A β-glucan-conjugate vaccine and anti-β-glucan antibodies are effective against murine vaginal candidiasis as assessed by a novel in vivo imaging technique

Donatella Pietrella, Anna Rachini, Antonella Torosantucci, Paola Chiani, Alistair J.P. Brown, Francesco Bistoni, Paolo Costantino, Paolo Mosci, Christophe d'Enfert, Rino Rappuoli, Antonio Cassone, Anna Vecchiarelli

ABSTRACT

The protective capacity of a parenterally administered β-glucan-conjugate vaccine formulated with the human-compatible MF59 adjuvant was assessed in a murine model of vaginal candidiasis. To monitor infection, an in vivo imaging technique exploiting genetically engineered, luminescent Candida albicans was adopted, and compared with measurements of colony forming units. The vaccine conferred significant protection, and this was associated with production of serum and vaginal anti-β-glucan IgG antibodies. Vaginal IgG molecules were the likely mediators of protection as inferred by the efficacy of passive transfer of immune vaginal fluid and passive protection by an anti-β-1,3-glucan mAb. Overall, the in vivo imaging technique was more reliable than vaginal CFU counts in assessing the extent and duration of the vaginal infection, and the consequent protection level.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Fungal infections have become increasingly common and represent a growing health problem in patients with weakened immune system [1–3]. In addition, some types of mucosal infection undergo multiple recurrences even in non-immunosuppressed subjects, of which a typical example is the recurrent or chronic vulvovaginal candidiasis (RVVC) [4–8]. It has been calculated that around 5% of women suffering an attack of vulvovaginal candidiasis (VVC) will acquire a chronic infection. Although maintenance chemotherapy regimens are useful to counteract any single recurrence, this infection remains substantially incurable [4.9–13].

Candida albicans is the major agent of VVC and RVVC. This fungus is found as relatively harmless commensal in the vagina of most women, and it is not clear which immunological mechanism(s) inhibits the shift from safe commensalism to vaginal infection. Indeed, there is no clear evidence of the type of immunological deficit that predisposes women to acute infection and, in some cases, to recurrent infection. Any deficit must, however, be local since subjects with RVVC do not show appreciable differences with respect to healthy counterparts in systemic immune responses to the fungus [14–16]. The perturbation of indigenous flora, genetic predisposition, imbalances or polymorphisms of some factors of innate immunity, high susceptibility to neutrophil influx are all thought to predispose to RVVC [15,17–20].

In an estrogen-dependent rat model of vaginal candidiasis, vaginal infection has been shown to be associated with hyphal growth and the expression of virulence factors, among which secretory aspartic proteinases play a major role [21–24]. This indirectly supports the reported relationship between severity of clinical symptoms of vaginal infection in women and amount of a secreted aspartic protease in the vaginal fluid [22]. Moreover, it has been found that both in rat and in mouse models, vaginal infection can be controlled by local or systemic vaccination, by immunotherapy with antibodies against virulence factors or adoptive transfer of various kinds of immune cells [25–33]. All of this raises the possibility that VVC and RVVC in women may be controllable by immunopreventive or immunotherapeutic interventions.

To assess the preclinical efficacy of such interventions, a reliable measure of the local infection is an obvious prerequisite. Valuable results have been obtained by counting vaginal colony forming units (CFU) of C. albicans both in the vaginal secretion and...
in the whole vagina [9,34–36]. However, these methods require repeated, invasive sampling of the vaginal cavity, or sacrifice of a high number of mice. These measurements can also be affected to a remarkable extent by the different growth forms of the fungus, mostly when hyphal mats are formed in the vaginal tissue. A method that allows continuous non-invasive monitoring of fungal growth in vivo in a small number of mice, without the need for frequent sampling and/or animal sacrifice would be very useful. Such a method would increase the sensitivity and reliability of infection monitoring, and would improve measurements of the efficacy of therapeutic and immunological interventions to combat vaginal infection.

In this paper, we have used a recently developed in vivo imaging technique, which exploits a novel cell surface luciferase as reporter gene [37], in order to measure the protection against vaginal infection by C. albicans in mice actively vaccinated with a β-glucan-conjugate vaccine or passive recipients of an anti-β-glucan monoclonal antibody. In addition, and for the first time in this vaccination, a human-compatible adjuvant, the oil-in-water emulsion MF59, was used instead of Freund’s adjuvant, and the immunization was performed by a parenteral, rather than mucosal (vaginal or intranasal) route. The live imaging data were compared with ordinary CFU measurements of the vaginal secretion to assess the degree of correlation between the two methods.

