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Genetic Heterogeneity of Mannose-Binding Proteins: The Jekyll and Hyde of Innate Immunity?

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Introduction

Innate immunity is the immediate ability of a host to prevent and limit an infectious challenge (Fearon and Locksley 1996; Medzhitov and Janeway 1997). The barriers to a noxious challenge take on many guises. As new knowledge accrues, a conceptual congruency in the spectrum of innate immunity in a variety of organisms, ranging from plants to humans, is emerging. The recognition of conserved proteins or domains of proteins has allowed insights into the evolution of nonclonal first-line host defenses, so that we are now able to draw analogies between tobacco plants, fruit flies, frogs, horseshoe crabs, mice, and humans.

Types of barriers to infection include the skin, the mucocillary escalator in the lung, antimicrobial peptides, bacterial-permeability inhibitors, antibodies, the complement system, lipopolysaccharide-binding proteins, certain classes of lymphocytes, and tissue macrophages. Although these molecules and cells undoubtedly act in concert, it is reasonable to assume that a versatile circulating effector molecule would be of added benefit to the host. Here, I discuss one such molecule, the mannose-binding protein (MBP), or mannan-binding lectin, which appears to play an important role in host defense but which, under certain circumstances, also may be deleterious to the host (Garred et al. 1994; Turner 1996).

MBP as an Ante-antibody: The Dr. Jekyll Role in Innate Immunity

If one had to design an idealized first-line host-defense molecule, several features might come to mind. These features include the ability to distinguish the self or the altered self from the nonself, a broad repertoire of recognition, appropriate regulation and distribution, adequate effector mechanisms, and the ability to trigger a specific immune response.

Overwhelming in vitro evidence indicates that human MBP has many of the features of an idealized first-line host-defense molecule. MBP's predominant role is as a so-called ante-antibody (Ezekowitz 1991). Like immunoglobulins, MBP binds microorganisms and enhances their uptake by phagocytic cells. However, as part of the acute-phase response, MBP is induced rapidly, in a period of minutes to hours (Ezekowitz et al. 1988), which is in contrast with the response period of days required by the humoral immune system. MBP is synthesized in the liver but is found in nasopharyngeal secretions, middle-ear fluid, inflamed joints, and amniotic fluid, as well as in the serum (Garred et al. 1993; Hoppe et al. 1994; Molhotra et al. 1994; Epstein et al. 1996; Turner 1996). Its overall structure resembles the first complement component C1q (Drickamer et al. 1986): like C1q, MBP has globular heads and collagen tails (Lu et al. 1990); and its monomers assemble as trimers, and its trimers form oligomers up to hexamers of trimers, which is the C1q-like configuration. Like C1q, MBP interacts with and initiates the complement cascade. MBP directly initiates the classic complement pathway, hence abrogating the need for an antibody. Two newly discovered, novel MPB-associated proteases, MASP1 (Matsushita and Fujita 1992; Madsen et al. 1994) and MASP2 (Thiel et al. 1997), directly activate the classic pathway convertase, and, hence, they mediate the cleavage of complement component C3 without requiring activation of the C1 complex. Cleaved C3 is an opsonin in which cleavage represents the common step between the traditionally defined antibody-dependent and -independent pathways. A consensus view is emerging that this MBP, or so-called lectin, pathway is the primordial mechanism of complement activation.

MBP recognizes a broad range of infectious agents, such as gram-positive and gram-negative organisms, yeast, parasites, mycobacteria, and certain viruses, such as HIV, the influenza virus, and the herpes virus (Epstein

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et al. 1996). Thus, MBP is like a broad-spectrum antibody that recognizes a range of structural patterns, rather than a single, defined epitope. The idea of pattern recognition in host defense was first raised by Janeway (1989), who postulated that lymphocytes express receptors for the "patterns" or the "antigenic arrays" common to multiple infectious agents. These lymphocyte receptors would then be expected to trigger co-stimulatory activity, an early step in the generation of a clonal immune response. The description of a human homologue of the *Drosophila Toll* (see Dushay and Eldon 1998 [in this issue]) is an important first step in validation of this concept, although the ligand and hence the pattern recognized by human TOLL remain to be defined. We have extended and expanded this concept of pattern recognition to include the circulating effector molecules, as well as the receptors expressed on the surface of macrophages. Accordingly, we consider MBP to be a pattern-recognition molecule.

