Molecular Immunology 45 (2008) 3934–3941

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Increased susceptibility of complement factor B/C2 double knockout mice and mannan-binding lectin knockout mice to systemic infection with *Candida albicans*

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ARTICLE INFO

Article history:
Received 22 April 2008
Received in revised form 16 June 2008
Accepted 19 June 2008
Available online 30 July 2008

Keywords:
Complement
Candida
Infection
Opsonophagocytosis

ABSTRACT

Candida albicans is the major cause of systemic fungal infections in immunocompromised patients. We investigated the susceptibility of mice deficient in complement factor B and C2 (Bf/C2$^{-/-}$), C1q (C1qa$^{-/-}$), and mannan-binding lectin (MBL)-A (MBL-A) and MBL-C (MBL-A/C$^{-/-}$) to systemic infection with *C. albicans*. Animals were infected i.p. with 10^8 *C. albicans* blastoconidia and monitored for mortality. Bf/C2$^{-/-}$ mice showed high mortality (over 90%) within the study period of 3 weeks. In contrast, mortality in C1qa$^{-/-}$ mice was below 15% whereas that of MBL-A/C$^{-/-}$ mice was 40% ($P < 0.001$). Intravenous infection of mice with 8×10^5 blastoconidia resulted in the same trend with Bf/C2$^{-/-}$ mice being highly susceptible compared to the other strains. Histology of kidney sections of infected Bf/C2$^{-/-}$ mice showed widespread mycelia confirming the high CFU counts from cultured tissue homogenates. In C1qa$^{-/-}$, MBL-A/C$^{-/-}$ and wild type C57BL/6 mice hyphal growth was limited. However, massive inflammatory infiltration was apparent, which was not seen in Bf/C2$^{-/-}$ mice. The ability of the mouse sera to opsonize *C. albicans* was determined by quantification of phagocytosis of *C. albicans* by peritoneal phagocytes. Whilst phagocytosis mediated by Bf/C2$^{-/-}$ mouse serum was low (10.6%), more phagocytosis could be seen in MBL-A/C$^{-/-}$ (19.9%), C1qa$^{-/-}$ mice (23.9%) and wild type mice (29%). Deficiency of classical pathway activation has only a low impact whereas the lectin pathway contributes to the host defence against candidosis. The more pronounced lack of complement activation in Bf/C2$^{-/-}$ mice leads to uncontrolled infection due to an opsonophagocytic defect.

1. Introduction

Candida albicans is a common constituent of the normal flora of the oropharyx and the gastrointestinal tract of healthy individuals and of the female lower genital tract. However, under predisposing conditions this colonization may lead to mucocutaneous and also invasive infection. *C. albicans* is the most frequent cause of systemic fungal infections in immunocompromised patients, especially in patients with granulocytopenia (Richardson, 2005). The phagocytic system, including neutrophilic granulocytes and monocytes/macrophages, plays an important role in the host defense mechanism against *C. albicans* infections. Phagocytosis can be enhanced substantially by opsonization with either immunoglobulin (IgG) or complement activation products (C3b) (Romani, 2002). The complement system may be activated via three pathways, i.e., the classical, the alternative, and the lectin pathway. The classical pathway is initiated by the C1q molecule which binds to IgM and IgG bearing immune complexes. Thus it bridges the innate and the adaptive immunity. In addition, antibody-independent binding of C1q to polyanions such as DNA, RNA, and lipopolysaccharide of Gram-negative bacteria has been shown (Loos and Clas, 1987; Petry and Loos, 1998). The alternative pathway is initiated by covalent binding of an activation product, C3b, of the third complement of component to a surface, e.g., a pathogen. Due to unbalanced control of the system on the surface of many microorganisms this leads to the deposition of more complement factors. The third pathway, the lectin pathway, is activated by binding of the mannan-binding lectin (MBL) (or members of the ficolin family) to microbes with a fitting pathogen associated molecular pattern (PAMP); in the case of MBL this is a pattern of carbohydrate structures (Thiel, 2007). Deficiencies of complement components may result in an increased susceptibility to infection. MBL deficiency in particular is associated with early childhood infections or serious infections following chemotherapy treatments (Turner, 2003). *C. albicans* can activate all three pathways of the complement system leading to opsonization of the yeast cells, enhanced complement receptor mediated phagocytosis and infiltration and activation of neutrophils (Kozel, 1996). Using experimental models the role of the complement system in