2. Materials and methods

2.1. Vaccine and adjuvant

The β-glucan-conjugate vaccine Laminarin-CRM (Lam-CRM) vaccine and MF59 have been obtained from Novartis Vaccines and Diagnostics, Siena, Italy.

2.2. Microorganisms

C. albicans CA1398 carrying the ACT1p–gLUC59 fusion (C. albicans gLUC59) or C. albicans CA1398 that did not express gLUC59 (control strain) was used in the models of vaginal Candida infections [37]. For experimental infections, cells from stock cultures in YPD agar (1% yeast extract, 2% peptone, 2% glucose, 1.5% agar, all w/v) with 50 µg/ml chloramphenicol were grown in YPD broth (1% yeast extract, 2% peptone, 2% glucose, all w/v) at room temperature for 24 h, then harvested by centrifugation, washed, counted in an hemocytometer, and resuspended to the desired concentration in sterile physiological saline.

2.3. Vaccination protocol

Seven-week-old CD1 female mice (Harlan) were immunized with three doses of the Lam-CRM vaccine (obtained from Novartis Vaccines and Diagnostics), each consisting of 7 µg polysaccharide/0.1 ml PBS. The priming was given subcutaneously in MF59 adjuvant; the boosters were given intraperitoneally 14 and 21 days later, without adjuvant. Control animals were injected with MF59 adjuvant only. Sera and vaginal fluids obtained from vaccinated and control animals were characterized for their composition by ELISA with laminarin as the coating antigen.

2.4. Mouse vaginal infection

CD1 female mice (Harlan), immunized as previously described, were maintained under pseudoestrus condition by subcutaneous injection of 0.2 mg of estradiol valerate in 100 µl of sesame oil (Sigma–Aldrich) 6 days prior to infection and weekly until the completion of the study. Mice anaesthetized with 2.5–3.5 (v/v) isofluorane gas were infected twice at a 24 h interval with 10 µl of 10^8 cell/ml C. albicans gLUC59 or the control strain. Cell suspensions were administered from a mechanical pipette into vaginal lumen, close to the cervix. To favour vaginal contact and adsorption of fungal cells, mice were held head down for 1 min following inoculation. Mice were then allowed to recover for 24–48 h, during which the Candida infection was established.

2.5. Monitoring of mouse vaginal infection

To monitor the infection and the degree of protection, every day post-infection (starting 48 h after challenge) 10 µl (1 mg/ml in 1:4 methanol:H2O) of coelenterazine was added to the vaginal lumen. Afterwards, mice were imaged in the IVIS-200TM imaging system under anaesthesia with 2.5% isoflurane. Total photon emission from vaginal areas within the images (Region Of Interest, ROI) of each mouse was quantified with Living Image® software package. In selected experiments mice were anaesthetized with 2.5% isoflurane and then held head down, the vaginal lumen was thoroughly washed with 150 µl of saline. To determine the fungal load in the vagina the lavage fluids from each mouse were plated on YPD agar plus chloramphenicol, then CFUs were evaluated.

2.6. Passive protection assay

Seven-week-old CD1 female mice (Harlan) were maintained under pseudoestrous condition by subcutaneous injection of 0.2 mg of estradiol valerate in 100 µl of sesame oil 6 days prior to infection and weekly until the completion of the study. Two hours before infection, mice were passively transferred with vaginal fluids from adjuvant plus Lam-CRM-vaccinated or adjuvant treated mice. The lavage fluids (150 µl), recovered at day 42 after the priming of vaccination, were lyophilised and resuspended in 10 µl of endotoxin free water. In parallel experiments mice were treated intravaginally with 100 µg/mouse of anti-β-glucan 2G8 antibody [33,38] or irrelevant IgG2b isotype control antibody (Sigma–Aldrich) prior to infection. Passive protection was evaluated through the monitoring of the infection by the IVIS-200™ imaging system and through the estimation of fungal CFUs in the vaginal lavages at different time points.

