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EDITORIAL REVIEW

Is there a role for mannan/mannose-binding lectin (MBL) in defence against infection following chemotherapy for cancer?

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It is now nearly 4 decades since the relationship between the degree of neutropenia and the risk of bacterial and fungal infections was first recognized in patients treated for cancer [1]. Bodey *et al.* [1] made the observation that as granulocyte counts fell, the frequency, duration and severity of infections dramatically increased. Infections were noted to be worse during relapse of the underlying disease and the failure of leucocytes to recover following an infection carried a very poor prognosis. It is now clear that this is largely determined by both the underlying disease and potency of chemotherapy. Interestingly, however, it is now also apparent that patients differ in their susceptibility to infection in the context of neutropenia. This indicates that other factors are operating to protect patients from infection in this immunocompromised state.

Mannan or mannose-binding lectin (MBL), is a member of the collectin subfamily of C-type lectins, which also includes pulmonary surfactant proteins A and D [2]. Amongst the collectins, however, MBL alone initiates the lectin pathway of complement activation following binding to mannose, N-acetylglucosamine, fucose and glucose residues presented in the orientations and densities commonly found on microorganisms [3]. A structurally similar group of molecules, the ficolins, also activate the lectin pathway but differ from the collectins in their carbohydrate recognition domain (CRD) structure. MBL has been shown to bind to gram-positive and gram-negative bacteria, to yeasts, some viruses and also to protozoa and other parasites. Once bound, MBL activates the complement system in an antibody and C1-independent manner. This is predominantly mediated through a serine protease, MASP-2, which cleaves C4 and C2 to generate a C3 convertase. MBL may also promote opsonophagocytosis by a complement independent pathway [4]. With these attributes it is logical to consider that MBL could be an important line of defence during episodes of chemotherapy induced immunodeficiency.

The human collectin genes are located in a cluster on chromosome 10. Of two MBL genes, only *MBL-2* encodes a protein product. The *MBL-2* gene comprises four exons. Three single point mutations have been identified in exon 1 each of which leads to a functional deficiency of the MBL protein. Several polymorphisms have also been identified in the promoter region of the MBL gene, namely H/l, X/Y, and P/Q at positions –550, –221, and +4. Of these two promoter haplotypes, HYP, which is associated with medium to high levels of MBL, and LXP, which is associated with low MBL levels, appear to be most important. Largely through a combination of structural gene and promoter polymorphisms, MBL concentration can vary a thousand-fold in apparently healthy individuals, and approximately a third of the Caucasian population has genotypes conferring concentrations sometimes deemed 'insufficient'. The correlation between genotype and phenotype is relatively strong and so many studies infer MBL deficiency or insufficiency using genotype or phenotype alone.

There is considerable debate about the importance of MBL in the general population. A recent large study of adults in Denmark failed to find MBL deficiency to be a major risk factor for morbidity or death [5]. However numerous studies have now shown that MBL deficiency is associated with an increased susceptibility to infection. The young, elderly and immunocompromised seem to be most at risk. In 2001, two studies were published in the Lancet, one concerning children and one concerning adults, both of which reported an effect of MBL deficiency on the course of infectious complications following chemotherapy for cancer. Peterslund *et al.* [6] described 54 adults treated with chemotherapy for a range of haematological malignancies. No differences were observed between the distribution of MBL levels in this population and an apparently healthy population of local blood donors. However, in 16 patients who developed either bacteraemia, pneumonia or both within 3 weeks of starting chemotherapy, MBL levels were significantly lower than in patients without serious infections. Further analysis revealed that it was patients with an MBL concentration of 0·5 g/ml or less that were particularly at risk of severe infection.

In the paediatric study, MBL phenotype and genotype were determined for 100 children receiving chemotherapy [7]. Their MBL status was then correlated with the causes, frequency and duration of febrile neutropenic episodes. Children with variant MBL alleles suffered from twice as many days of febrile neutropenia compared to patients with a wild type genotype. In addition the mean duration of each febrile neutropenic episode was significantly higher in patients who were MBL deficient compared to

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MBL-wildtype patients. However, this study did not find a strong relationship between the frequency of infections and MBL status or between promotor haplotype and any of the measured clinical parameters.

Subsequently, another study with a much longer follow-up period reported that MBL gene polymorphisms were associated with major infections following allogeneic haemopoietic stem cell transplantation, with invasive bacterial, viral, and fungal infection occurring significantly more frequently among those with variant MBL alleles. Curiously, both the donor and recipient MBL status was found to be important. Even more curiously, the association between mbl-2 mutations and major infections was stronger and much more statistically significant for donors. The presence of the HYA promotor (high MBL level-producing) haplotype in either donor or recipient appeared to protect against infection [8].

While these studies, although not entirely consistent, fostered optimism that MBL replacement therapy could be a viable therapeutic modality in preventing infection in patients receiving chemotherapy, two later studies failed to confirm these findings. Both examined adults with a range of malignancies. Bergmann *et al*. [9] studied 80 patients with acute myeloid leukaemia (AML) and found no differences in frequency, severity or duration of fever in MBL replete or deficient patients. Kilpatrick *et al*. [10] studied 128 patients with more than half diagnosed with lymphoma or AML and in whom two thirds were receiving intensive conditioning prior to bone marrow transplantation. A major influence of MBL levels on rates or severity of infection was not detected, but a modest influence of MBL concentrations ≤100 ng/ml on susceptibility to infections was confirmed.

