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THE MANNOSE-BINDING LECTIN: AN INFECTION SUSCEPTIBILITY GENE

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1. INTRODUCTION

A critical but unanswered question is what defines each individual's pre-morbid susceptibility to infection? We propose that individuals must have an "immune haplotype" that shapes their response to infectious agents. Infection is a balance between the intrinsic virulence of the infectious agent and the host defenses. Recent viral outbreaks of SARS and influenza serve to illustrate this point as these viruses cause severe disease in certain individuals, yet there are others in whom the same infectious challenge results in minimal symptoms. On the other hand it might be that those self same people who are resistance to one particular viral infection might be susceptible to other infection challenges. Similar rules can apply to susceptibility to bacterial infections.

We hypothesize that individual variations in a set number of genes that regulate both innate and adaptive immune responses might explain this individual variation in response to an infectious challenge. The mannose-binding lectin (MBL) serves as a broad first line host defense molecule and presents an interesting opportunity to explore this hypothesis further. MBL appears to be a prototypic pattern recognition molecule that is able to recognize the molecular patterns that decorate a wide range of microorganisms. Infectious agents that are recognized by MBL include certain Gram positive and Gram negative bacteria, yeast, parasites, mycobacteria, and viruses [1-3]. The idea that a relative lack of MBL might predispose the host to infection was based on the description of an MBL-dependent opsonic defect in human serum that correlated with a phenotype of recurrent infection [4]. These patients were found to have one of three substitution single nucleotide polymorphisms (SNPs) in exon 1 of the MBL gene that disrupt the collagen helix [5]. It appears that the disordered collagen chain acts a dominant negative fasion, resulting in a decrease in circulating levels of MBL that do not activate complement. More detailed analysis of the MBL gene has revealed at least ten distinct MBL

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haplotypes in humans, four of which (LYPB, LYQC, HYPD and LXPA) dictate low serum levels [6]. Interestingly, there is a high rate of haplotype variation in various human populations with a range of heterozygosity from 15% in Caucasians to 30% in certain African populations [7,8].

A basic function of innate immunity is a concerted response by numerous molecules and effector cells that conspire to restrict the initial spread of an infectious focus. First line host defense molecules include antimicrobial peptides, natural antibodies, complement proteins, lipopolysaccharide binding protein (LBP), soluble receptors and collectins [9-11]. The collectins are multimeric lectin-containing molecules with collagen stalks that include the pulmonary surfactant proteins-A and -D, conglutinin, CL-43, CL-46, and MBL [1,12-14].

Importantly, MBL seems to be able to distinguish species self or altered self from non-self though it is able to recognize dying cells [15]. The specificity that allows the distinction of surfaces of virally infected cells and transformed cells from normal host cells depends on both fine recognition of molecular micro patterns, and on the spatial geometry of macro pattern of these molecules on the surface of the cell. The cognate ligands that are recognized by MBL appears to be dictated by the spatial orientation of the carbohydrate binding domains and the differences in geometry of the sugars that adorn microorganisms versus host glycoproteins exposed on viable cells. MBL is able to activate complement via a novel mechanism that co-opts the mannose-binding lectin associated serine protease (MASP) [16,17]. There is a family of three related MASP genes, but it is MASP-2 that utilizes the classical pathway convertase to cleave the third complement component (C3) [17]. MBL therefore activates complement in an antibody independent manner. The analogy of MBL to antibodies extends MBL's function as an opsonin [18-20].

