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Mannose-Binding Lectin Deficiency and Respiratory Tract Infection

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Complement system · Infectious diseases · Innate immunity · Lectins · Lung · Mannose-binding lectin · Pneumococcus · Pneumonia · Sepsis

Abstract
Mannose-binding lectin (MBL) is an innate immune system pattern recognition protein that kills a wide range of pathogenic microbes through complement activation. A substantial proportion of all human populations studied to date have MBL deficiency due to *MBL2* polymorphisms, which potentially increases susceptibility to infectious disease. MBL binds numerous respiratory pathogens but the capsule of *Streptococcus pneumoniae* abrogates its efficient binding. Clinical studies in humans have shown that MBL deficiency appears to predispose to severe respiratory tract infection. A recent meta-analysis shows that MBL deficiency was associated with death in patients with pneumococcal infection after adjusting for bacteraemia and comorbidities. Human clinical studies have also shown associations between MBL deficiency and various less common respiratory infections. Intracellular infections like tuberculosis may be less common with MBL deficiency because of reduced opsonophagocytosis. Lung secretions contain small amounts of MBL that are potentially sufficient to activate complement, but their measurement is confounded by dilution inherent in collection techniques. Therefore, if this protein does play a role in pulmonary immunity it is presumably through prevention of haematogenous dissemination of respiratory pathogens while adding to mucosal defences. Ficolins are collectins that are structurally and functionally related to MBL and are either present in serum or expressed in tissues including the lung. Limited variation in serum levels of L- and H-ficolin result from the presence of *FCN2* and *FCN3* polymorphisms. Initial studies on the impact of *FCN2* polymorphisms or low L-ficolin levels do not seem to show major associations with respiratory infection. MBL is being developed as a new immunotherapeutic agent for prevention of infection in immunocompromised hosts. The available literature suggests that it may also be of benefit in MBL deficient patients with severe pneumonia. This review concentrates on clinical associations between MBL deficiency and susceptibility to respiratory tract infection.

Introduction

Physical, cellular and soluble innate immune defences against lower respiratory tract infection are crucial in maintaining the lungs in a healthy state. Mannose-binding lectin (MBL) is a soluble pattern recognition protein that contributes to killing of a broad range of pathogenic micro-organisms via the lectin complement pathway and through opsonophagocytosis [1]. Deficiency states arise from polymorphisms in the structural and promoter sequences of the *MBL2* gene. A large number of associations

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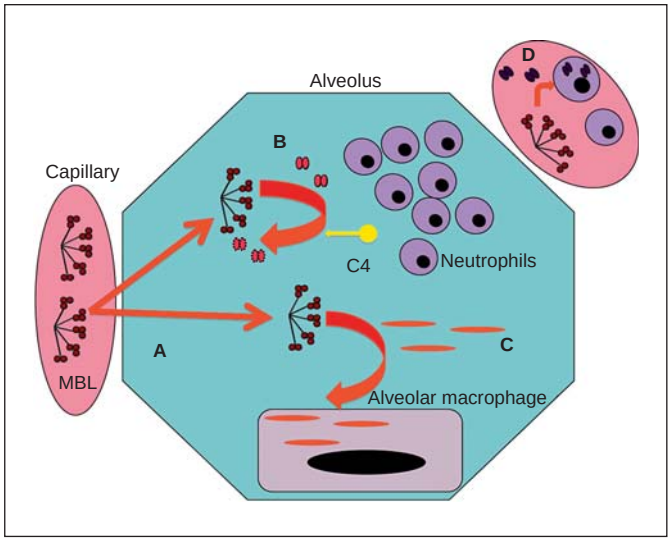

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Fig. 1. Schematic representation of the alveolus and capillaries illustrating the potential roles of MBL in modulating respiratory tract infection. **A** As there is no local production of MBL, small amounts of the protein 'leak' into inflamed airways. **B** Lectin pathway-mediated killing may contribute to mucosal defences against pathogens such as *Haemophilus influenzae*. **C** MBL-facilitated phagocytosis of intracellular pathogens such as *Mycobacterium tuberculosis* may predispose to tuberculosis in MBL replete individuals. **D** MBL-facilitated opsonophagocytosis of *Streptococcus pneumoniae* may predispose to severe invasive disease in MBL deficient individuals.

