Resistance of MBL gene-knockout mice to experimental systemic aspergillosis

Karl V. Clemons, Marife Martinez, Ann-Jay Tong, David A. Stevens

1. Introduction

Several classes of host proteins are important in innate immunity, including the collectins. These proteins specifically bind to certain patterns of carbohydrate moieties on the surface of micro-organisms, acting as opsonins to enhance interaction with phagocytic cells [1–5]. The collectins include molecules such as the surfactant proteins SP-A and SP-D, and mannose binding lectin (MBL).

MBL is of particular interest as a collectin that binds to the cell wall mannose of various fungi including Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus [4,5]. MBL recognition of fungal pathogens stimulates the immune system through the activation of complement, promoting the killing of pathogens either directly by complement activation through the lectin pathway or by opsonization, as well as effects on the pathogen by targeting the ligands and the action of linked enzymes [5–9]. Because MBL deficiency has been associated with a higher risk for infection by various pathogens including bacterial, fungal and viral etiologic agents in patients undergoing chemotherapy or immunosuppressive therapy [6], defects in MBL have been proposed to contribute to increased susceptibility to certain infections by further reducing the effectiveness of the host's innate response [3]. In particular, MBL deficiency has been associated with recurrent episodes of vaginal candidiasis [10–12], as well as chronic necrotizing pulmonary aspergillosis and chronic pulmonary aspergillosis [4]. In light of these data, it has been proposed that MBL therapy has potential in the clinical setting for reducing immunosuppression- or chemotherapy-associated infections [6,8,13].

The possible protective role of MBL against experimental pulmonary aspergillosis has been studied using a corticosteroid-suppressed murine model of disease [3,14]. Those authors found that administration of recombinant MBL prolonged the survival of mice. Even so, the magnitude of the role played by MBL in innate resistance to Aspergillus is not clear. The aim of our current study was to determine the comparative susceptibility of MBL gene-knockout (KO) mice with that of MBL-sufficient wild-type control mice to systemic aspergillosis.

2. Methods

A murine model of experimental systemic aspergillosis was established in 5- to 6-week-old female mice as previously described [15–18] and with the approval of the Institutional Animal Care and Use Committee of the California Institute for Medical Research. Female wild-type (WT) control mice were MBL-sufficient C57BL/6 and female MBL gene-knockout (KO) mice. KO mice were homozygous congenic B6.129S4 – Mb11tm1Kata Mb12tm1Kata/J, originally backcrossed seven times on a C57BL/6 background prior to being made homozygous, were supplied by Jackson Laboratories (Bar Harbour, ME). MBL-sufficient C57BL/6 (WT) mice and B6.129S4 – Mb11tm1Kata Mb12tm1Kata/J MBL A and C gene-knockout (KO) mice were infected intravenously with different inocula of Aspergillus fumigatus conidia. WT and KO mice were dose-responsively susceptible. In no instance were the KO mice more susceptible than WT. At the highest inoculum, all WT and 90% of KO mice died on day 4 (P < 0.015). Reduction of the inoculum to 5.5 × 10⁵ conidia was lethal, but comparison showed KO mice less susceptible to lethal infection (P < 0.015). At the lowest inoculum used, deaths of KO mice were delayed, but survival was not significantly different than WT (P > 0.05). These results suggest MBL may play a deleterious role in systemic aspergillosis.

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Harbor, Maine); C57BL/6 WT mice are the recommended controls for these KO mice. WT and KO mice were age matched between 5 and 6 weeks of age at the initiation of the experiment and were not immunosuppressed. Humans have one functional MBL gene, whereas mice have two, Mbl1 and Mbl2 (also known as MBL A and MBL C, respectively) [4,6–9]. Both Mbl1 and Mbl2 genes are knocked out in the mice we used. In brief, A. fumigatus strain 10AF was grown on potato dextrose agar and conidia were harvested in 0.05% Tween 80–saline. Viability was assessed by plating of serial dilutions. On day 0 groups of 10 each of KO and WT control mice were infected intravenously with 3 × 10^6, 5.5 × 10^6, or 8 × 10^6 conidia. Survival was tallied through 18 days and comparative survival evaluated statistically using a log rank test. No residual fungal tissue burdens were determined due to few surviving animals.

