The C-terminal antibody binding domain of Candida albicans mp58 represents a protective epitope during candidiasis

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Abstract

The 58-kDa surface mannoprotein of Candida albicans (mp58) elicits strong antibody responses during infection. Epitope mapping with sera from patients with candidiasis and control individuals indicated the presence of multiple IgG-reactive continuous epitopes on the protein, expanding both the amino- and carboxy-terminal domains and several internal regions. These immunoreactive regions were similar to the ones previously identified using sera from immunized animals. Two of the epitopic regions (including the C-terminal domain) showed increased reactivity with antibodies present in sera from patients with candidiasis as compared to control individuals. Patients who survived the infection displayed increased antibody reactivity towards the C-terminal epitope as compared to those succumbing to candidiasis. A monoclonal antibody directed towards this epitopic region conferred protection in serum therapy experiments in a murine model of hematogenously disseminated candidiasis. Together, these observations indicate the carboxy-terminal antibody binding domain of Candida albicans mp58 represents a protective epitope during candidiasis.

Keywords: Candida albicans; Protective epitope; Monoclonal antibody

1. Introduction

Candida albicans is a dimorphic fungus that can be either commensal or an opportunistic pathogen with the ability to cause a variety of infections, ranging from superficial to life-threatening. Predisposing factors for C. albicans infections include immunosuppressive therapy, antibiotic therapy, use of indwelling devices and intravenous catheters, HIV infection, diabetes and old age. Nosocomial infections due to C. albicans are becoming increasingly important [1,2]. The most recent surveys have shown yeast to be the third or fourth most commonly isolated bloodstream pathogen from US hospitals. The lack of an early and accurate diagnostic procedure, the limited arsenal of therapeutic weapons to combat candidiasis, and the emergence of resistant strains due to empirical prophylactic treatment are responsible for the high morbidity and mortality rates associated with infections caused by C. albicans.

As the outermost part of the cell, the cell wall of C. albicans is the structure that mediates most host–fungal interactions [3]. The cell wall of C. albicans is a significant source of antigens that elicit both cellular and humoral immunity [4]. Although innate defenses and cell-mediated immunity play preponderant roles in protection against candidiasis, there is now convincing evidence that humoral immunity can modify the course of active infection to benefit the host [4–8]. For example, different groups of investigators have described protective antibodies against C. albicans, including those with specificity for mannans, hsp90, enolase, an immunosuppressive protein and anti-idiotype antibodies with yeast killer toxin activity [9–14].

Our group has identified 58-kDa mannoprotein (mp58) in the cell wall (surface) of C. albicans and experiments in our laboratory highlighted the important immunobiologi-
2.2. Epitope mapping

Analysis of continuous B-cell epitopes on *C. albicans* mp58 was carried out by means of the multipin peptide technology (PepScan) as previously described [19]. Briefly, the deduced amino acid sequence of the protein portion of mp58 was used to synthesize a complete set of overlapping dodecapeptides (overlap 7, offset 5) covalently attached to the surface of derivatized polyethylene pins in a format compatible with standard enzyme-linked immunosorbent assays (ELISA). And the reactivity of different preparations containing anti-mp58 antibodies (serum samples from individuals and monoclonal antibodies, see below) with the pin-bound peptides was detected by a modified enzyme immunoassay. A second Pepset was constructed that included both a ‘window net’ and a ‘replacement net’ synthesis to further analyze the epitope recognized by monoclonal (mAb) antibody 3H3 (see below) within the C-terminal domain of the protein. The ‘window net’ consists of synthesizing all of the shorter overlapping sequences covering an identified antibody binding peptide, which in this particular case were all 4-mers, 5-mers, 6-mers, and so on. The ‘replacement net’ consists of synthesizing peptide analogs with single amino acid substitutions in this mimetic sequence in a larger number of human serum samples. In its final format the keyhole limpet hemocyanin (KLH)-conjugated peptide was used to coat Immulon 2 plates in a carbonate buffer (pH 9.6) at a concentration of 1 μg per well. Then the different serum samples from patients with candidiasis and controls (1:1000 dilution in phosphate-buffered saline with 0.05% Tween 20 (PBST) and 1% bovine serum albumin (BSA)) were added to the wells and incubated for 1 h at 37°C. After washings the plates were incubated with peroxidase-conjugated goat anti-human IgG (1:2000 dilution in PBST with 1% BSA) for 1 h at 37°C. After washings, the plates were developed (o-phenylenediamine in citrate buffer) and read at 490 nm in a microtiter plate reader.