between MBL deficiency and lower respiratory tract infection have been documented through clinical studies in humans [2–5]. While these have used different definitions of MBL deficiency, the commonality of association suggests that MBL does contribute to defences in the lung. Mechanistically it can be seen how MBL can contribute to respiratory mucosal and bloodstream defences as it can be shown to bind to numerous important respiratory pathogens, promoting C4 deposition (fig. 1). If a cut-off serum level of 0.5 μg/ml is used as a definition, the frequency of MBL deficiency is approximately 25% [5]. This common immunodeficiency state may therefore have a general role in predisposing to respiratory tract infection, an equally common infectious disease. Equally, it may be that in individuals with additional risk factors, MBL deficiency assumes a more significant role in predisposition to infection. This may apply to those with diseased respiratory mucosa such as cystic fibrosis patients [6] or lowered immunity as with infants in the 'window of vulnerability' [7] between the disappearance of maternal antibody and maturation of adaptive immune responses.

MBL Deficiency Definitions

MBL initiates complement activation via the lectin pathway and promotes opsonophagocytosis. Both these actions can be measured but most work has focused on the complement pathway. *MBL2* structural gene polymorphisms affect the assembly of this complex molecule and clearly interfere with its complement activating function. The wild-type *MBL2* allele is referred to as *A* with variants being *B, C* and *D* or collectively, *O* [8]. The promoter polymorphisms *H/L* and *X/Y* influence the serum level of the protein [8]. As such there is a correlation between the *MBL2* haplotype and MBL level and function. This is not precise as many individuals with wild-type MBL genes still have low or unmeasurable MBL levels and function [8]. Consequently, studies that explore infectious diseases associations with low producing *MBL2* genotypes alone may underestimate these. It may be that MBL definitions based solely on low MBL level (<0.5 μg/ml) [5] or, ideally, MBL function (<0.2 U/μl C4 deposition) [9] are most appropriate for determining such associations. In this regard it is important to state that MBL levels do not alter by more than 3–4 times baseline levels during inflammation [10]. To date, most MBL-disease association studies have assessed *MBL2* genotypes with or without MBL levels. Where MBL levels or function were used to explore infectious disease association this will be specifically noted in this review. Less common is the entity of mannose-binding lectin associated serine protease 2 (MASP2) deficiency, which also leads to MBL functional deficiency [11]. MASP2 deficiency is less studied than MBL at a population and disease association level.

Tissue Distribution of MBL in the Lung

MBL is a serum protein produced in the liver but it is also found at the sites of inflammation, albeit at levels substantially less than in the blood [12]. No appreciable MBL transcription was found by real-time PCR on lung tissue [13], so MBL present in respiratory secretions most likely represents 'leakage' from the serum (fig. 1). A number of case series have investigated levels of MBL in the lungs and essentially shown that it is present in bronchoalveolar lavage (BAL) fluid of patients with pneumonia but not where the lungs were not inflamed [14–16]. Median lung MBL levels were 0.015–0.019 μg/ml [14, 16] with a maximal lung level of MBL from a case of *Pneumocystis jiroveci* pneumonia was 0.078 μg/ml [14] which compares with serum levels that range from 0 to 7.9 μg/

ml [8]. These studies all measure MBL levels in BAL fluid and do not make allowance for dilution or comparison with lung proteins of know concentrations. It is estimated though that lung MBL levels are likely to be between 1 and 2 logs higher than those measured in BAL [16] and, therefore, to be at least equal to the minimum range of 0.3–0.4 μg/ml required for complement activation [17]. Further data are required on levels and function of MBL in respiratory secretions. The severity of pulmonary inflammation is probably also a factor controlling the amount of MBL present in the lungs. Animal studies have documented that MBL was present in lung secretions of laboratory mice only after prolonged infection with virulent influenza virus. No MBL was found in respiratory secretions in mice infected with an influenza strain that replicated poorly in the lungs [18].