2. MBL NULL MICE ARE HIGHLY SUSCEPTIBLE TO INFECTION WITH *STAPHLYOCOCCUS AUREUS*

In order to provide formal proof that MBL is indeed important in host defense *in vivo*, we set out to create a mouse model of MBL deficiency. Humans and new world monkeys have a single MBL gene, whereas rodents have two homologous forms of MBL that are designated MBL-A and MBL-C, the respective gene products of the *mbl1* and *mbl2* genes, and are 50% homologous [21-23]. These two homologues proteins have distinct and overlapping binding specificities, are found predominantly in serum, and are able to bind MASPs to activate complement [23-25]. The relative physiological role of these two proteins *in vivo* has not been clearly defined. In order to address some of these questions, we created MBL-A and MBL-C double KO (MBL null) mice. We verified that the MBL null mice lack MBL in serum and therefore have a nonfunctional MBL complement pathway. We chose to infect these mice with *S. aureus*, as this organism is a significant cause of human infection worldwide. The emergence of widespread antibiotic resistance to *S. aureus* poses new therapeutic challenges and so identification of host factors that play a role in resistance to infection with this Gram-positive infection is of great interest. We found that (1) all MBL null mice died two days after i.v. inoculation of *S. aureus* compared with 55% survival of wild type mice; (2) pretreatment of the mice with recombinant MBL reversed the phenotype; (3) there were significantly more bacteria in the blood of MBL mice compared to wild type mice at 24 hours; (4) the viscera of MBL null mice accumulated significantly more bacteria than wild type mice

24 hours post infection; (5) there was a decrease in phagocytosis of bacteria in blood and peritoneal cavity in MBL null mice. In contrast to intravenous infection, i.p. inoculation of *S. aureus* did not result in enhanced infectious complications in MBL null mice compared with wild type mice. However, when the MBL mice were rendered neutropenic, these neutropenic MBL null mice displayed enhanced bacterial accumulation in organs and had persistent bacteremia 10 days post inoculation.

3. CONCLUSIONS

MBL appears to fulfill the criteria as an important host defense molecule against initial infection with *S. aureus*. The animal data indicate that MBL acts in serum as an opsonin. The effector mechanism appears to be mediated in part by MBL-dependent complement lysis of bacteria and in part, via MBL-dependent phagocytosis by leukocytes. Based on these studies, it is not clear whether MBL dependent clearance of *S. aureus* is mediated via complement receptors or MBL (collectin) receptors. Unpublished observations from our laboratory indicate that there is indeed an MBL-dependent, complement independent clearance mechanism. What remains an open question is the consequence of MBL dependent clearance versus clearance via complement receptors.

It thus appears that MBL is part of the initial response to infection, which is a complex interaction between a variety of pattern recognition molecules that trigger the downstream physiological cascades of complement, clotting, cytokine, and chemokine release and interface with effectors cells such as neutrophils [26,27]. Furthermore, the effector action of MBL appears intimately tied to circulating phagocytes. Neutrophils and monocytes express complement receptors, MBL receptors (collectin receptors) [28,29] and the receptor for lipopolysaccharide binding protein (LBP) [30]. Wright and colleagues linked humoral and cellular interactions and drew attention to the importance of co-operative interactions between neutrophils and opsonins in combating infection [31,32]. More recent examples have exploited the use of null animals to explore such interactions and are germane to this present study, including the interaction of LBP and neutrophils in resistance to intraperitoneal *Salmonella* infection [33,34]. A similar synergistic interaction between neutrophils and MBL is suggested by clinical observations that chemotherapy-induced neutropenic patients with haplotypes that specify low serum MBL levels [14,35,36] appear more susceptible to infection [37]. These clinical observations together with *in vitro* studies suggest that MBL plays a key role as an ante-antibody in first line host defense [38,39] and supports a role for MBL in combating infection *in vivo*.

What has not been clearly determined is the role of MBL against a variety of pathogens. Does MBL play a role against other Gram-positive and Gram-negative bacteria, mycobacteria and viruses *in vivo*? What is the relative role of MBL, complement and antibody in first line host defense? Finally, what is the real selective pressure for MBL haplotypes that specify low levels of MBL in humans? One speculation is that low levels of MBL might be protective against infection with intracellular pathogens like tuberculosis and malaria. While there might be some merit in this suggestion, it seems that it might well be that low MBL levels decrease the activity of the MBL complement pathway, resulting in a response that is less proinflammatory, and therefore less injurious to the host. Accordingly, low MBL levels might be protective against reperfusion injury. Overall, we are entering an exciting new chapter in this saga.

