Association of Mannose-Binding Lectin Deficiency with Acute Invasive Aspergillosis in Immunocompromised Patients

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(See the editorial commentary by Kalil, on pages 1492–5.)

Background. Invasive aspergillosis is a devastating infection with attributable mortality of 40% despite antifungal therapy. In animal models of aspergillosis, deficiency of mannose-binding lectin (MBL), a pattern recognition receptor that activates complement, is a susceptibility factor. MBL deficiency occurs in 20%–30% of the population. We hypothesized that MBL deficiency may be a susceptibility factor for invasive aspergillosis in humans.

Methods. Serum MBL concentrations were measured by enzyme-linked immunosorbent assay in 65 patients with proven or probable acute invasive aspergillosis and 78 febrile immunocompromised control subjects. MBL concentrations and the frequency of MBL deficiency were compared.

Results. The median serum MBL level was significantly lower in patients with aspergillosis than in control subjects (281 ng/mL vs 835 ng/mL; P = .007). MBL deficiency (MBL concentration, <500 ng/mL) was significantly more common in patients with aspergillosis than control subjects (62% vs 32%; P < .001). Frequency of MBL deficiency was similar among patients with aspergillosis irrespective of response to antifungal therapy (P = .10).

Conclusions. This study is the first, to our knowledge, to show an association between MBL deficiency and acute invasive aspergillosis in humans. Further study is required to investigate the causal nature of this association and to define whether diagnosis of MBL deficiency may identify immunocompromised patients at increased risk of invasive aspergillosis.
forming MBL polymers that bind to conserved carbohydrate arrays that are commonly found on the outer surface of microorganisms but that are rarely found on the surface of normal human cells [6, 7]. Once bound to its carbohydrate targets, the MBL polymer initiates activation of the complement cascade via the lectin pathway [8] and directly enhances phagocytosis, signaling via MBL receptors on the surface of phagocytic cells [9].

Interestingly, mutations in exon 1 of the gene coding for MBL (mbl2) are present in up to 40% of the population [10]. These mutations prevent effective cross-linking of MBL monomers and result in secretion of nonfunctional MBL [11]. Individuals are classified as A/A (normal homozygotes), A/O (heterozygotes), and O/O (compound heterozygotes and mutant homozygotes).

The mean serum MBL concentrations recorded for each of these groups are 2660 ng/mL (range, 40–6790 ng/mL), 830 ng/mL (range, 0–4460 ng/mL), and 40 ng/mL (range, 0–300 ng/mL), respectively [10]. Although genotype is the primary determinant of serum MBL concentration, MBL levels vary significantly among individuals with the same MBL genotype, and the serum MBL concentration more accurately reflects downstream effector function than genotype [10]. Acute infection with pathogens known to bind MBL does not consistently cause a reduction in serum MBL levels [12, 13], despite the fact that MBL is consumed during pathogen binding [14]. It is likely that increased MBL gene transcription during an acute-phase reaction [15, 16] maintains MBL levels during infection. MBL levels do not vary with respect to age, sex, physical exercise, season, time of day, or hepatic function [17, 18] and are stable during storage at −80°C through a number of freeze-thaw cycles [19]. The dynamics of circulating MBL during acute invasive aspergillosis have, to our knowledge, not been investigated.

MBL deficiency is defined by a low serum MBL concentration, although there is no universally accepted concentration cutoff [20]. If defined by a serum concentration of ≤500 ng/mL, MBL deficiency is present in 20%–30% of the population, making MBL deficiency the most common hereditary immunodeficiency [11, 21].