MBL Binds to Many Respiratory Pathogens

MBL binding and complement deposition can be demonstrated for respiratory pathogens including *Haemophilus influenzae* [19], *Mycoplasma pneumoniae* [20], *Mycobacterium tuberculosis* [21], *Mycobacterium avium* complex (MAC) [22], *Legionella pneumophila* [23], influenza virus [24], *Nocardia farcinica* [23], and such recently described respiratory pathogens as severe acute respiratory syndrome coronavirus. [4] *Chlamydia pneumoniae* and *Chlamydia psittaci* are bound by MBL with inhibition of their growth in cell culture, albeit at much higher inhibitory concentrations than for *Chlamydia trachomatis* [25]. *Pseudomonas aeruginosa*, an important pathogen in cystic fibrosis (CF), was initially not thought to be bound by MBL in studies using flow cytometry [19]. Analysis of binding of MBL from mouse serum showed that it did in fact bind to *P. aeruginosa* and that FACS was insensitive at detecting this [26]. MBL binding has been shown to bind to *Burkholderia cepacia*, a Gram-negative pathogen previously included in the pseudomonads, that is also a CF pathogen [27].

Pneumococcus is one crucial exception, where the most definitive information indicates that the organism's polysaccharide capsule abrogates MBL's binding [28]. Furthermore, mouse studies indicate that the classical complement pathway plays the paramount role in anti-pneumococcal defences, with MBL contributing little in this model [29].

In addition to mediating lectin-pathway complement killing, MBL promotes opsonophagocytosis [30]. The nature of the phagocyte receptor for MBL remains contentious but is most likely a C1q receptor [31, 32] or complement receptor type 1 [33]. MBL enhanced phagocytosis has been demonstrated for *Staphylococcus aureus* [34] and *Neisseria meningitidis* [35]. A recent study, that used similar methodology to the preceding, did not show any increase in opsonophagocytosis of *S. aureus* in the presence of MBL [36]. This was the first study to assess phagocytosis of *Streptococcus pneumoniae*, and, as for *S. aureus*, it failed to demonstrate any increase mediated by MBL [36]. This important result requires further verification.

Clinical Studies Exploring Lower Respiratory Tract Infection Associations with MBL

Community-Acquired Pneumonia

Pneumococcal infection-MBL associations have now been sought in a number of studies, initially concentrating on invasive infection. Here pneumococcal blood stream infection was more common in patients with *O/O MBL2* genotype [2]. Subsequent investigation of a similar, Caucasian, patient population did not confirm this finding [37]. Spanish patients with pneumococcal pneumonia were more likely to be bacteraemic if they had wild-type *MBL2* genotype. The *MBL2* genotype did not otherwise predict pneumonia severity as measured by the Fine score [38]. A recent study that combined individual patient data from published studies that included MBL level as well as genotype found that MBL levels <0.5 μg/ml were associated with increased death in patients with pneumococcal sepsis after adjusting for pneumococcal bacteraemia and patient comorbidities [5]. Overall, with the available data, it seems that there is an association between MBL deficiency and pneumococcal infection, but more clinical studies and information on pneumococcal pathogenesis such as MBL's contribution to phagocytosis are required before concluding on the certainty of this association.

Legionellosis has been shown to be associated with MBL deficiency, this finding being somewhat unexpected given that this is due to an intracellular pathogen. Two reports have identified low MBL function as a risk factor for legionellosis in outbreaks of this infection in Australia [39] and the Netherlands [40]. MBL binds to *Legionella pneumophila*, albeit with low efficiency [23]. Normal MBL levels may be expected to promote macrophage uptake, but as intracellular killing is routinely impaired, potentially increase the frequency or severity of legionellosis. However, MBL may contribute to increased intracellular killing by as yet undefined mechanisms or through more efficient complement killing.

Individuals with primary antibody deficiency had their MBL genotype determined to assess susceptibility to *Mycoplasma* infection. Structural gene variants of *MBL2* were significantly more common among these patients with proven mycoplasma infections, including respiratory tract *Mycoplasma pneumoniae* disease, than among healthy controls [20]. There are no data on associations between *M. pneumoniae* or *Chlamydia* respiratory tract infections and MBL deficiency in immunocompetent hosts.

Other studies of community-acquired pneumonia have not concentrated on specific pathogens but have still shown major associations with MBL deficiency. A large, prospective study conducted at 3 Spanish university hospitals has shown that patients with deficient *MBL2* genotypes had increased ICU admission, septic shock and 90-day death rates than *MBL2*-wild-type community-acquired pneumonia patients [3]. The commonest pathogen in this study was *S. pneumoniae* (195 patients, 23% of the total) but no clear association was identified between MBL deficiency and severe outcomes in pneumococcal pneumonia in this study. No link was found between MASP2 deficiency and pneumonia outcomes. A considerable number of patients (61%) did not have a respiratory pathogen identified, as is common in pneumonia series [3]. Another recent study has linked low MBL level promoter haplotypes with increased susceptibility to chest infection in a prospective study of Finnish military recruits [41].