2.7. Serological assays

At different time points after vaccination 3 mice for each group of treatment were sacrificed, the vaginal lumen was thoroughly washed with 150 µl saline and blood was recovered from the heart. Immune sera and vaginal fluids were assayed for their reactivity with laminarin (β-glucan) by ELISA as previously described [33]. Immunoglobulin classes of murine anti-β-glucan antibodies were evaluated using alkaline phosphatase-conjugated goat anti-mouse IgA or IgG antibodies (Sigma–Aldrich). Vaginal fluids were considered positive for a determined antibody when their ELISA readings (OD, 405 nm) were at least twofold those from control wells reacted with fluids from mock-vaccinated mouse. Serum titers were defined as the highest dilution of sera producing an OD of 405 nm, at least twofold that obtained from sera of mock-vaccinated animals assayed at the same dilution.

2.8. Histological assessment

For histological assessment, the vagina was removed, fixed in 10% (v/v) formalin for 24 h, dehydrated, embedded in paraffin, sectioned into 3–4-μm-thick sections, and stained with periodic acid–Schiff reagent.
2.9. Statistical analysis

Differences between adjuvant plus Lam-CRM and adjuvant treated mice were evaluated by non-parametric Mann–Whitney U-test. The alpha value was set at 0.05, $P<0.05$ were considered significant.

3. Results

3.1. Performance of the luciferase reporter gene in assessing the vaginal infection by C. albicans and the protective ability of the Lam-CRM vaccine

Recently, a novel reporter gene, PGA59::gLUC also referred to as gLUC59, has been developed for C. albicans [37]. This reporter system takes advantage of the C. albicans PGA59 gene encoding a GPI-linked cell wall protein [39,40] and the naturally secreted G. princeps luciferase [37] in order to permit direct measurement of gene expression through luciferase assays on intact C. albicans cells. Indeed, the Pga59::gLUC protein localized to C. albicans cell surface and was therefore fully accessible to its substrate coelenterazine [37]. This new construct was adapted for in vivo imaging of vaginal infection by C. albicans, and in this particular study we assessed whether this new tool could be effectively used to assess the efficacy of a parenterally administered glycoconjugate vaccine against C. albicans.

Naive mice were injected with adjuvant (MF59) or Lam-CRM conjugate at days 0, 14, and 21. Thirty days after the first dose of vaccine, the animals were intravaginally challenged with an infectious system.
dose of the fungus. The infected mice were monitored continuously in real-time for several days after challenge using the imaging system. The vaginal infection was detected clearly (as emitted total photon flux) in the mice infected with *C. albicans* cells expressing gLUC59, but not in those animals challenged with a control *C. albicans* strain lacking the luciferase gene (Fig. 1A and B).

We then monitored the progress of vaginal infections in animals that were vaccinated with the Lam-CRM vaccine, and compared these infections with those in control animals that were administered adjuvant only (Fig. 1A). Variation was evident between individual animals. Nevertheless, the luminescence signal intensity was significantly lower in the vaccinated mice compared with the control animals. In particular, very low signal was detected in vaccinated animals 11 days after the challenge, whereas luminescence was still detectable in the control animals on day 25 (Fig. 1B). This suggests that vaccinated animals displayed lower levels of infection than unvaccinated control animals (Fig. 1A).

### 3.2. Emitted luminescence, CFU counts and morphology of *C. albicans* in vivo

We then tested whether there was a correlation between the emitted luminescence by gLUC59-expressing *C. albicans* cells and the fungal counts in the vaginal fluid, both in vaccinated and non-vaccinated mice. Our results showed high individual variations in the CFU counts, particularly in the first week of infection, when the counts could differ by >2 logs within the individual animals, independently of the immunization status (Fig. 2, right side). However, the CFU counts were less variable at the later time point (see CFU on day 13, *P* = 0.047). Overall, based on these CFU counts, there was a small but statistically significant difference between the two groups of animals only on day 13 (Fig. 2). This was consistent with the idea that the Lam-CRM vaccine provides a degree of protection against *C. albicans* infection in this model.

The CFU data were compared with the luminescence data. A good correlation between the two methods was noticed at each time point measured, particularly in the vaccinated mice (Fig. 2). Nonetheless, the luminescence metric provided a more uniform and sensitive method in the two groups of animals, as also indicated by the differences between vaccinated animals and controls which were statistically significant on days 11 and 13 when fungal colonization was measured by photon emission, and only on day 13 when measured by CFU (Fig. 2). Again this was consistent with the view that the Lam-CRM vaccine provides some protection against vaginal *C. albicans* infections.