To our knowledge, there have been no human studies investigating MBL deficiency in acute invasive aspergillosis. Some studies have demonstrated an association between serum MBL deficiency and an increased incidence of fever or serious infection in immunocompromised children [22, 23] and adults [24, 25]. Other studies have not demonstrated this association [26, 27]. The discrepancy is probably explained by the specificity of MBL binding. These studies enrolled patients with infections due to a heterogeneous mix of pathogens, but MBL does not bind equally well to all organisms [28, 29], and it is likely that MBL does not play a role in defense against all pathogens. MBL binds to Aspergillus species [28], presumably to the mannan-rich outer wall [30]. Binding is associated with a dose-dependent activation of the complement cascade [28] and increased phagocytosis of Aspergillus conidia by neutrophils [31]. Studies involving a neutropenic mouse model of invasive aspergillosis have shown that MBL-deficient mice are more susceptible to invasive aspergillosis and that MBL replacement therapy returns the risk of invasive infection in deficient mice to that of their wild-type littermates [31]. The number of cases of probable or proven aspergillosis reported in the studies of MBL in immunocompromised patients is too small to draw strong conclusions [32]. The results of the 2 studies investigating chronic pulmonary aspergillosis in immunocompetent patients are discrepant. A case series of 11 patients demonstrated that haplotypes associated with low serum MBL concentration were more common in case patients than in control subjects [33]. However, this association was not identified in a more recent study of 23 patients [34].

In light of this evidence, we hypothesized that low serum levels of MBL may be an additional risk factor for the development of acute invasive aspergillosis in immunocompromised patients. We investigated the role of MBL deficiency in susceptibility to acute invasive aspergillosis by measuring serum MBL levels in a large cohort of well-characterized patients with proven or probable invasive aspergillosis and in control subjects with comparable underlying illness but no evidence for acute invasive aspergillosis. The identification of MBL deficiency as a potentially reversible risk factor [35] for acute invasive aspergillosis could justify diagnosis of MBL deficiency in individuals susceptible to invasive aspergillosis.

PATIENTS, MATERIALS, AND METHODS

The present study used fully anonymized serum samples from 65 adult patients with proven or probable invasive aspergillosis and 78 immunocompromised control subjects with a febrile illness. Of the case patients, 36 were enrolled in a multicenter trial of antifungal therapy [1], 23 were seen in Strasbourg, France, from 1997 through 2002 by 2 of the authors (R.H. and V.L.-B.), and serum samples before, during, and after acute Aspergillus infection were collected from 6 patients recruited via the University of Wales in Cardiff. Of the control subjects, 18 patients were from Strasbourg; 18 from St George’s Hospital in London, England; 15 from St Bartholomew’s Hospital in London; and 27 from the UK National Public Health Service Mycology Reference Laboratory in Bristol, England. The study was approved by the Research Ethics Committee of St George’s University and was conducted in accordance with the Helsinki Declaration (revised in 1983).

Proven and probable invasive aspergillosis was initially de-
Mannose-binding lectin (MBL) levels in patients with invasive aspergillosis (cases) and immunocompromised control subjects (controls). Median MBL levels: 281 ng/mL and 835 ng/mL for 65 cases and 78 controls, respectively ($P = .007$, by Mann-Whitney $U$ test). For differences in MBL levels across control groups, $P = .40$, by Kruskal-Wallis test (bacterial infection, 24 patients; viral infection, 11 patients; and fever of unknown origin, 43 patients). Error bars indicate interquartile range.

Figure 2. Frequency of mannose-binding lectin (MBL) deficiency in patients with invasive aspergillosis (cases) and immunocompromised control subjects (controls). The figure shows the percentage of cases and controls with MBL deficiency defined using serum MBL concentrations of $\leq 1000$ ng/mL ($P = .40$), $\leq 500$ ng/mL ($P < .001$), $\leq 300$ ng/mL ($P < .001$), $\leq 200$ ng/mL ($P = .003$), and $\leq 100$ ng/mL ($P = .01$). $P$ values were determined using the Fisher exact test.
of 6 patients, who had been enrolled in an investigation of polymerase chain reaction for the diagnosis of invasive aspergillosis [39]. For each of these patients, we analyzed, on average, 3.2 serum samples obtained before infection and 4.5 samples obtained during acute invasive Aspergillus infection. Preinfection samples were obtained, on average, 35 days before the diagnosis of invasive aspergillosis (range, 15–62 days).

The control subjects were patients with a febrile illness and at least 1 risk factor for invasive fungal disease, including prolonged neutropenia (neutrophil count of $<0.5 \times 10^9$ cells/L for $>10$ days) in the preceding 60 days, significant immunosuppression (including treatment with steroids or T cell suppressors) in the previous 30 days, acute (grade 2) or chronic extensive graft-versus-host disease, or symptomatic AIDS. Microbiological and radiologic investigation of febrile illness in control subjects did not identify Aspergillus species, and the cause was classified as bacterial, viral, protozoal, or fever of unknown origin. The average time from onset of fever to sample retrieval was 4 days.

