Infusion of plasma-derived mannan-binding lectin (MBL) into MBL-deficient humans

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Abstract
Our first experience of mannan-binding lectin (MBL)-replacement therapy was with a patient experiencing recurrent erythema multiforme associated with reactivation of herpes simplex virus; his erythematous eruptions could be controlled with infusions of fresh frozen plasma containing MBL, but not with plasma lacking MBL. Some years later, we treated a young girl with recurrent, debilitating infections with purified MBL; this was also followed by a dramatic clinical improvement. We have now carried out a phase I clinical trial on 20 MBL-deficient, but healthy, adult volunteers. The MBL was prepared by the State Serum Institute in Copenhagen, Denmark, from blood donor plasma. Each volunteer received a total of 18 mg of MBL in three 6-mg doses given intravenously once a week over 3 weeks. The volunteers were monitored closely after each infusion and no adverse clinical or laboratory effects were observed. Laboratory parameters included C-reactive protein, various complement components, and antibodies to MBL, HIV and hepatitis viruses. C3a (the anaphylatoxin derived from complement component C3) was monitored for signs of complement activation, but no significant infusion-associated fluctuations were observed. Serum levels of MBL after each 6-mg infusion ranged between 1200 and 2500 ng/ml. The half-life of the infused MBL was about 70 h, or 3 days. It was concluded that infusion of purified MBL manufactured by the Danish State Serum Institute is a safe procedure. However, adults may have to be given 6 mg or more at least twice weekly to maintain protective plasma MBL levels in MBL-deficient individuals.

Background
The detection of an opsonic defect, first reported by Miller et al. [1] in 1968 and confirmed by Soothill and Harvey [2] in 1976, was based on microscopic evaluation of ingestion of baker’s yeast particles by neutrophils. As this method is subjective and cumbersome we attempted to devise more objective and quantitative assays, including a radiometric method based on incorporation of [3H]uridine by a fast-growing strain of brewer’s yeast. As only extracellular yeast can incorporate uridine, the rate of phagocytosis shows a linear and inverse relationship with the amount of radioactive uridine that the yeast particles incorporate over a defined period [3]. Measured in this way, phagocytic uptake of the yeast increases in a linear fashion with concentrations of serum ranging from 0.5% to about 5%. This opsonic activity was independent of antibodies and activation of C1q, the first component of the classical complement pathway. About 7% of apparently healthy individuals were subnormal in this assay compared with 30% of individuals with recurrent or abnormal infections [3]. After it was demonstrated that this defect is due to deficiency in mannan-binding lectin (MBL) [4], a close correlation was observed between MBL levels and the opsonic activity measured by the radiometric assay. Furthermore, addition of purified MBL to a concentration of 1 µg/ml fully restored the opsonic activity of defective sera [5].

In 1979, using this assay, we detected an opsonic defect in a 25-year-old man with several years’ history of recurrent attacks of erythema multiforme that were consistently associated with reactivation of herpes simplex virus (cold sores). The erythematous eruptions could be prevented by infusing the patient with about 1000 ml of fresh frozen plasma with strong opsonic activity within 3–4 days after the first symptoms of the herpes simplex reactivation. However, the erythematous attacks could not be prevented by infusing plasma that was defective in opsonic activity. The patient had high titres of serum antibodies to herpes simplex, and plasma with low levels of such antibodies could prevent the attacks of erythema multiforme. Prior to the plasma treatment, the erythema attacks had been treated with relatively high doses of prednisolone (up to 40 mg/daily), but the steroids could be discontinued after the plasma treatment was instituted, and the attacks gradually became less frequent. When last contacted the patient had not experienced any episodes of erythema multiforme for more than 1 year compared with typically 5 attacks every year before the initiation of plasma treatment.

When HIV infections were recognized in the early 1980s, experimental treatment with plasma became highly questionable, and it was not until MBL deficiency had been identified as the cause of the defective yeast opsonization, and MBL became available in therapeutic quantities, that reconstitution of patients with opsonic defects and abnormal infections
became a feasible option. It was then decided to treat a 2-year-old girl who had suffered debilitating and recurrent infections from the age of 4 months, with purified MBL. The MBL was isolated from plasma of Danish blood donors by the State Serum Institute in Copenhagen, Denmark [5]. The only immunological abnormality that could be detected in this girl was an opsonic defect determined by the radiometric assay, and she had a very low level of MBL. No evidence of complement activation could be detected in vitro when purified MBL, which normalized her opsonic activity, was added to her serum.

The girl was given daily infusions (2 mg) of MBL for 3 consecutive days and this treatment was repeated after 10 days. No adverse reactions were observed, the MBL concentration in her blood reached normal values after each infusion, and the opsonic activity of her plasma was temporarily restored to normal. Furthermore, a striking clinical improvement coincided with the MBL infusions [5] and she has remained free of recurrent or abnormal infections during the 8 years since she received this treatment (M. Stefansson, personal communication). The apparent clinical benefits of the MBL infusion in this patient could of course have been coincidental, simply reflecting a maturation of her immune system including the expanding repertoire of her immunological memory. However, it is also possible that the MBL infusion may have broken a vicious circle initiated and maintained by a combination of relatively late immunological maturation, MBL deficiency and the recurrent infections. It should be emphasized in this context that persistent or recurrent infections can be considered immunosuppressive [6]. These possibilities can only be evaluated by controlled clinical trials and it was therefore decided to carry out a phase I trial to assess the safety and pharmacokinetic aspects of MBL infusions.

**Phase I trial**

The trial will be described in detail elsewhere. The protocol, including the subject information sheet, was approved by the National Bioethics Committee and the State Committee on Pharmaceuticals in Iceland. The MBL was purified by the State Serum Institute in Copenhagen from the plasma of blood donors. The 20 volunteers who participated in the trial were mostly recruited from individuals who had previously been investigated for suspected food allergy or intolerance. Interestingly, two of the participants reported, spontaneously and independently, when they were interviewed for possible delayed adverse reactions 24 weeks after the trial, that they had been experiencing a persistent semi-purulent nasal discharge for a number of years. They had not reported this at the recruitment to the trial as they had learnt to live with it and assumed this to be normal. However, the discharge stopped completely for 3–4 days after each of the three MBL infusions but reappeared before the next infusion in both of these participants.

**Further development**

There are at least two clinical conditions that appear to be feasible for further clinical trials with MBL. Firstly, MBL-deficient patients with transient neutropenia caused by treatment of malignancies have been reported to be at risk of significant infections [7–9]. Another group of patients that might benefit from temporary MBL reconstitution are children who are referred to specialized centres for immunological assessment due to debilitating infections. We and others have reported that a substantial proportion of such children have opsonin or MBL deficiency [3,10]. Although the improvement in the child that we infused with MBL could obviously have been fortuitous, it is well established, but often forgotten, that recurrent or persistent infections can be immunosuppressive and thereby initiate and maintain a vicious circle of disease, especially in individuals with immature or aging immune systems. It can therefore be envisaged that temporary reconstitution with MBL might have a significantly beneficial impact on MBL-deficient children who are immunocompromised in this way.

**References**


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