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Characteristics of Fc-independent human antimannan antibody-mediated alternative pathway initiation of C3 deposition to *Candida albicans*

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ABSTRACT

The complement system has an important role in host resistance to systemic candidiasis but regulation of complement activation by *Candida albicans* remains poorly defined. Previous studies have identified a requirement for naturally occurring antimannan IgG antibody in initiation of C3 opsonization of *C. albicans* through either the classical or alternative pathway. This study characterized antibody-dependent initiation of the alternative pathway using the recombinant human monoclonal antimannan Fab fragment M1 and its full-length IgG1 antibody M1g1. Kinetic analysis of C3b deposition onto *C. albicans* with flow cytometry demonstrated the ability of M1g1 to restore the activity of either the classical or alternative pathway to the yeast-absorbed normal human serum, but the Fc-free M1 Fab restored only the activity of the alternative pathway. This Fc-independent, antimannan Fab-mediated C3 deposition through the alternative pathway was also observed in a serum-free assay containing the six alternative pathway proteins and in C1q- or C2-depleted serum but not in factor B-depleted serum. M1- or M1g1-dependent alternative pathway initiation of C3b deposition occurred in an asynchronous manner at discrete sites that expanded to cover the entire cell surface over time as revealed with immunofluorescence microscopy, in contrast to a uniform appearance of initial C3 deposition through the classical pathway. Furthermore, antimannan Fab M1 promoted the assembly of the alternative pathway convertase on the cell surface seen as colocalization of C3 and factor B with immunofluorescence microscopy. Thus, human antimannan antibody has a distinct Fc-independent effector function in regulation of C3 deposition to *C. albicans*.

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1. Introduction

Candida albicans is an opportunistic yeast-like pathogen and may cause life-threatening hematogenously disseminated candidiasis. A critical role for the complement system has been demonstrated in host resistance to *Candida* infections. Chemically induced deficiency in C3 (Gelfand et al., 1978), congenital deficiency in C5 (Hector et al., 1982; Lyon et al., 1986), or genetically induced deficiency in C3 (Han et al., 2001) produces a significant increase in susceptibility to candidiasis in experimental animals. In addition, administration of human mannan-binding lectin required for lectin pathway-mediated complement activation enhances the resistance of mice to hematogenously disseminated candidiasis (Lillegard et al., 2006), whereas blockage of initiation of complement activation in mice deficient in mannan binding lectin A/C or in factor B and C2 is associated with reduced resistance to systemic candidiasis (Held et al., 2008). Furthermore, studies with the mouse model of hematogenously disseminated candidiasis showed that protection by a murine antimannan IgM antibody or its IgG3 variant requires an intact complement system (Han et al., 2001). However, regulation of complement activation by *C. albicans* has not been well understood.

The cell surface of *C. albicans* is naturally resistant to complement opsonization (Kozel et al., 1996; Zhang et al., 1997; Zhang and Kozel, 1998). Previous studies have established a requirement for antimannan antibody in initiation of C3b deposition onto the cell surface of *C. albicans*. Absorption of normal human serum with either *C. albicans* yeast cells (Kozel et al., 1996; Zhang et al., 1997) or immobilized chemically purified *Candida* mannan essentially abolished the serum complement activity (Zhang et al., 1997; Zhang and Kozel, 1998). Addition of affinity-purified naturally occurring polyclonal antimannan IgG antibody restored classical pathway activity to the absorbed serum (Zhang et al., 1997). Furthermore, antimannan antibody was found to initiate the alternative pathway under

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conditions where normal human serum was rendered free of Ca^{2+} with EGTA chelation to inhibit classical pathway initiation or where the alternative pathway was reconstituted from the six alternative pathway proteins (Zhang and Kozel, 1998). The requirement for antimannan antibody in complement activation by *C. albicans* is further supported by studies that revealed a significant correlation between the amounts of naturally occurring antimannan antibody in individual sera and the ability of the sera to initiate either the classical (Kozel et al., 2004; Zhang et al., 1997) or the alternative pathway (Kozel et al., 2004). Thus, antimannan IgG antibody modulates complement opsonization of *C. albicans* through both the classical and alternative pathways.

The conventional view is that initiation of the classical complement pathway begins with the attachment of C1q to the Fc region of antibody–antigen complex and thus is antibody-dependent. In contrast, initiation of the alternative complement pathway is typically independent of antibody. The influence of antibody on alternative pathway activities has not been well understood. While antimannan antibody is required for alternative pathway activation by *C. albicans* as described above, some anti-capsular antibodies can suppress alternative pathway-mediated C3 opsonization of encapsulated *Cryptococcus neoformans* (Kozel et al., 1998). These opposing effects of the adaptive immunity on the alternative pathway of complement activation may influence host innate resistance to fungal infections. Our ability to dissect the molecular mechanisms of antimannan antibody-mediated activation of the alternative pathway by *C. albicans* has been limited by the polyclonal nature of naturally occurring antimannan antibody.