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doi:10.1016/j.molimm.2008.06.021

the host defense against candidiasis has been clearly demonstrated. Guinea pigs that had been made complement C3 deficient by cobra venom factor injections show higher susceptibility to lethal infection than untreated controls. In contrast, C4 deficient guinea pigs are as resistant to infection as wild type animals, suggesting that the classical pathway of complement activation has no influence on the systemic infection (Gelfand et al., 1978). Mice congenitally deficient in C5, such as DBA/2 or A/J, are highly susceptible to *C. albicans* infection (Hector et al., 1982; Ashman et al., 1996). This indicates that the C5a mediated chemotaxis and activation of neutrophils play an important role in the inflammatory response to organ infection. Also, C5 deficiency prevents the formation of the membrane attack complex C5b-nC9. A role of the terminal complement components for the immune defense against *Candida* has been suggested (Triebel et al., 2003). *C. albicans* has evolved mechanisms of immune evasion including proteins that bind the complement regulators C4 binding protein, factor H and FHL-1 (Meri et al., 2002, 2004). These are co-factors of the serine protease factor I which in a normal situation reduces the formation of C3 convertase and an increased number of such regulators on the surface may thus lead to less complement deposition.

The aim of this study was to investigate the susceptibility to *C. albicans* infection in different complement deficient mouse strains. We compared mice with targeted disruption of the C1qa gene ($C1q^{-/-}$), a MBL-A and MBL-C double knockout mouse (MBL-$A/C^{-/-}$) and a strain that lacks both factor B and C2 ($Bf/C2^{-/-}$). All strains of mice had the genetic background of the C57BL/6 mouse and could therefore be compared directly with no confounding factors other than the different complement defects, in contrast to several of the animal studies mentioned above.

2. Materials and methods

2.1. Mice

Three complement deficient mouse strains MBL-$A/C^{-/-}$ (Shi et al., 2004), $C1qa^{-/-}$ (Botto et al., 1998) and $Bf/C2^{-/-}$ (Taylor et al., 1998) on the C57BL/6 genetic background and the wild type strain were used to assess the role of the complement system in systemic *C. albicans* infections. Breeding colonies of the mouse strains were kept under specific pathogen-free conditions at the central animal facilities of the University of Mainz. Each animal experimentation was licensed by the regional health authorities.

2.2. C. albicans infection

C. albicans strain SC5314 was grown in Sabouraud medium (2% Glucose, 1% Pepton, pH 5.6) in a shaker at 30 °C for 72 h. The yeast cells were harvested by centrifugation and washed twice in phosphate-buffered saline (PBS). The mice were matched by gender and age and groups of 8–20 mice were used for each experiment. Mice aged 6–12 weeks were infected i.p. with 1×10^8 *C. albicans* blastoconidia or i.v. via the tail vein with $2–16 \times 10^5$ blastoconidia.

2.3. Survival curves and determination of tissue fungal burden

Mice were checked daily for signs of disease. Animals suffering from infection were sacrificed. Initially, survival was monitored for 28 days but as no mice died after the third week the time course was limited to 21 days p.i. Organs were harvested from deceased or sacrificed mice, weighed and homogenized in 2 ml PBS with an Ultra Turrax T-25 homogenizer (IKA Labortechnik, Staufen, Germany). Serial dilutions of the homogenates were plated on Sabouraud agar plates and after cultivation for 48 h at 30 °C yeast colonies were counted. The fungal load was expressed as colony forming units (CFU)/g organ.