2.4. Generation of mAb 3H3

All animal experiments were performed in accordance with Institutional regulations in an AAALAC-certified facility at UTHSCSA. Electrophoretically purified *C. albicans* mp58 (25 μg) [19] was used to immunize two (BALB/c) mice. Mice were killed, their spleens removed aseptically and antibody-secreting cells isolated and mixed with myeloma cells (NS1). After the fusion, cells were diluted in selective medium and plated at low densities in multiwell tissue culture dishes. Hybridoma cell lines secreting antibodies against *C. albicans* mp58 were screened by ELISA and single-cell subcloned. Among these, a hybridoma cell line producing a mAb designated 3H3 (an IgG1 with κ light chain as determined by an isotyping kit, Zymed) was established. Ascites was generated in BALB/c mice given i.p. injections of mAb 3H3 hybridomas.

2.5. Passive transfer of protection experiments with mAb 3H3

Female BALB/c mice, 6–7 weeks old, were housed in groups in biolean hoods and provided with water and food ad lib. Six mice were used for each experimental group. mAb 3H3 (approximately 1.8 mg of IgG) was administered i.p. as ascites 2 h before infection. Control animals received the same volume of saline. All mice received an i.v. challenge with 1 × 10⁶ cells of *C. albicans* clinical strain 412 [20] grown overnight at 24°C in Lee medium [21].

3. Results

3.1. Epitope mapping with antibodies in sera from candidiasis patients

PepScan analysis was carried out to identify continuous epitopes recognized by IgG antibodies present in serum samples from patients with systemic candidiasis (*n* = 6) and control patients (*n* = 5). As shown in Fig. 1A, the
different antisera were capable of recognizing multiple dodecapeptides, thus indicating a complex polyclonal response in humans to the protein moiety of mp58. Results indicated that the IgG-reactive continuous epitopes fell into multiple regions of the protein that included both the amino- and carboxy-terminal domains and several internal regions which were similar to the reactive regions identified before using sera from immunized animals [19]. Overall levels of reactivity were similar for both sera from patients and the control population. However, two dodecapeptides exhibited increased reactivity with serum from patients with disseminated candidiasis as compared to serum samples from control individuals (Fig. 1A, asterisks). These included the C-terminal dodecapeptide 288HCHTHADGEVHC299 and the internal epitope 221YYALDVAYDVT232.

3.2. Synthetic peptide-based capture ELISA to study antibody responses during candidiasis

To further investigate the potential relevance of the epitopic region at the C-terminus of the protein a 10-mer synthetic peptide encompassing the last 10 amino acid residues at the C-terminus of the mp58 sequence (the 290HTHADGEVHC299 epitope [19] plus the C-terminal cysteine residue which is already present in the native protein and served as a spacer arm for conjugation to the carrier protein) was synthesized. This decapeptide was coupled to KLH and used in an antibody capture ELISA to further investigate antibody reactivity of a larger number of serum samples from patients with disseminated candidiasis (n = 13) and control individuals (n = 22). These experiments confirmed the increased levels of antibodies against
this peptide in serum samples from patients with disseminated candidiasis as compared to control individuals (Fig. 1B). Moreover, levels of anti-peptide antibodies were significantly higher in surviving patients compared to those with fatal outcomes (Fig. 1C), thus indicating that this peptide may represent a protective epitope during candidiasis.

### 3.3. Characterization of the mAb 3H3 and assessment of its protective effects

We established a hybridoma cell line producing a mAb designated 3H3. Immunoblotting experiments indicated that mAb 3H3 recognized mp58 with high specificity among other materials present in the cell wall extracts of

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C. albicans (not shown). When used in PepScan analysis with the dodecapeptide set, reactivity of mAb 3H3 was limited to the peptide corresponding to the C-terminal region of the amino acid sequence (Fig. 2A). Delineation of the specific boundaries of the epitope recognized by mAb 3H3 was performed by a window-net analysis, which identified the nonapeptide 290HTHADGEVH298 as the minimal region that retained most of the mAb 3H3 binding activity, whereas a pronounced decrease in binding was already observed for all derived octapeptides (Fig. 2B). The contribution of individual amino acid residues to antibody binding was analyzed by a replacement-net approach, which revealed the prominent role of all three histidine residues in the recognition by mAb 3H3 (Fig. 2C).