4. REFERENCES

1. Fraser, I.P., H. Koziel, and R.A. Ezekowitz. 1998. The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity. *Semin Immunol* 10:363-372.
2. Epstein, J., Q. Eichbaum, S. Sheriff, and R.A.B. Ezekowitz. 1996. The collectins in innate immunity. *Curr Op Immunol.* 8:29-35.
3. Eisen, D.P., and R.M. Minchinton. 2003. Impact of mannose-binding lectin on susceptibility to infectious diseases. *Clin Infect Dis* 37:1496-1505.
4. Super, M., S. Thiel, J. Lu, R.J. Levinsky, and M.W. Turner. 1989. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Lancet* 2:1236-1239.
5. Sumiya, M., M. Super, P. Tabona, R.J. Levinsky, T. Arai, M.W. Turner, J.A. Summerfield, S. Thiel, and J. Lu. 1991. Molecular basis of opsonic defect in immunodeficient children. *Lancet* 337:1569-1570.
6. Madsen, H.O., M.L. Satz, B. Hogh, A. Svejgaard, and P. Garred. 1998. Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. *J Immunol* 161:3169-3175.
7. Super, M., S.D. Gillies, S. Foley, K. Sastry, J.E. Schweinle, V.J. Silverman, and R.A. Ezekowitz. 1992. Distinct and overlapping functions of allelic forms of human mannose binding protein. *Nat Genet* 2:50-55.
8. Madsen, H.O., P. Garred, S. Thiel, J.A. Kurtzhals, L.U. Lamm, L.P. Ryder, and A. Svejgaard. 1995. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. *J Immunol* 155:3013-3020.
9. Hoffmann, J.A., F.C. Kafatos, C.A. Janeway, and R.A. Ezekowitz. 1999. Phylogenetic perspectives in innate immunity. *Science* 284:1313-1318.
10. Tobias, P.S., and R.J. Ulevitch. 1994. Lipopolysaccharide-binding protein and CD14 in the lipopolysaccharide-dependent activation of cells. *Chest* 105:48s-50s.
11. Lu, J., C. Teh, U. Kishore, and K.B. Reid. 2002. Collectins and ficolins: sugar pattern recognition molecules of the mammalian innate immune system. *Biochim Biophys Acta* 1572:387-400.
12. Holmskov, U., S. Thiel, and J.C. Jensenius. 2003. Collections and ficolins: humoral lectins of the innate immune defense. *Annu Rev Immunol* 21:547-578.
13. Holmskov, U., R. Malhotra, R.B. Sim, and J.C. Jensenius. 1994. Collectins: collagenous C-type lectins of the innate immune defense system. *Immunology Today* 15:67-74.
14. Turner, M.W., and R.M. Hamvas. 2000. Mannose-binding lectin: structure, function, genetics and disease associations. *Rev Immunogenet* 2:305-322.
15. Ogden, C.A., A. deCathelineau, P.R. Hoffmann, D. Bratton, B. Ghebrehiwet, V.A. Fadok, and P.M. Henson. 2001. C1q and mannose binding lectin engagement of cell surface calreticulin and cd91 initiates macropinocytosis and uptake of apoptotic cells. *J Exp Med* 194:781-796.
16. Matsushita, M., and T. Fujita. 1992. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J Exp Med* 176:1497-1502.
17. Thiel, S., T. Vorup-Jensen, C.M. Stover, W. Schwaeble, S.B. Laursen, K. Poulsen, A.C. Willis, P. Eggleton, S. Hansen, U. Holmskov, K.B. Reid, and J.C. Jensenius. 1997. A second serine protease associated with mannan-binding lectin that activates complement. *Nature* 386:506-510.
18. Kuzu, I., R. Bircknell, A.L. Harris, M. Jones, K.C. Gatter, and D.Y. Mason. 1992. Heterogeneity of vascular endothelial cells with relevance to diagnosis of vascular tumors. *Journal of Clinical Pathology* 45:143-148.
19. Neth, O., D.L. Jack, M. Johnson, N.J. Klein, and M.W. Turner. 2002. Enhancement of Complement Activation and Opsonophagocytosis by Complexes of Mannose-Binding Lectin with Mannose-Binding Lectin-Associated Serine Protease After Binding to Staphylococcus aureus. *J Immunol* 169:4430-4436.
20. Super, M., R.J. Levinsky, M.W. Turner, S. Thiel, and J. Lu. 1990. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Clin Exp Immunol* 79:144-150.
21. Mogues, T., T. Ota, A.I. Tauber, and K.N. Sastry. 1996. Characterization of two mannose-binding protein cDNAs from rhesus monkey (Macaca mulatta): structure and evolutionary implications. *Glycobiol* 6:543-540.
22. Laursen, S.B., T.S. Dalgaard, S. Thiel, B.L. Lim, T.V. Jensen, H.R. Juul-Madsen, A. Takahashi, T. Hamana, M. Kawakami, and J.C. Jensenius. 1998. Cloning and sequencing of a cDNA encoding chicken mannan-binding lectin (MBL) and comparison with mammalian analogues. *Immunology* 93:421-430.
23. Hansen, S., S. Thiel, A. Willis, U. Holmskov, and J.C. Jensenius. 2000. Purification and characterization of two mannan-binding lectins from mouse serum. *J Immunol* 164:2610-2618.
24. Lee, R.T., Y. Ichikawa, M. Fay, K. Drickamer, M.C. Shao, and Y.C. Lee. 1991. Ligand-binding characteristics of rat serum-type mannose-binding protein (MBP-A). Homology of binding site architecture with mammalian and chicken hepatic lectins. *J Biol Chem* 266:4810-4815.