Fungal morphology could significantly affect the accuracy of the CFU measurements as a measure of fungal load because *C. albicans* forms mixed populations of yeast, pseudohyphal and hyphal cells during infections in animals. In contrast the luciferase reporter system generates similar levels of photon emission for yeast and hyphal cells [37]. This issue is particularly important in assessing the efficacy of the vaccine used here in view of the report that the anti-β-glucan antibodies elicited by this glycoconjugate confer protection by affecting hyphal growth and adherence to epithelial cells [38]. For these reasons, we performed a histological examination of the infected vaginal epithelium in vaccinated or non-vaccinated mice (Fig. 3). For each experimental group, three or four low magnification sections were analyzed for the presence of *C. albicans* morphology and morphology of vaginal epithelium. *C. albicans* cells with mixed morphology and many hyphal filaments were present in the vaginal lumen and epithelial cell surface in animals administered with the adjuvant only and challenged with either the luminescent or the control strain. In contrast, the few fungal cells present on the vaginal cell surface of vaccinated mice only displayed yeast-like

![Fig. 2. Measurement of Candida albicans in the vaginal fluids of vaccinated mice.](image-url)

**Fig. 2.** Measurement of *Candida albicans* in the vaginal fluids of vaccinated mice. Mice, previously treated with adjuvant (MF59) or vaccinated with adjuvant plus Lam-CRM conjugate (MF59 + LAM), under pseudoestrus condition, were infected for 2 consecutive days with 10 μl of a 10⁶ cell/ml suspension of *Candida albicans* cells (gLUC) into the vaginal lumen. After 4, 7, 11 and 13 days post-infection 7–8 mice groups were anaesthetized, imaged in the IVIS-200TM imaging system and the vaginal lumen was thoroughly washed with 150 μl of saline using a mechanical pipette. The fungal burden of vaginal lavage fluids was determined by evaluating the colony forming units (CFU) assay. For CFU assay 50 μl of the lavage fluids were diluted and seeded in YPD agar plus chloramphenicol. Results were reported and the statistical significance was evaluated by the non-parametric Mann-Whitney U-test. *P* < 0.05 (MF59 + LAM treated mice versus MF59 treated mice). The correlation between the two methods was also assessed in vaccine recipients by the Pearson’s correlation statistics, and both correlation coefficients and *P* values are shown.
Fig. 3. Histological evaluation of the vaginal tissues of *C. albicans*-infected vaccinated mice. Mice, previously treated with adjuvant (MF59) or vaccinated with adjuvant plus Lam-CRM conjugate (MF59 + LAM), under pseudoestrus condition, were infected for 2 consecutive days with 10 μl of a 10⁶ cell/ml suspension of *Candida albicans* cells (gLUC or control strain) into the vaginal lumen. After 17 days post-infection vagina was recovered and histological analysis was performed. Formalin-fixed tissues were sectioned and stained with periodic acid–Schiff reagent using standard procedures.
organization experiments with the anti-
unimmunized mice. Finally, we performed direct passive immu-
vaginal fluids from immunized mice provide passive protection in
ally with the Lam-CRM vaccine. We tested whether the immune
serum and vaginal fluid of mice which were administered parenter-
between 30 and 40 days (roughly corresponding to the challenge
dilution factor 1:2) in these immunized mice reached a maximum
els of anti-laminarin IgG (serum dilution factor 1:10, vaginal fluid
immunization, but not in adjuvant-only recipient mice. The lev-
the serum and vaginal fluid of Lam-CRM recipients 3 weeks after
it anti-
appearance. Importantly, the architecture of the vaginal epithelium
was almost unaltered in vaccinated animals, whereas in the control
animals, the normal architecture of the vaginal epithelium was
destroyed or significantly altered in about 40% of the vaginal tissue.

3.3. Passive protection conferred by immune vaginal fluids and
anti-β-glucan antibodies

The protection conferred by the Lam-CRM vaccine appears to be
mediated by IgG antibodies, as demonstrated by passive trans-
fer of protection by immune sera and elevated protection by passive
vaccination with an IgG2b with restricted recognition of a β-1,3-
glucan epitope (mAb 2G8; 33,38). In other immunizations with
subunit protein vaccines, vaginal IgA antibodies were also protec-
tive [22,30,41]. Thus, we measured anti-β-glucan IgG and IgA in
serum and vaginal fluid of mice which were administered parenter-
ally with the Lam-CRM vaccine. We tested whether the immune
vaginal fluids from immunized mice provide passive protection in
unimmunized mice. Finally, we performed direct passive immu-
nization experiments with the anti-β-1,3-glucan mAb2G8.