3. Results

The results of this study are shown in Fig. 1. As is evident, both WT and MBL KO mice were dose-responsively susceptible to lethal infection with A. fumigatus. At the highest inoculum, all WT and 90% of KO mice died on day 4 (P > 0.05). Reduction of the inoculum to 5.5 × 10^6 conidia per mouse also proved highly lethal to the WT mice, with all animals succumbing to infection on days 4 and 5. Although at the same inoculum fewer KO mice died on days 4 and 5 (only 40% of KO versus 100% of WT), all KO mice died by day 11. However, comparison of the survival curves showed MBL KO mice to be less susceptible to lethal infection (P < 0.015). At the lowest inoculum, 3 × 10^6 conidia per mouse, deaths were delayed, with 50% of WT and 70% of KO mice surviving on day 7. Ten percent of WT survived through day 18, whereas 30% of KO mice survived. Although more KO mice survived there was no statistical difference in the survival of WT or KO mice given the lowest inoculum (P > 0.05). The KO survival trend across the different inocula also argues for lack of KO susceptibility.

4. Discussion

The results of this study are to some extent surprising, as we might have predicted that MBL KO mice would be more susceptible to infection with Aspergillus. However, Hogaboam et al. [19] reported no lethality of MBL A<sup>−/−</sup> (MBL A deficient, but MBL C sufficient) and MBL A sufficient control mice after intravenous inoculation with up to 5 × 10^6 conidia of A. fumigatus. These authors concluded MBL A is not required for survival after exposure to conidia [19]. It should be noted that those authors used an inoculum >10-fold lower than we did in our studies, which in our experience would be below the threshold number of conidia to result in death. Thus, the lower inoculum very likely contributes to the differences in mortality between our study and that in the Hogaboam study [19]. In addition, the knockout mice they used were deficient only in MBL A, whereas those used in our study were deficient in both forms of murine MBL, which may contribute to an altered susceptibility. Those authors report decreased Th2 cytokines and increased interferon-γ in Aspergillus-challenged MBL A<sup>−/−</sup> mice, which may correlate with the resistance we observed. MBL A<sup>−/−</sup> mice have also been observed to be resistant to bacterial sepsis [20], and not more susceptible to C. albicans [21]. In examining the role of an immune mediator such as MBL to host-resistance in vivo, the challenge inoculum becomes critical. Experimental design using a dose-escalation of inoculum is necessary to result in a range of disease severity to be evaluated. A high inoculum could cause an overwhelming infection even in immune competent animals, whereas with too low an inoculum an immune deficient host may be able to control or clear the infection similar to the immunocompetent controls. In neither of these situations would one be able to demonstrate a significant difference between the control mice and the KO mice, which would lead to an incorrect conclusion that the gene or immune mediator plays no role in resistance to that infection [22].

MBL genotype mutations (single nucleotide polymorphisms [SNPs]) have been associated with increased susceptibility to invasive pulmonary aspergillosis (IPA) in humans and types of chronic pulmonary aspergillosis [3,4,23–26]. However, high levels of MBL have been found in allergic bronchopulmonary aspergillosis (ABPA) patients and have been correlated with increased IgE, airway hypersensitivity, and increased levels of Th2 cytokines, all associated with asthma [25]. It is possible that the choice of model being that of systemic disease in nonimmunosuppressed mice contributes to the appearance of MBL KO mice being less susceptible than WT
mice. The lack of MBL would likely reduce the influx of PMNs due to the reduction of chemotactic factors arising from the MBL activation of the lectin pathway of complement. Less influx of PMNs and subsequent host-induced damage to the tissues may be a plausible explanation for these results, as some authors have indicated that PMN-induced tissue damage is the cause of death rather than from the hyphae of the infecting Aspergillus [27–29]. Indeed, decreased neutrophilic infiltration has been noted in MBL A−/− pulmonary aspergillosis [19]. Additional studies would be required to determine whether this may be the case in this model. It is possible that MBL may block glycan sites on the pathogen, thereby reducing the triggering of a protective response. There are several examples where MBL may dampen the inflammatory response, and where MBL deficiencies may be protective against infection [30–33].

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References