One challenge has been to define the biochemical basis by which MBP is able to recognize a range of apparently disparate structures but does not bind self-glycoproteins. In fact, the pattern recognized by MBP is remarkably simple. The equatorial orientation of the C3-OH and C4-OH groups of a sugar moiety defines the pattern recognized by MBP (Weis et al. 1991; 1992). The pattern, as defined by structural analysis, is represented in *N*-acetylglucosamine, glucose, and fucose, as well as in mannose and the carbohydrate moieties found in the cell walls of many infectious agents. Importantly, galactose and sialic acid, the penultimate and ultimate sugars, respectively, that decorate human self-glycoproteins, are not accommodated within the MBP binding site. In addition, the crystal structures of the human and the rat MBP carbohydrate-recognition domains (CRDs) and neck regions illustrate that the MBP neck region forms a triple α-helical coil that orients each CRD of human MBP 45 Å or 53 Å apart (Sheriff et al. 1994; Weis and Drickamer 1994). The organization of self-oligosaccharides appears to be unable to span this distance, unlike that of the complex-carbohydrate arrays that decorate the surfaces of microorganisms (R. Putzer, R. A. B. Ezekowitz, and T. Stehle, unpublished data).

MBP in Human Immunity

The importance of MBP in first-line host defense is supported further by the description of patients who lack MBP and who have an apparent phenotype of recurrent infections (Super et al. 1989). Turner et al. (1993) found an association between a phenotype of recurrent infections and a polymorphism at codon 54 (B allele; table 1) of the *MBP* gene that results in the replacement of a glycine by an aspartic acid, within the collagen region of MBP (Sumiya et al. 1991). Turner (1996) postulated that MBP may be particularly important in children <18 mo of age, since competent antibody responses to carbohydrate antigens are developed only after this age (Garred et al. 1992*a,* 1992*b,* 1996; Lipscombe et al. 1992; Turner et al. 1993; Madsen et al. 1994, 1995; Turner 1996). Subsequent studies have revealed two additional alleles, the C allele and the D allele. The B allele results in a G54D substitution at the sixth collagen repeat, resulting in an unstable protein and, hence, in very low to undetectable serum levels of MBP. MBP containing only the B-type chains can be expressed in vitro, but it does not bind MASP1 and is unable to activate the complement. AB heterozygotes have a 20-fold reduction in MBP levels. The B chain appears to act as a dominant negative and disrupts the assembly of the collagen helix. The C allele results in a G57E substitution. AC heterozygotes have intermediate levels of MBP. The D allele results in a C52D substitution and appears to cause a milder defect. The frequency of the C allele is ~33% in African populations (Garred et al. 1992*a,* 1992*b,* 1996; Lipscombe et al. 1992; Turner et al. 1993; Madsen et al. 1994, 1995; Turner 1996), whereas the frequency of the B allele is 13% among Caucasians (table 1).

Table 1

MBP-Allele Mutations and Frequencies in Three Different Ethnic Groups

	MBP Allele[a]			
	A	B	C	D
Mutation:				
Location	Wild-type	Exon 1	Exon 1	Exon 1
Codon	Wild-type	54	57	52
Amino acid change	None	Gly→Asp	Gly→Glu	Arg→Cys
Frequency:[b]				
Africans (n = 56)	.69	.03	.23	.05
Caucasians (n = 123)	.80	.13	.02	.05
Eskimos (n = 73)	.87	.13	NF	NF
Activates complement?	Yes	No	ND	ND

Note.—Adapted from Garred et al. 1992*a*.
[a] NF = not found; and ND = not determined.
n = the number of unrelated individuals investigated.

MBP as Mr. Hyde?

The high frequency of MBP mutations led Garred et al. (1994) to suggest that, under certain circumstances, MBP may be protective, whereas under other circumstances there may be a selective advantage for the het-

erozygous state that results in low MBP levels. According to this hypothesis, because wild-type MBP can opsonize parasites or mycobacteria, it actually may assist in the hematogenous spread of these infectious agents, or it may enhance the survival of intracellular pathogens. If this idea is carried a step further, the B and C alleles would be expected to confer some protection against the spread of the disease, hence causing the selective advantage of the heterozygote state.

MBP also may function in autoimmune disorders: It has been found in the fluid isolated from patients with rheumatoid arthritis, and it appears to be able to bind agalactosyl antibodies, which bear exposed *N*-acetylglucosamine moieties—patterns that are recognized by MBP (Holmskov et al. 1993). When these altered antibodies accumulate, MBP would be expected to bind to them and activate the complement, thereby stimulating the inflammatory process. In this regard as well, MBP alleles that result in low expression levels or those that fail to activate the complement may be seen as protective.

In host defense, MBP appears to have a role as an ante-antibody, serving as a primitive pattern-recognition molecule that confers protection to the host during the lag time required for the generation of a specific clonal immune response. However, under certain circumstances, MBP may lead to autoimmune disease or may facilitate infection by intracellular pathogens. The availability of suitable mouse models will cast more light on the insights already gained from the human experience.

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