The protective effects of mAb 3H3 were assessed in a murine model of disseminated candidiasis. mAb 3H3 ascites was passively administered prior to infection with a lethal i.v. challenge of C. albicans and animals were monitored for survival. Results indicated that passive administration of mAb 3H3 significantly increased survival (P = 0.0048 by Kaplan–Meier test) compared to a control group receiving saline (Fig. 2D).

4. Discussion

Epitope mapping with antibodies present in sera from patients with candidiasis indicated the complexity of the polyclonal IgG response to C. albicans mp58. The immunoreactive regions were similar to those detected with animal sera. C. albicans is a commensal and anti-C. albicans antibodies circulate in the serum from most individuals in the absence of active infection [4]. Thus, it was not surprising that the same antibody binding regions were detected when serum samples from a control population were used in epitope scanning experiments. However, two individual dodecapeptides, one of them corresponding to the C-terminal domain of the protein, exhibited increased reactivity with sera from patients with candidiasis compared to control individuals (Fig. 1A).

There have been numerous attempts to develop serodiagnostic tests for candidiasis; however, to date no such test has been successfully established [22]. Clearly, the use of synthetic peptides may provide a reliable alternative for the development of such tests and possibly circumvent some of the inherent problems encountered in the past. Thus, based on results of the epitope mapping analysis, a synthetic peptide corresponding to the C-terminal epitope of mp58 was used in an antibody capture ELISA to further investigate the antibody responses to this particular epitopic region with a larger number of samples. Results confirmed the increased antibody recognition of this epitope by sera from patients with candidiasis as compared to control individuals (Fig. 1B). However, the diagnostic value of this particular technique was still low since there was a great degree of overlap in the reactivities observed for patient and control populations. As a result of this overlap a cut-off value could not be established to provide both good sensitivity and specificity. Another important observation was made when results of the synthetic-based antibody capture ELISA were compared according to outcome of infection: sera from patients who survived the infection displayed significantly higher levels of reactivity as compared to those succumbing to candidiasis (Fig. 1C). Together these results seemed to indicate that the C-terminal epitope of C. albicans mp58 may not have diagnostic value but rather represents a protective epitope during candidiasis. Importantly, levels of reactivity against C. albicans cytosolic and cell wall extracts of antibodies in sera from patients with good or fatal outcome were similar (not shown), thus indicating that higher levels of reactivity against the C-terminal peptide are not due to overall higher antibody levels in survivors versus those who succumbed to the infection, further supporting the hypothesis that this epitope represents a specific protective epitope during candidiasis. Two major limitations of these analyses were the use of a relatively small number of serum samples and the heterogeneity of the patient population with candidiasis. Current experiments in the laboratory are aimed at validating these results by evaluating antibody responses in a large cohort of patients with candidiasis.

Passive transfer of protection experiments in a murine model of hematogenously disseminated candidiasis provided further evidence supporting the protective nature of the C-terminal epitope of C. albicans mp58. mAb 3H3 recognizing this epitope was protective when administered to animals as a single dose prior to infection. The mechanisms by which this mAb exerts its protective action are currently under investigation. These observations add to a growing body of convincing evidence that humoral immunity contributes to host defense against disseminated disease with C. albicans [5–8].

In conclusion, the C-terminal B-cell epitope of C. albicans mp58 that was previously identified by our group in epitope mapping experiments in vitro is also immunodominant during C. albicans infection in vivo. Increased antibody levels against this epitope correlate with protection in patients with C. albicans infection. A mAb with specificity towards this epitope was protective in serum therapy experiments in a murine model of candidiasis. Together, these data indicate that the C-terminal antibody binding domain of C. albicans mp58 represents a protective epitope during candidiasis.

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