There is no universally recognized serum MBL concentration that defines MBL deficiency. Different studies have used a range of cutoffs of 1000–200 ng/mL [20, 24]. In this study, cutoffs of 1000, 500, 300, 200, and 100 ng/mL were investigated.

Samples were obtained during routine clinical care of enrolled patients and were separated according to the standard operating procedures of the diagnostic laboratories involved. After separation, serum aliquots were stored at $\sim80^\circ$C until required. Serum MBL concentration was determined by solid-phase enzyme-linked immunosorbent assay (HK323-ELISA; Hycult Biotechnology) [40] using a monoclonal antibody specific for functional MBL (131–01). The lower limit of detection of the assay is 0.4 ng/mL, and the average coefficient of variation was 6%.

Median serum MBL levels of case and control patients were compared using the 2-tailed Mann-Whitney rank-sum test and the Kruskal-Wallis test because MBL levels were not normally distributed in either group. Fisher’s exact test was used to compare the frequency of MBL deficiency in cases and controls. The Wilcoxon signed-rank test was used to investigate differences in MBL levels before and during acute invasive Aspergillus infection in the subset of patients for whom sequential samples were available. Receiver operating characteristic (ROC) curves were analyzed to determine the discriminatory potential of MBL deficiency. $P<.05$ was considered to represent a significant difference between groups in all analyses. Data were analyzed using Excel, version XP (Microsoft); GraphPad Prism, version 5 (GraphPad Software); and SPSS, version 16 (SPSS).

RESULTS

Case patients were older (49 vs 42 years; $P=.01$) and more likely to be male (74% vs 57%; $P=.06$), compared with control subjects. However, MBL levels and the frequency of MBL deficiency in case and control subjects were not associated with age, sex, ethnicity, or underlying disease, which is consistent with the literature [10, 17]. Most patients in each group were white (100% of case patients and 94% of control subjects; $P=.007$). The cause of immunodeficiency in case and control subjects was similar ($P=.40$) and included hematologic malignant neoplasms (55% vs 64%), hematopoietic cell transplantation (25% vs 25%), and other immunosuppressive conditions, such as AIDS, solid organ transplantation, and chemotherapy for solid organ malignancy (20% vs 11%).

The median serum MBL concentration was significantly lower in patients with invasive aspergillosis than in control subjects (281 vs 835 ng/mL; interquartile ranges [IQRs], 44–1541 ng/mL and 312–1798 ng/mL; $P=.007$). In the control subjects, the cause of febrile illness was identified as bacterial in 31%, viral in 14%, protozoal in 1%, and fever of unknown origin in 54%. MBL levels were similar between individuals with these different agents identified as the cause of their fever ($P=.40$) (Figure 1).

MBL deficiency (MBL level, $<500$ ng/mL) was significantly more common in case patients than in control subjects (62% vs 32%; odds ratio, 3.4; 95% confidence interval, 1.7–6.8; $P<.001$). The significant difference in frequency of MBL deficiency between case and control patients was observed at all MBL levels $\leq500$ ng/mL (Figure 2). Comparing case and control patients, the area under the ROC curve was 0.63 (95% confidence interval, 0.54–0.73; $P=.007$). The most discriminatory point on the curve corresponded to an MBL value $\approx502$ ng/mL (corresponding likelihood ratio, 1.92).

In the case patients, MBL levels were similar between individuals with proven (25 patients [38%]) and probable (40 patients [62%]) invasive aspergillosis (359 vs 327 ng/mL; IQR, 46–1296 vs. 45–1847 ng/mL; $P=.90$). Initial antifungal therapy was voriconazole in 31 patients (48%), amphotericin in 29 (45%), itraconazole in 4 (6%), and caspofungin in 1 (1%). MBL levels did not differ according to the antifungal drugs administered ($P=.60$). The number of patients with extrapulmonary invasive aspergillosis was small ($n=10$). With this in mind, no significant difference was found in MBL levels in patients with pulmonary versus extrapulmonary disease ($P=.40$).

