ml DPBS. Prolonged neutropenia were maintained for 50 days by giving each animal a 150mg/kg dose of CY by i.p. every 10 days after infection. **For passive immunization**, neutropenic mice were treated with 0.5 ml of MAb M2-4 (0.5 μ g/ μ l) or MAb E2-9 (0.2 μ g/ μ l) by i.p. 4hr before i.v. challenge. Control mice received 0.5 ml of the DPBS diluents or absorbed MAb. After challenge, some experimental groups of mice were given the same dose of antibodies (MAb E2-9 or MAb M2-4) as above or control materials on every other day for two weeks as we described before (19). All mice were sacrificed on day 50, when experiments were terminated.

Fig. 2. The effect of CY treatment on neutrophil counts in peripheral blood

The experiment was set up to test the regimen to maintain vaccinated mice in neutropenic condition for the entire experimental procedure (50 days). In the CY-treated group, BLAB/c mice were made neutropenic by intraperitoneal receipt of a 200 mg/kg dose of cyclophosphamide (CY; Sigma-Aldrich) at day -3 prior to infection, and the dose of CY (150 mg/kg) was given i.p. on every 10 day after infection in order to maintain low neutrophil counts for the entire experimental procedure. Control mice received saline. Blood samples were collected from tail veins every 203 days during 50 days for quantification of neutrophil cells. This regimen was able to render mice neutropenic (the absolute neutrophil count is <500 cells/mm³) within 3–4 day of the first CY injection, and neutropenia lasts until the termination of the experiments (day 50).

Fig. 3. Vaccination with peptide vaccines significantly improved survival and decreased fungal burden in neutropenic mice challenged intravenously with lethal dose of *C. albicans*

We used the same antigen-pulsed DC-based immunization strategy favoring production of antibodies as described before (14, 15). Vaccinated BALB/c Mice were rendered severe neutropenic (polymorphonuclear leukocyte count, $<500/\text{mm}^3$) 3 days prior to the beginning of the challenge study (Day 0). Immunized and control neutropenic mice were infected i.v. with a lethal dose of live *C. albicans* yeast cells (5×10^4 in 0.1 ml of DPBS). (A) Peptide vaccines Fba and Met6, each was able to induced good protection in neutropenic mice as evidenced by significantly improved survival ($p < 0.01$). (B) In vaccinated group, fungal burdens (colony forming units, CFUs) were significantly reduced in kidneys ($P < 0.001$), a target organ in disseminated candidiasis, in mice challenged with the fungus.

Fig. 4. Passive immunization with MAbs E2-9 and M2-4 enhanced resistance of neutropenic mice to disseminated candidiasis

(A) Neutropenic mice received 0.5ml of either MAb M2-4 or MAb E2-9 (one dose) had significantly prolonged survival as compared to control animals that received DPBS or absorbed MAbs, $p < 0.05$; (B) Consistently, the groups received treatment of either protective MAb (M2-4 and E2-9) had significantly reduced CFUs in kidneys compared with control mice, $p < 0.001$. (C) After challenge, some groups of mice were given the same dose of antibodies (MAb E2-9 or MAb M2-4) as above or control materials on every other day post-challenge for two weeks. The survival of recipients was significantly improved ($p < 0.01$). As compared to one-dose treatment, treatment with MAb M2-4 for two weeks was able to increase protection from 20% to 40%; with MAB E2-9, from 20% to 60%. (D) Groups received MAb treatment for two weeks had significantly reduced CFUs in kidneys as compared with control animals that received DPBS or absorbed MAbs ($p < 0.001$).