Our earlier work generated a monoclonal human recombinant antimannan Fab fragment known as M1 and converted it to a full-length IgG1 antibody designated as M1g1 (Zhang et al., 2006). M1g1 was found to activate the mouse complement system and to enhance the resistance of mice to systemic candidiasis (Zhang et al., 2006). M1g1 and M1 are identical in the epitope specificity but differ in the presence of the Fc region of IgG1 (Zhang et al., 2006). They were utilized in the present study to characterize the patterns of antimannan antibody-mediated alternative pathway initiation of C3 opsonization of *C. albicans*. We found that (i) the Fc-free antimannan Fab fragment M1 is unable to activate the classical pathway but is necessary and sufficient for alternative pathway initiation, (ii) M1-dependent alternative pathway initiation of C3b deposition occurs in an asynchronous manner at discrete sites that expand rapidly to the entire cell surface, and (iii) M1 promotes the formation of the alternative pathway C3 convertase on the cell surface. These results establish an Fc-independent effector function for human antimannan IgG antibody that modulates activities of the complement system.

2. Methods

2.1. Preparation of yeast cells

Yeast cells of *C. albicans* 3153A were used for all experiments and prepared as described with minor modifications (Zhang et al., 1997). Briefly, yeast cells were passaged daily for three times at 37 °C in 3 ml broth containing 2% glucose, 1% peptone, 0.3% yeast extract and then used to initiate a large overnight broth culture. Yeast cells from the large culture were inactivated by 1 h treatment with 1% formaldehyde at room temperature, harvested by centrifugation, washed, resuspended in PBS (pH 7.2, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, 154 mM NaCl), and stored in aliquots at −80 °C. No discernible difference between live and formaldehyde-treated cells was observed in C3 activation and binding in a previous study (Kozel et al., 1987) or in the binding of M1g1 or M1 in preliminary experiments of the current study.

2.2. Human recombinant antimannan Fab fragment (M1) and full-length IgG1 antibody (M1g1)

The gene construct of M1g1 contains the DNA sequences for the light chain (V_L–C_L) and the heavy chain (V_H–C_H1) of M1 and the DNA sequence for the C_H2–C_H3 of IgG1 (Zhang et al., 2006). Therefore, M1g1 and M1 are expected to have the same epitope specificity but only M1g1 has the Fc region. The IgG1 subclass specificity was chosen for the recombinant antibody because IgG is the main isotype for naturally occurring antimannan antibody (Jones, 1980; Kozel et al., 2004) and our analysis of normal human sera found IgG1 to be a common subclass. Soluble M1g1 was produced in a Chinese hamster ovarian cell line and affinity-purified (Zhang et al., 2006); the concentration was estimated by absorbance at 280 nm with a molar absorption coefficient adjusted for the amino acid composition of M1g1 (Pace et al., 1995). For this project, the M1 gene construct was modified to include a six-histidine tag at the C-terminus of the heavy chain gene using QuikChange XL Site-Directed Mutagenesis (Stratagene, La Jolla, CA) and subsequently cloned into pET28b (EMD Biosciences, Madison, WI) at the sites of *EcoR*I and *Not*I. The light chain gene in pET28b was oriented in frame behind T7 promoter by removal of extraneous N-terminal DNA (Nadkarni et al., 2007) with QuikChange II XL Site-Directed Mutagenesis (Stratagene, La Jolla, CA). The resulting plasmid construct pET28b-M1 was transformed into Rosetta 2(DE3) bacteria for production of soluble M1 with Overnight Express Autoinduction System 1 (EMD Biosciences, Madison, WI). Soluble M1 was collected by centrifugation and concentrated with Pellicon XL tangential flow filtration system (Millipore, Billerica, MA). M1 Fab was captured on a HisTrap FF column with AKTA chromatography (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and eluted with 150 mM NaCl, 20 mM phosphate, 0.5 M imidazole, pH 7.5. Purified M1 was dialyzed against PBS, passed through a 0.45-μm filter, and stored at −20 °C. The concentration of purified M1 Fab was determined by absorbance at 280 nm with an adjusted molar absorption coefficient (Pace et al., 1995). The purity and structural integrity of M1 Fab were confirmed with sodium dodecyl sulphate-polyacrylamide gel electrophoresis under reducing and non-reducing conditions and Coomassie Blue staining; the identity was confirmed with Western blot using goat anti-human κ chain antibody (SouthernBiotech, Birmingham, AL). The binding specificity of M1 Fab for the mannan of *C. albicans* 3153A was verified with ELISA and HRP-conjugated goat anti-human κ antibody (SouthernBiotech, Birmingham, AL). The pattern of M1 binding to the cell surface was visualized with FITC-conjugated goat anti-human κ (SouthernBiotech, Birmingham, AL) and immunofluorescence microscopy by a digital imaging system described below. His-tagged M1 was found qualitatively and quantitatively similar to non-tagged M1 in binding to *C. albicans* yeast cells or in the pattern of M1-mediated alternative pathway of complement activation by analysis with immunofluorescence microscopy and flow cytometry.

2.3. Kinetic analysis of C3 deposition onto the cell surface by flow cytometry

Three different sources of complement were utilized to analyze the influence of M1g1 or M1 Fab on the activity of the classical or alternative pathway in initiation of C3b deposition to the surface of *C. albicans* yeast cells: pooled normal human serum (NHS), an alternative pathway reconstituted from the six alternative pathway proteins, and normal human serum depleted of C1q, C2 or factor B. Complement activity was measured in a Veronal-buffered saline (5 mM sodium Veronal, 142 mM NaCl, pH 7.3) containing 0.1% gelatin (GVB).

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