Is mannan-binding lectin (MBL) detectable on monocytes and monocyte-derived immature dendritic cells?

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Abstract
MBL (mannan-binding lectin; also called mannose-binding lectin) is a circulating C-type lectin with a collagen-like region synthesized mainly by the liver. MBL may influence susceptibility to infection in recipients of stem cell transplants, and it has even been suggested that the MBL status of a donor can influence the recipient’s susceptibility to post-transplant infections. We have previously reported that MBL can be detected on human monocytes and monocyte-derived dendritic cells, based on detection using biotinylated anti-MBL, suggesting that those cells could synthesize MBL. If true, permanent MBL replacement therapy could be achieved by stem cell infusions. However, two other groups independently failed to find mbl-2-encoded mRNA in monocytes. Therefore, to confirm or refute our previous observations, we used an alternative experimental strategy. Instead of using biotinylated antibody and labelled streptavidin, detection of surface MBL was attempted using MBL-specific primary antibodies (131-1, 131-10 and 131-11) followed by fluorescein-labelled anti-IgG, and controlled by the use of non-specific IgG as primary antibody. Monocytes were counterstained with anti-CD14-PE before FACS analysis. Adherent monocytes were also cultured for 48 h in serum-free medium or converted into immature dendritic cells by culture with IL-4 (interleukin-4) and GM-CSF (granulocyte/monocyte colony-stimulating factor). During FACS analysis, the dendritic cells were gated after counter-staining with anti-CD1a-PE. MBL was readily detected on the surface of fresh monocytes using all three specific anti-MBL monoclonal antibodies, but specific anti-MBL binding was greatly diminished after monocytes had been cultured for 2 days in serum-free medium. Moreover, we could not detect any MBL present on the surface of monocyte-derived dendritic cells. We therefore conclude that MBL is indeed present on the surface of fresh human monocytes. However, in view of the mRNA findings of others and our own previous observation that no secretion of MBL took place in culture, we presume that the surface-bound MBL is derived from autologous plasma and not synthesized by the cells. This conclusion is consistent with our in vivo findings in stem cell transplant patients which provided evidence against significant extra-hepatic production of serum MBL. It provides no ready explanation for the remarkable observation of Mullighan, Heatley, Szabo, Grigg, Hughes, Schwarer, Szer, Tait, Bik To and Bardy [(2002) Blood 99, 3524–3529] that the presence of variant alleles of mbl-2 in stem cell donors can influence susceptibility to serious infections in their recipients.

Background
MBL (mannan-binding lectin; also called mannose-binding lectin) is a plasma protein synthesized mainly by the liver. High molecular mass, multimeric forms of MBL can opsonize various micro-organisms, both directly and through activation of complement via the lectin pathway, contributing to innate immunity. Several point mutations in the first exon of the structural gene markedly influence the formation of multimers and therefore both carbohydrate-binding and complement-activating activities. Mutations in the promoter region also influence serum MBL concentration and strong linkage disequilibria exist between promoter region and structural gene polymorphisms. There is a strong correlation between the seven common haplotypes (and 28 common genotypes) and functional MBL protein concentration [1,2]. Many disease association studies, at the DNA and/or protein level, support the concept that relative MBL deficiency predisposes to infection and can influence the course of numerous conditions complicated by infections [2,3]. Consequently, MBL replacement therapy is currently under development and evaluation.

Several studies investigating the possible influence of MBL on susceptibility to infections following chemotherapy have recently been published ([3–5]; Table 1). In these series, treatment was given either to induce remission or as conditioning before stem cell transplantation. MBL deficiency or insufficiency was variously defined either by a protein level (with cut-off values ranging from 100 to 1000 ng/ml) or at the DNA level (presence of

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Key words: monocyte-derived dendritic cell, mannan-binding lectin (MBL), mannose-binding lectin, stem cell transplantation, mannan-binding lectin replacement therapy.

Abbreviations used: MBL, mannan-binding lectin; PE, phycoerythrin.

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mutant alleles or variant haplotypes). Follow-up periods have varied enormously, ranging from 3 weeks to several years (Table 1). Patients both within and between series were often heterogeneous with regard to diagnosis and therefore treatment. It is therefore unsurprising that the results of these investigations have been very inconsistent [3].

One particular observation arising from this confusing literature was most curious and remarkable. Mullighan et al. [6], studying stem cell transplantation patients, reported that the presence of variant alleles of \textit{mbl}-2 in recipients or donors increased susceptibility to serious infections post-transplant, and that the \textit{mbl}-2 status of the donor had a stronger influence than that of the recipient. Donor \textit{mbl}-2 coding mutations were independently associated with infection and therefore could not be explained by genotype sharing between siblings. It was most surprising that a donor’s MBL status could have any influence at all. Nevertheless, these findings have received some support from a subsequent prospective study by the same group [7]: there was a trend to-wards infection susceptibility with donor \textit{mbl}-2 genotype, of borderline statistical significance. Moreover, a relationship between donors’ low MBL-conferring \textit{mbl}-2 genotypes and susceptibility to invasive fungal infections following allogeneic stem cell transplantation has been independently reported [8]. On the other hand, a recent as yet unpublished study found no influence of donors’ \textit{mbl}-2 genotype on recipients’ serum MBL or infection incidence (O. Neth, personal communication).