2.4. Histological analysis

The right kidney of each animal was fixed in neutral buffered 4.5% formaldehyde (Roti-Histofix, Roth, Karlsruhe, Germany) for a minimum of 2 days and kept at 4 °C. The histofixative was replaced every week until embedding in paraffin. Serial 4 μm thick sections were examined microscopically after staining with periodic acid Schiff (PAS) or hematoxylin-eosin (HE).

2.5. Recruitment of neutrophils

To investigate the recruitment of neutrophils into the peritoneal cavity after i.p. infection with 1×10^8 *C. albicans* blastoconidia, mice were sacrificed 3 h p.i. by CO_2 asphyxiation and peritoneal cells were harvested by intraperitoneal lavage. To do this 7 ml of ice-cold PBS was injected with a 23-gauge needle through the abdominal fat pad to the right of the bladder. Peritoneal cells were detached by shaking the mice for 30 s. The peritoneal fluid was withdrawn with the same syringe and transferred to siliconized 12 ml plastic tubes. Peritoneal cells were quantified using a haemocytometer. To determine the fungal load in the peritoneal cavity, the peritoneal fluid was plated in serial dilutions onto Sabouraud agar plates.

2.6. Immunofluorescence analysis

C. albicans were cultured in Lee's Medium (Lee et al., 1975) for 72 h in a shaker at 23 °C. To obtain blastoconidia the fungi were cultured at pH 4.5 (30 min; 37 °C) or at pH 6.5 (24 h; 37 °C) to induce hyphal growth. Cells were washed twice with PBS and adjusted to 1×10^7 blastoconidia per ml PBS. Hyphae were adjusted to an optical density of 0.4 at 600 nm. Ten microliters of these solutions were applied onto a chamber of poly-L-lysine coated slides (Adcell, Roth, Karlsruhe, Germany) air-dried and fixed with ice-cold acetone. The cells on the slide were then incubated with 10 μl mouse sera (MBL-$A/C^{-/-}$, $C1qa^{-/-}$, $Bf/C2^{-/-}$ or C57BL/6) for 1 h at 37 °C in a wet chamber and then washed twice with PBS. Cells were fixed again with ice-cold acetone to maintain the present state of the specimen. Thereafter, the slides were incubated for 1 h at room temperature with primary rat monoclonal antibodies: anti-MBL-A (8G6; Hycult biotechnology, Uden, Netherlands), anti-MBL-C (14D12; Hycult biotechnology) or anti-C3b/iC3b/C3c (3/26; Hycult biotechnology). Mouse C1q was detected using an IgG fraction of a goat anti-mouse C1q antiserum (Petry et al., 1991). Slides were then washed twice in PBS. Binding of the rat monoclonal antibodies was detected by incubation with the secondary antibody goat anti-rat IgG-Alexa Fluor 488 (Molecular Probes, Invitrogen, Karlsruhe, Germany) for 1 h. For the detection of bound anti-C1q, donkey anti-goat IgG-Alexa Fluor 488 (Molecular Probes, Invitrogen) was used. Unbound antibody was washed away twice with PBS and the slides were covered in glycerin for microscopy. Slides were examined under epifluorescence and differential interference contrast (Axioskop 2 equipped with an AxioCam HRc digital camera, and fluorescence filter EX BP 450-490, BS FT 510, EM LP 515, Zeiss, Oberkochen, Germany).

2.7. Phagocytosis of C. albicans by neutrophils

For recruitment of neutrophils to the peritoneum, C57BL/6 mice were injected i.p. with 1 ml thioglycolate medium (Merck, Darmstadt, Germany) and after 4 h cells were obtained by intraperitoneal lavage (White et al., 2002). The obtained peritoneal cells were counted in a hemocytometer, washed in PBS, resuspended in RPMI