As shown in Fig. 4A and B, anti-laminarin IgG was found both in
the serum and vaginal fluid of Lam-CRM recipients 3 weeks after
immunization, but not in adjuvant-only recipient mice. The lev-
els of anti-laminarin IgG (serum dilution factor 1:10, vaginal fluid
dilution factor 1:2) in these immunized mice reached a maximum
between 30 and 40 days (roughly corresponding to the challenge
period) and persisted for a further 3 weeks. No anti-β-glucan IgA
was found in the serum (data not shown) or vaginal fluid of these
animals at any time post-immunization (Fig. 4C).

The passive transfer of vaginal fluid obtained by the vaginal
lavage from mice immunized with the Lam-CRM vaccine signifi-
cantly reduced photon emission in the first week after transfer,
and helped the resolution of infection in 2 out of 3 mice (day 9) as
compared to the luminescence emitted by the animals transferred
with the vaginal fluid of adjuvant-only administered mice (Fig. 5).

We then tested the impact of passive immunization with the
anti-β-glucan mAb2G8. In an experiment with a similar format as
above, 3 mice were passively immunized with this mono-
clonal antibody intravaginally, while 3 other mice received an
isotype-matched IgG2b control. As shown in Fig. 6, the lumines-
cence emission from the vaginas of the animals recipients of the
mAb2G8 was significantly lower than that emitted by the control
mice, demonstrating protection by the locally administered anti-
β-glucan antibody.

4. Discussion

In a preliminary and limited investigation using the rat model
of vaginal infection, it was found that a glycoconjugate vaccine
consisting of a common and highly conserved fungal polysaccha-
ride, β-glucan, plus the diphtheria toxoid CRM197 as a protein
carrier, could confer protection against vaginal infection by C. albi-
cans [33]. In that study, besides the use of ovariectomized rats
rather than mice, as in the present study, the vaccine was adminis-
tered intravaginally. Freund’s oil mixture (a human-incompatible
adjuvant) was used as the adjuvant and the protection was mea-
sured by classical CFU counts. In other experiments with a mouse
model of vaginal infection [9,42–44], quantitative assessment of
infection by C. albicans under immunomodulating conditions has
been performed by sacrificing animals, dissecting their vaginas and
measuring total vaginal CFU counts.

In the present study, we exploited the recently demonstrated
capacity to monitor vaginal infection in conservative, non-invasive,
animal-sparing and non-sacrificing conditions by using a C. albi-
cans strain that bears a new luciferase gene expression system
and measuring photon emissions from live mice. This has allowed
us to re-assess the protective ability of the Lam-CRM vaccine and
its mechanism of protection, which has been putatively attributed
to anti-β-glucan antibodies [33]. Importantly, we used here the
human-compatible adjuvant MF59 instead of Freund’s adjuvant.
In addition, we tested whether protection against vaginal infection
could be achieved by parenteral rather than local administration
of the vaccine, with the supposition that both systemic and mucosal
antibodies would be induced, thereby achieving a stronger and
more persistent protection.

Using this approach, we made the following original obser-
vations. Firstly, the use of ordinary CFU counts to assess vaginal
infection in mice by sampling the vaginal cavity was a relatively
insensitive measure of the true fungus growth (Fig. 2). In contrast,
luminescence emission gave relatively consistent and reproducible
measurements of Candida growth in the vaginal cavity. Accord-
ingly, the protection offered by the Lam-CRM vaccine could be more
easily established based on luminescence emission measurements
(Figs. 2 and 5). Secondly, vaccine protection could be achieved at
the vaginal level following parenteral immunization (Fig. 1).
Thirdly, protection was associated with the presence of specific
anti-β-glucan IgG antibodies at the vaginal level and in serum, with
the level of protection being time-dependent with rising levels of
IgG production in the vaginal fluid (Fig. 4). Fourthly, protection
was probably mediated by these antibodies as shown by passive
transfer of immune vaginal fluid and passive vaccination with an
Fig. 5. Course of the experimental vaginal candidiasis in mice administered with vaginal fluids of vaccinated animal. CD1 mice were inoculated intravaginally with 10 μl of concentrated lavage fluids of mice treated with adjuvant or vaccinated with adjuvant plus Lam-CRM conjugate (adjuvant + LAM) before intravaginal infection with Candida albicans cells (gLUC). After 2, 5 and 9 days mice were treated intravaginally with 10 μg of coelenterazine and imaged in the IVIS-200™ imaging system under anaesthesia with 2.5% isoflurane. Line plot shows the total photon emission from the infected regions of mice reported in the figure. Results are expressed as mean values of photons (n = 3). Statistical significance is as indicated in each panel. NS, not significant.