Two other recent studies may also be relevant. Rocha *et al*. [11] found no relationship between MBL structural mutations and post-transplant infections in over 100 donor-recipient pairs after HLA-identical sibling bone marrow transplantation. All of their transplants were HLA identical and non – manipulated, and this was proposed as an explanation for the difference between theirs and the Mulligan study [8]. Tacx *et al*. [12] conducted a prospective observational study on 177 hospitalized patients with new onset fever. In those febrile adults, microbial infection induced complement activation independently of MBL, and the authors concluded that MBL deficiency does not predispose to serious microbial infection in unselected adults.

Obviously, the magnitude of the influence of MBL in the context of chemotherapy-induced neutropenia must be considered uncertain, but could there be a rational explanation for these conflicting results? Firstly, most of the studies included patients with a variety of underlying malignancies. While it is practical to lump patients together on the premise that most will become neutropenic, in reality, the degree of immunodeficiency due to the disease itself and that which results directly from the selected chemotherapy regime will differ from patient to patient. This could be pertinent to all of these studies, most of which only enrolled around 100 patients. In the only paediatric study, the majority of patients were diagnosed with acute lymphoblastic leukaemia (ALL), which reflects the higher frequency of this condition in childhood. The chemotherapeutic regimes used in ALL are generally less 'toxic' than those used in AML. Secondly, different assays were used in the various studies. Some relied solely on genotype while others used a range of phenotypic assays. Only one study analysed both genotype and phenotype. While all of the assays used have been validated it is becoming clear that that the levels of MBL protein measured can differ significantly between assay systems [13]. Thirdly, the outcome measures also differed between the studies. In the paediatric study, MBL status was associated with the duration of fever, a parameter that has been used in related paediatric studies. Time to defervescence is shorter in children than in adults [14] and may reflect the underlying disease or response to chemotherapy in children. The definition of serious infection also differed slightly between the different adult studies. Fourthly, any febrile episode would have been treated, and with antimicrobial regimes, given both as prophylaxis and treatment. Each of the centres will have used different combinations of antimicrobial agents and GCSF and their use differs markedly between adults and children and between institutions. This could have a major bearing on any additional antimicrobial effect attributed to MBL. While any or all of these variables could have been responsible for the different conclusions drawn from these studies, there may also be a unifying physiological explanation.

Inevitably, the majority of bacterial pathogens encountered in neutropenic patients are normally prevented/controlled by phagocytes. Few organisms appear to be predominantly killed by the complement system alone. The exception to this is *N. meningitidis,* which is seen more commonly in terminal complement component deficiencies and for which MBL has been shown to enhance killing in the absence of phagocytes [15]. This organism is rarely seen in neutropenic patients. This suggests that MBL may not influence the susceptibility/course of bacterial infection in the complete absence of phagocytes. A recent study in MBL deficient mice found that MBL deficiency alone increased the fatality rate following an intravenous inoculation of *Staphylococcus aureus*. However when the bacteria were introduced into the peritoneum, it was only in the absence of neutrophils that MBL was seen to limit infection [16]. Therefore the route/nature of infection may be critical in exposing the importance of MBL in neutropenic patients.

In the studies in which MBL status did not influence infectious susceptibility, the patients are likely to have received more intensive chemotherapy. The majority of the patients had AML which is associated with a particularly high rate of bacterial infectious complications. In the Peterslund and Neth studies [6,7], AML was less common. Support for the view that some phagocytic function is required for MBL to be functionally effective against bacteria comes from Mulligan who found that MBL coding mutations in stem cell donors only influenced infections following phagocytic recovery. Optimizing phagocytic function through MBL could also be the explanation for the reduction in fever described by Neth *et al*. It has been shown that MBL can enhance the uptake of staphylococci by phagocytes both directly and through an increase in opsonic C3 fragments [4]. Such mechanisms could explain how MBL could be operating when phagocytes are present at low numbers or are functionally impaired. In this regard it is important to note that not all phagocytes are completely destroyed by chemotherapy. Shi *et al*. [16] show that MBL significantly enhanced the function of resident peritoneal macrophages in mice rendered neutropenic by administration of Cyclophosphamide. Clearly other mechanisms may also influence nonbacterial causes of infection. MBL has been shown to inhibit viral infectivity and has also been implicated in susceptibility to aspergillus infection [17].

The studies published to date indicate that MBL does not play a universal role in modulating infections in patients receiving chemotherapy for underlying malignancy. While it seems likely

that patients with AML, who are at a high risk of bacterial infections, are unlikely to benefit from MBL therapy, the studies are also consistent with the possibility that under some circumstances, MBL could be important. Paradoxically, MBL could prove to be most beneficial in those patients receiving chemotherapy associated with a lower risk of infectious complications. A number of studies have attempted to identify subsets of patients with febrile neutropenia at lower risk of significant infection and/or complications who can be managed safely at home or following early discharge from hospital. This approach potentially reduces the risk of nosocomial infection and the development of bacterial resistance, and has obvious cost benefits. It would also enable patients to spend more time at home with their families with the aim of improving quality of life. Further studies are required to identify the role of MBL in this patient population and determine whether MBL replacement therapy could be cost effective in an appropriately targeted population.

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