25. Ng, K.K., K. Drickamer, and W.I. Weis. 1996. Structural analysis of monosaccharide recognition by rat liver mannose-binding protein. *J Biol Chem* 271:663-674.
26. Matsukawa, A., C.M. Hogaboam, N.W. Lukacs, P.M. Lincoln, H.L. Evanoff, and S.L. Kunkel. 2000. Pivotal role of the CC chemokine, macrophage-derived chemokine, in the innate immune response. *J Immunol* 164:5362-5368.
27. Chinnaiyan, A.M., M. Huber-Lang, C. Kumar-Sinha, T.R. Barrette, S. Shankar-Sinha, V.J. Sarma, V.A. Padgaonkar, and P.A. Ward. 2001. Molecular signatures of sepsis: multiorgan gene expression profiles of systemic inflammation. *Am J Pathol* 159:1199-1209.
28. Holmskov, U.L. 2000. Collectins and collectin receptors in innate immunity. *APMIS Suppl* 100:1-59.
29. Wright, J.R. 1997. Immunomodulatory functions of surfactant. *Physiol Rev* 77:931-962.
30. Wright, S.D., R.A. Ramons, P.S. Tobias, R.J. Ulevitch, and J.C. Mathison. 1990. CD14 serves as the cellular receptor for complexes of lipopolysaccharide with lipopolysaccharide binding protein. *Science* 249:1431-1433.
31. Wright, A.E., and S.R. Douglas. 1904. Opsonins. *Proc Roy Soc Ser* 73:128-142.
32. Silverstein, A.M. 2003. Cellular versus humoral immunology: a century-long dispute. *Nat Immunol* 4:425-428.
33. Yang, K.K., B.G. Dorner, U. Merkel, B. Ryffel, C. Schutt, D. Golenbock, M.W. Freeman, R.S. Jack, J. Fierer, M.A. Swancutt, and D. Heumann. 2002. Neutrophil influx in response to a peritoneal infection with Salmonella is delayed in lipopolysaccharide-binding protein or CD14-deficient mice. *J Immunol* 169:4475-4480.
34. Fierer, J., M.A. Swancutt, D. Heumann, and D. Golenbock. 2002. The role of lipopolysaccharide binding protein in resistance to Salmonella infections in mice. *J Immunol* 168:6396-6403.
35. Garred, P., H.O. Madsen, J.A. Kurtzhals, L.U. Lamm, S. Thiel, A.S. Hey, and A. Svejgaard. 1992. Diallelic polymorphism may explain variations of the blood concentration of mannan-binding protein in Eskimos, but not in black Africans. *Eur J Immunogenet* 19:403-412.
36. Summerfield, J.A., M. Sumiya, M. Levin, and M.W. Turner. 1997. Mannose-binding protein gene mutations are associated with childhood infection in a consecutive hospital series. *Br Med J* 314:1229-1232.
37. Peterslund, N.A., C. Koch, J.C. Jensenius, and S. Thiel. 2001. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. *Lancet* 358:637-638.
38. Turner, M.W. 1998. Mannose-binding lectin (MBL) in health and disease. *Immunobiology* 199:327-339.
39. Ezekowitz, R.A.B. 1991. Ante-antibody immunity. *Curr Biol* 1:60-62.