Fig. 6. Kinetics of the experimental vaginal candidiasis in mice administered with a murine anti-β-glucan 2G8. CD1 mice were inoculated intravaginally with 100 μg of murine anti-β-glucan 2G8 or isotype control antibody IgG2b 24 h before intravaginal infection with Candida albicans cells (gLUC). After 2, 5 and 9 days mice were treated intravaginally with 10 μg of coelenterazine and imaged in the IVIS-200™ imaging system under anaesthesia with 2.5% isoflurane. Line plot shows the total photon emission from the infected regions of mice reported in the figure. Results are expressed as mean values of photons (n = 3). Statistical significance is as indicated in each panel. NS, not significant.

anti-β-glucan monoclonal antibody (Figs. 5 and 6). These findings markedly extend what has been reported previously in a quite different model of vaginal infection, which was made in rats using a different vaccine formulation and a different route of immunization [33].

Protective immunity against C. albicans at the mucosal level in humans has long been attributed to cell-mediated immunity. Following this line of thought, a protective response against C. albicans has been obtained by using dendritic cells pulsed with fungal antigens, or with fungal DNA, or by transferring Candida antigen-activated vaginal CD4+ T cells or dendritic cells from vaccinated to naive animals [32,43–45]. However, protection against mucosal infection by C. albicans has also been achieved by using antibodies against specific cell wall or secretory components of the fungus [22,28,38,46]. In particular, it was previously shown in a rat model of Candida vaginal infection that vaginal antibodies in animals locally immunized with a secretory aspartyl protease enzyme or a glycoconjugate vaccine, or locally administered monoclonal antibodies against these components, could exert some protection [22,27,33,38]. Interestingly, the protection in this rat model could
be shown by CFU measurements in samples of vaginal fluid from rat vaginal cavity, a method of measurement which has proven rather inconsistent, because of low sensitivity and with large variability, in the mouse model of vaginal infection studied here. Presumably this variability arose at least partly as a result of the morphological variation of the fungal cells infecting the vaginal cavity, both free in the vaginal fluid and adherent to squamous cells of the vaginal epithelium (Fig. 3). Others have used measurements of total Candida counts in dissected, whole vaginas [34,41,42,44]. However, to gain reliable results they had to analyze and sacrifice relatively large numbers of mice. This could be avoided with the technology used in this study because gLUC59 expression levels are similar in yeast and hyphal cells [37].

The data in this paper confirm that passive vaccination with anti-β-glucan monoclonal antibody can be used to combat Candida infections in the vaginal cavity. It has been shown recently that this antibody (mAb2G8) recognizes a linear epta- or octa-β-1,3-glucan epitope [38] which is present in GPI cell wall proteins such as AlS3 and Hylr1 which are thought to play roles in hyphal growth, invasion and adherence [47–49]. Thus, it is conceivable that the vaginal anti-laminarin IgG raised by the Lam-CRM vaccine systemically administered in this study exerts a similar protective ability. However, these antibodies could also exert local protection by mechanisms requiring host cell intervention such as for instance the opsonization of yeast cells and/or by enhancing extrahyphal killing by polymorphonucleocytes or other phagocytes. In fact, polymorphonuclear cells are recruited into the vaginal cavity of Candida-infected mice [15] which was detected in our model (data not shown), and this might have contribute significantly to the pathology of vaginal infection.

An additional novelty of this paper is the use of a human-compatible adjuvant, the MF59, to raise anti-Candida protective antibodies. This adjuvant is an oil-in-water emulsion, comprising Candida compatible adjuvant, the MF59, to raise antibodies. This adjuvant is an oil-in-water emulsion, comprising Candida compatible adjuvant, the MF59, to raise anti-

References