On the basis of published criteria [1, 38], 36 patients (55%) were judged to have had a successful response to antifungal therapy. Median MBL levels and the frequency of MBL deficiency did not differ significantly between patients successfully and unsuccessfully treated for acute invasive aspergillosis (147 ng/mL vs 446 ng/mL; [IQR, 14–1207 vs 111–2054 ng/mL; $P=.06$] and 69% vs 52%; deficiency cutoff, 500 ng/mL; $P=.20$). Using ROC curve analysis, MBL deficiency is not a significant predictor of treatment outcome ($P=.10$). For the
subset of 6 cases for whom multiple serum samples were available at different time points, MBL levels before and during invasive Aspergillus infection were not significantly different (2306 vs 2250 ng/mL; IQR, 1737–2808 vs 1414–3096 ng/mL; \( P = .78 \), by Wilcoxon signed-rank test).

DISCUSSION

We have demonstrated that MBL deficiency is associated with acute invasive aspergillosis in immunocompromised patients. It is possible that MBL deficiency renders immunocompromised individuals more susceptible to invasive aspergillosis or that MBL levels decrease during invasive aspergillosis. The preliminary finding that MBL levels do not decrease during invasive aspergillosis requires validation in a larger, prospective study. On the basis of current published data demonstrating stable MBL levels during infections with pathogens to which MBL is known to bind [12, 13]; increased MBL gene transcription during infection [15, 16]; the lack of variability in MBL levels with respect to multiple variables, including time of day, season, physical exercise, and impaired hepatic function [17, 18]; and the fact that MBL deficiency confers susceptibility to invasive aspergillosis in an animal model [31], we believe that it is appropriate to postulate that MBL deficiency may render immunocompromised individuals more susceptible to invasive aspergillosis.

Exposure to Aspergillus is universal. After exposure, Aspergillus appears able to penetrate alveolar epithelial and endothelial cells within 14–16 h [41], before the adaptive immune system has time to mount an effective local response. Neutrophils, dendritic cells, and pulmonary macrophages have been identified as important innate effector cells in the elimination of Aspergillus within the alveolus [41–43]. Each of these cells possesses receptors for MBL [44] and cell membrane bound pattern recognition receptors similar in structure and function to MBL, such as dendritic cell–specific intercellular adhesion molecule 3–grabbing non-integrin [43]. Neutrophil effector function in response to Aspergillus is influenced by Toll-like receptor 2 (TLR2) [42]. It has recently been demonstrated that TLR2 and MBL receptors colocalize on the surface of neutrophil phagosomes and their costimulation results in modulation of TLR–associated signaling cascades, leading to enhanced organism clearance [45]. Given that MBL is present in bronchoalveolar lavage fluid [34, 46], we suggest that MBL recognizes inhaled Aspergillus, enhances phagocytosis, and modulates the early innate immune response within the alveolus, resulting in the clearance of organisms before invasion occurs. The lack of an association between MBL deficiency and response to antifungal therapy may imply that once infection has become established, MBL plays only a limited role.

DNA samples were not available from case or control subjects for genetic analysis. However, MBL levels vary considerably between individuals with the same genotype, in part because of mutations in the promoter region of the MBL gene [10]. Differences in promoter region splicing sites, transcription response elements, and individual variation in the degree of extrahepatic MBL gene transcription may also play a role [16]. Serum concentration of MBL represents a reliable correlate of in vivo functional activity, and serum MBL concentration rather than genotype may be a more convenient basis for defining MBL deficiency [20]. Our data suggest that the most clinically relevant cutoff for identifying immunocompromised patients at increased susceptibility to aspergillosis is 500 ng/mL.

The patients for whom sequential samples were available had MBL levels greater than the other patients. We believe this to be due to random variation, and these patients are likely to possess the wild-type MBL gene. Previous studies report that it is this group of patients in whom changes in MBL levels during infection are most readily observed [22].

We have demonstrated an association between MBL deficiency and invasive aspergillosis, raising the possibility that MBL deficiency may render immunocompromised patients more susceptible to invasive aspergillosis. Diagnosis of MBL deficiency may be of benefit in the diagnostic workup of immunocompromised patients. Further elucidation of the causal nature of this association is required before recommendations can be made about the potential utility of MBL replacement therapy.

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