MBL Deficiency Appears to Protect Against Tuberculosis

Numerous studies using *MBL2* genotype to divide patients into low- and high-producing MBL groups have shown that MBL deficiency protects against disease caused by *M. tuberculosis*. Carriage of a single variant *MBL2* structural allele in South African adults [42] and Gambian children [43], or full promoter haplotypes indicating low MBL production in Danish patients were all protective against tuberculosis [44]. Not all studies are concordant with these findings as a large study in southern India found that patients with pulmonary tuberculosis were more likely than healthy controls to have homozygous variant alleles and therefore have no circulating MBL [45]. Furthermore, a large study of Tanzanian patients showed no association between *MBL2* alleles and tuberculosis susceptibility [46]. There are not obvious differences in the overall frequency of MBL alleles between these diverse study populations or methodological differences to account for these discrepancies. The Indian group have followed up their study with another that documents decreased *M. tuberculosis* phagocytosis in wild-type MBL patients with pulmonary tuberculosis despite them having higher MBL levels than healthy controls [47]. This observation has not been repeated for *M. tuberculosis* to date. There are also conflicting results regarding MBL-mediated phagocytosis in the case of MAC. Initially, MBL was shown to increase neutrophil phagocytosis of MAC [22] but more recently, while MBL binding to MAC was confirmed, it has been shown to not increase macrophage phagocytosis, unlike SP-A and SP-D [48]. There are no clinical data on the association between MBL and MAC to assist in resolution of this conflict.

Respiratory Viral Infections

MBL status has been assessed in children with respiratory syncytial virus infection. In a small Brazilian study, respiratory syncytial virus patients had lower MBL levels than healthy controls and significantly more patients with severe disease requiring hospital admission had MBL levels <0.5 μg/ml [49]. However, there are no data on MBL binding to or inactivation of respiratory syncytial virus by which to judge the biological significance of these measured associations.

SARS was an emergent respiratory viral infection in 2003 that had mortality of approximately 10% and which temporarily paralysed global travel. Two case control studies of Chinese patients showed an association between carriage of the *B* variant *MBL2* allele, the only *MBL2* structural variant found in the Chinese population, and predisposition to SARS [4, 50]. There was no association between low *MBL2* haplotypes and death due to SARS. In vitro experiments show that MBL binds to SARS coronavirus, mediating C4 deposition and inhibiting viral infectivity in cell culture [4].

Although there are numerous in vitro data to indicate that MBL is able to inactivate influenza virus [24, 51], including in the absence of complement [52], there are no clinical studies that show an association between MBL deficiency and influenza. Interestingly, carriage of the *MBL2 B* variant allele was found to predict inadequate response to influenza vaccination, while TNF and IL-10 promoter polymorphisms did not [53].

MBL Deficiency and Fungal Respiratory Tract Infections

Aspergillus fumigatus is bound by MBL [19, 54]. In addition to activation of the lectin complement pathway, MBL also increases phagocytosis of *A. fumigatus* conidia

and oxidative burst by polymorphs [54]. Data on the role of MBL in protecting against invasive aspergillosis are available from animal models [54, 55]. Here, steroid-treated BALB-C mice were inoculated intranasally with *A. fumigatus* conidia. Intra-nasal recombinant human MBL was as effective as amphotericin B in protecting mice against lethal invasive aspergillosis.

Although not a human correlate of this mouse model of invasive aspergillosis, a small study of 11 patients with chronic necrotising pulmonary aspergillosis showed increased carriage of the *MBL2 D* variant in cases when compared with healthy controls [56]. One small study of non-HIV infected patients with cryptococcosis showed a trend towards MBL functional deficiency in those with CNS rather than pulmonary cryptococcosis. This result suggests that MBL is important in protecting against dissemination from a pulmonary cryptococcal focus rather than its establishment [57].