This surprising relationship could most easily be explained if some component of the stem cell donation could synthesize MBL or could differentiate into cells capable of synthesizing MBL. A few years ago, we investigated this possibility and indeed found MBL on monocytes and monocyte-derived dendritic cells [9], although no secretion of MBL into the medium of such cell cultures could be detected. However, two other groups failed to detect \textit{mbl}-2-derived mRNA in monocytes or whole blood preparations, although one of them found traces of \textit{mbl}-2 mRNA in cord blood leucocytes (stem cells?) and differentiated THP-1 cells, a monocytic cell line [10,11].

A further concern was a report that biotinylation can alter the binding properties of C-reactive protein [12]. This consideration raised the worrying possibility that biotinylated MBL may also behave differently from native MBL and therefore that our previous results may have been artefactual.

**Objective**

Our previous results were based on MBL detection using biotinylated anti-MBL. We therefore determined to confirm or refute our previous observations by an alternative experimental strategy.

**Strategy**

Detection of surface MBL was attempted using MBL-specific monoclonal antibodies (clones 131-1, 131-10 and 131-11, kindly given by Claus Koch, State Serum Institute, Copenhagen, Denmark). Cells [human peripheral blood mononuclear cells or dendritic cells derived from them by culturing in the presence of IL-4 (interleukin-4) and GM-CSF (granulocyte/macrophage colony-stimulating factor)] were washed three times in a serum-free medium and suspended in Tris-buffered saline containing 5 mM calcium, 1% BSA and 0.1% sodium azide. Cells were incubated with anti-MBL antibodies or with non-specific murine IgG1 antibodies at a concentration of 9000 ng/ml for 30 min at room temperature. After washing, the cells were further incubated under similar conditions with fluorescein-conjugated goat anti-mouse IgG. Finally, cells were counterstained with PE (phycoerythrin)-conjugated anti-CD14 (to identify monocytes) or PE-conjugated anti-CD1a (to identify dendritic cells) before application to a FacScan flow cytometer and analysis using Cell Quest software.
**Figure 1 | The presence of MBL on the surface of monocytes**

Flow cytometric histograms demonstrate the presence of MBL on the surface of monocytes after incubation with three specific anti-MBL monoclonal antibodies (clones 131-1, 131-10 and 131-11 recognizing different epitopes in C, D and E respectively). Each histogram is overlaid in grey with the non-specific murine IgG1κ profile (B). An immunoglobulin-free negative control (A) and an anti-CD11b positive control (F) were also included. (i) Freshly prepared monocytes, (ii) the same monocytes after culture for 2 days in serum-free medium.

**Cell-surface localization?**

No MBL could be detected on the surface of immature dendritic cells with any of the three anti-MBL monoclonal antibodies used. The profiles obtained were superimposable on that obtained with non-specific murine IgG or with buffer used instead of immunoglobulin during the first incubation. In contrast, an anti-CD11b antibody used as a positive control caused a significant shift, as expected.

However, MBL was clearly detected on the surface of monocytes with all three specific monoclonal antibodies (recognizing three distinct epitopes) utilized (Figure 1). There was also some indication of modest non-specific antibody
binding. Similar results were obtained when the procedure was modified by pre-incubating cells with non-specific IgG before the addition of specific antibody. However, this specific anti-MBL binding was substantially diminished after the same monocytes had been cultured in a serum-free medium for 2 days.

Significance

Our previous observations were based on the use of biotinylated anti-MBL detected with a streptavidin conjugate. The alternative immunochemical strategy described here only partially confirms those earlier observations. No MBL was detected on monocyte-derived dendritic cells, but there was good evidence that MBL is indeed present on the surface of fresh monocytes. Moreover, it seems to be very tightly bound, since it was present even after several washes and even (to some extent) after days of culture in a serum-free medium.

Our original interpretation of the presence of cell surface MBL, that it had been synthesized by the monocytes, no longer seems plausible. In view of the mRNA findings of others and our own observation that no secretion of MBL took place in culture, we now presume that surface-bound MBL has been acquired from autologous plasma.

It is nonetheless surprising that the MBL appears to be bound so tightly, resisting the attempts of removal by numerous washes. No reason for this behaviour was apparent, and is not known if any of this binding is due to the sugarsensitive surface receptor [9] or whether it is mediated by membrane phospholipids [13].

Implications for MBL replacement therapy

Direct evidence, from this group [14] and others [15], leads us to believe that stem cell preparations do not contribute to circulating MBL, and indeed any appreciable extrahepatic production of MBL seems unlikely, except perhaps at inflammatory loci. Moreover, cultured human CD34⁺ cells do not produce MBL (O. Neth, personal communication). It seems unlikely therefore that stem cell preparations could be used as a source of MBL for prophylactic or therapeutic purposes. That is unfortunate because stem cell transplantation would have provided a one-off cure, in contrast to the need for regular intravenous infusions of the protein which has a rather short biological half-life ($t_{1/2}$) [16].

How, then, can we account for Mullighan et al.’s finding that the presence of variant alleles of mbl-2 in stem cell donors can influence susceptibility to serious infections in their recipients? One possibility is that the association is with another gene in linkage disequilibrium with mbl-2, and therefore nothing to do with MBL at all. Alternatively, MBL might induce some kind of maturation of autologous cells. If so, an MBL-deficient stem cell donor could be contributing an immunologically naïve preparation less advantageous than that from MBL-sufficient/normal individuals. If the latter be true, MBL from replacement therapy could still have beneficial effects lasting much longer than would be predicted from its short biological $t_{1/2}$.

References

12 Taylor, K.E. and van den Berg, C. (2006) Structural and functional comparison of native pentameric, denatured monomeric and biotinylated C-reactive protein. Immunology 120, 404-411

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