An increase in invasive fungal infections has been found among allogeneic stem cell transplant patients who either received donor stem cells bearing low-producing *MBL2* genotypes or where the recipient MASP2 genotype contained a variant allele. This study included only a small number of patients with probable invasive pulmonary aspergillosis but still found a strong association with the above MBL/MASP variants on multivariable analysis [58]. The issue of association of increased infection risk in haematopoietic stem cell transplant recipients from donors with variant *MBL2* genotype remains unresolved but more likely, the major association is with recipient *MBL2* genotype as MBL is produced in the liver. Additionally, haematopoietic stem cell transplant using wild-type *MBL2* cells has been shown to not correct low MBL levels in recipients with variant *MBL2* genotype [59].

MBL and Chronic Lung Diseases

MBL deficiency appears to be an important modulator of the severity of lung disease in cystic fibrosis. Danish CF patients with MBL-deficient alleles had greater decline in lung function and lower survival than controls with normal *MBL2* genotypes [60]. As with other humans studied, a small minority of these CF patients had low levels of MBL detected in expectorated respiratory secretions. The high levels of neutrophil enzymes found in the sputum of CF patients were also able to degrade MBL in vitro [60]. A large study of Canadian CF patients confirmed this association, showing that those with low-producing MBL genotypes were colonised with *P. aeruginosa* at an earlier age. There was also a significant interaction between another variant gene, transforming growth factor beta 1, which was previously shown to affect CF progression [61], and MBL deficiency in this study with a higher rate of decline in lung function demonstrated [6]. Other groups have also found associations between MBL status and CF disease parameters. Accelerated reduction of lung function was found in North American CF patients with MBL levels <0.2 μg/ml [62]. MBL deficiency can now be regarded as a significant indicator of the risk of progression of CF.

The association between MBL status and chronic obstructive pulmonary disease (COPD) is being actively investigated. Patients with COPD who carried the *MBL2 B* variant alleles were shown to be more likely to have hospital admissions due to infective exacerbations of their airway disease [63]. Pulmonary MBL levels measured in BAL, along with surfactant protein and mannose receptor levels were found to be reduced in patients with COPD, more markedly so in those actively smoking [64]. This study confirmed that azithromycin produced improved phagocytosis by alveolar macrophages and sought a link with collectin activity. No differences in MBL or mannose receptor levels were induced in COPD patients by azithromycin treatment although the numbers studied were small [64]. The contribution of MBL to improved phagocytosis seen in azithromycin-treated COPD patients was not proven and it will not be mediated by mannose receptors as these are not involved in the interaction between MBL and alveolar macrophages.

Replacement Therapy

Recombinant human MBL that has the same complement-activating function as plasma-derived MBL has been produced [65] and is in early phase clinical studies in cancer chemotherapy and post-liver transplantation settings. If further data substantiating the role of MBL in the pathogenesis of severe bacterial pneumonia accumulate, it would be reasonable to consider this as an attractive target condition for MBL replacement given its prevalence and high mortality. Evidence of a survival decrement in CF patients with variant *MBL2* genotypes suggest that these patients with their chronic life-limiting condition could also benefit from intermittent MBL supplementation.

Molecular and Clinical Comparisons between Ficolins and MBL

Ficolins are lectin recognition molecules, structurally and functionally homologous to MBL (table 1). Three ficolins have been described: L-ficolin encoded by the

Table 1. Comparison of MBL and the ficolins emphasising their contribution to respiratory infection defences

Lectin	Tissue expression	Location	Function	Binding to respiratory organisms	Association with RTI
MBL	liver	serum; sites of inflammation including respiratory epithelial surface	lectin pathway activation; mediates opsonophagocytosis; clearance of apoptotic cells	*H. influenzae* *M. pneumoniae* influenza virus *M. tuberculosis* *L. pneumophila*	invasive pneumococcal disease; community-acquired pneumonia; tuberculosis; Legionnaires' disease; SARS; progression of cystic fibrosis
L-ficolin	liver	serum	lectin pathway activation; mediates opsonophagocytosis; clearance of apoptotic cells	*S. pneumoniae* serotypes 11A 11D 11F	recurrent RTI in children
M-ficolin	monocytes; lung type II alveolar cells; spleen	PBMC surface; possibly respiratory epithelial surface	mediates opsonophagocytosis	not detected	not studied
H-ficolin	lung type II alveolar cells and bronchial epithelial cells; liver	respiratory epithelial surface; serum	lectin pathway activation; clearance of apoptotic cells	not studied	not studied

RTI = Respiratory tract infection; PBMC = peripheral blood mononuclear cell.

FCN2 gene [66], M-ficolin *(FCN1)* [67] and H-ficolin *(FCN3)* [68]. L-ficolin is a liver-expressed serum molecule, M-ficolin is purely a tissue ficolin expressed in the lung [67], monocytes and the spleen [69], and H-ficolin is also a liver-expressed serum ficolin but is additionally expressed in the lung by bronchial and type II alveolar epithelial cells [70]. When ficolin gene expression was compared by real-time quantitative PCR, *FCN3* was found to be more highly expressed in the lung than *FCN1* [71]. A direct comparison of C4 deposition between MBL and the ficolins indicated that H-ficolin had the highest complement-activating capacity [71].

While the ficolins share the collagenous structure of MBL with homogenous subunits assembling into oligomeric proteins, the carbohydrate recognition domain of MBL is replaced by a fibrinogen-like domain. This region of the ficolins mediates binding to the microbial cell wall carbohydrate residues with some overlapping specificity to MBL. L-ficolin has also been shown to bind to lipoteichoic acid and peptidoglycan, important constituents of Gram-positive bacteria. Thus, binding of L-ficolin can be demonstrated to *S. pneumoniae* and *S. aureus* in a serotype-specific manner, with this binding not being reduced by the presence of capsule [28]. M-ficolin binds to *S. aureus* but H-ficolin does not bind to either *S. pneumoniae* or *S. aureus* [28].

Promoter and structural sequence polymorphisms have been identified in all the ficolin genes [72], but particularly *FCN2* [73] and *FCN3* [74]. Unlike *MBL2* polymorphisms, which produce substantial variation in the concentration and function of MBL over a 1,000-fold range, ficolin levels in the presence of these *FCN2* and *FCN3* polymorphisms vary at most by only a factor of 20 [74, 75], with no absolute deficiency identified. Associations between *FCN2* [76] and *FCN3* [74] single nucleotide polymorphisms and low or high serum levels are becoming apparent.

In contrast to the wide range and number of infectious disease associations shown for MBL, there are limited studies of the significance of low L-ficolin levels or the presence of L-ficolin single nucleotide polymorphisms. In a cohort of Polish children with recurrent respiratory tract infection, a group of patients with underlying atopic disorder were significantly more likely to have L-ficolin levels less than the lower range seen in healthy controls. The same did not apply to the group of children with recurrent infection but no other detectable laboratory abnormalities [77]. No association between *FCN2* polymorphisms and disease susceptibility [78] was found in the UK invasive pneumococcal disease cohort previously studied for MBL deficiency association [2]. MBL status did not appear to alter the negative disease association with *FCN2* polymorphism [78]. To date, the *FCN1* and *FCN3* single nucleotide polymorphisms have not been sufficiently characterised to comment on their potential disease associations and clinical significance [72]. Overall, it can be said that ficolins play an overlapping role in mediating lectin pathway complement killing with MBL but the presence of ficolin-deficiency states that predispose to infectious diseases is uncertain.

Unresolved Issues in MBL's Role in Susceptibility to Respiratory Tract Infection

The location of the *MBL2* gene lies close to the SP-A and SP-D loci on chromosome 10q [79]. Potentially, some of the associations recorded above between *MBL2* polymorphism and lower respiratory tract infection are reflective of associated variation in the surfactant protein genes. There are substantial differences seen in the pulmonary and serum levels of SP-A and SP-D but few associations between either protein levels or genetic polymorphisms and susceptibility to respiratory tract infection [80]. Most of the above studies that demonstrate associations between MBL deficiency and respiratory infection do not additionally test for surfactant protein variation so the interdependence of these factors remains unresolved. One animal model study of invasive aspergillosis did compare the anti-aspergillus activity of collectins showing MBL and recombinant SP-D to have equivalent protective efficacy [55].

Ultimately, it may be that the presence of MBL in blood in the alveolar capillaries is more pertinent than the measured low concentrations of MBL in the respiratory secretions to the pathogenesis of severe pneumonia. This would be an appropriate explanation for the recent observations of increased mortality in bacterial pneumonia [5] and the reduced susceptibility to severe tuberculosis in patients with low MBL levels [44]. Future investigation of the interaction of the MBL present in respiratory secretions with pneumonic pathogens may substantiate an active role for the molecule in respiratory mucosal defences. Information from clinical studies available to date shows that MBL appears to play an important role in reducing susceptibility to severe chest infection as soon as respiratory pathogens enter the blood stream.

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