Q J Med 1998; **91**:13–18

Original papers

QJM

Mannose binding protein deficiency is not associated with malaria, hepatitis B carriage nor tuberculosis in Africans

R. BELLAMY[1], C. RUWENDE[1], K.P.W.J. MCADAM[3], M. THURSZ[4], M. SUMIYA[4], J. SUMMERFIELD[4], S.C. GILBERT[1], T. CORRAH[3], D. KWIATKOWSKI[2], H.C. WHITTLE[3] and A.V.S. HILL[1]

From the [1]Wellcome Trust Centre for Human Genetics and [2]Institute of Molecular Medicine, Oxford University, Oxford, UK, [3]MRC Laboratories, PO Box 273, Banjul, The Gambia, and [4]Department of Medicine, Imperial College School of Medicine at St Mary's Hospital, London, UK

Received 27 October 1997

Summary

We retrospectively studied MBP genotypes in patients with malaria, tuberculosis (TB), and persistent hepatitis B virus (HBV) carriage, in clinics and hospitals in The Gambia. Children under 10 years with cerebral malaria and/or severe malarial anaemia, were compared with children with symptomatic, mild malaria, and controls of the same age and ethnicity. Adult TB cases with smear-positive pulmonary TB were compared with healthy blood donors from the same ethnic groups. Malaria cases and controls were tested for hepatitis B core antibody (anti-HBc) and surface antigen (HBsAg). TB patients were tested for HIV antibodies. Genotyping used sequence-specific oligonucleotide analysis to identify MBP variant alleles. Overall, 46% (944/2041) of patients and controls were homozygous for the wild-type MBP allele, 45% (922/2041) were carriers of a single variant allele and 8.6% (175/2041) had two variant alleles. Neither homozygotes nor heterozygotes for MBP variants were at increased risk of clinical malaria, persistent HBV carriage or TB. The most common mutation in Africans, the codon 57 variant allele, was weakly associated with resistance to TB (221/794 in TB cases and 276/844 in controls, $p = 0.037$). MBP deficiency is not a significant risk factor for persistent HBV, severe malaria nor pulmonary TB in West Africa.

Introduction

Low serum mannose-binding protein (MBP) has been described as the world's commonest immune deficiency.[1] MBP is a calcium-dependent serum lectin which acts as an opsonin to promote phagocytosis, and which activates complement via the classical pathway independently of C1q.[2,3] Three different co-dominant mutations in the MBP gene produce reduced plasma MBP concentrations and those with two mutant alleles (functional mutant homozygotes, FMHs) have extremely low plasma MBP.[4–6] These individuals have been found to be at increased risk of recurrent childhood infections[5,7,8] and possibly infections in adult life,[10,11] and it has been suggested that heterozygotes may also be immunocompromised.[10]

MBP variant alleles are extremely common and occur in over 30% of Africans.[4,6] This seems paradoxical, as infections are a frequent cause of premature

Address correspondence to Professor A.V.S. Hill, Wellcome Trust Centre for Human Genetics, Windmill Road, Oxford OX3 7BN. e-mail: adrian.hill@well.ox.ac.uk

death throughout Africa. Routine screening for MBP deficiency in adults with recurrent infections has been recommended[11] despite the fact that MBP deficiency occurs in up to 5% of the healthy Caucasian population.[12–14] No large case-control studies have compared the frequency of homozygotes and heterozygotes for MBP variant alleles in patients with serious infections and healthy subjects. We have investigated the relationship between variant MBP alleles and malaria, TB and HBV. These infections were selected as they are three of the most important infectious causes of morbidity and mortality in Africa, and there is significant evidence that host genetic factors are important determinants of outcome of infection.[15–18] Furthermore, macrophage phagocytosis of virulent *Mycobacterium tuberculosis* is mediated by mannose receptors,[19] and a lectin-like receptor is used by *Plasmodium falciparum* to invade erythrocytes.[20] Previous studies have suggested that MBP deficiency is associated with increased risk of infections generally rather than with any specific pathogens. However, these observations have, with a single exception, been based on small series of patients, and the true association between MBP variants and risk of infection remains unknown.

Methods

Patients

Patients with severe malaria (defined as parasitaemia $>2\,500/\mu l$, cerebral malaria and/or severe anaemia, haemoglobin <5 g/dl), patients with mild malaria and non-malaria controls were recruited from hospitals and clinics in The Gambia as described previously.[15,16] Controls were patients with non-malaria-related illnesses taken from the same hospitals and clinics, who were of the same age (<10 years) and ethnic groups as the malaria patients.[15] These patients and controls were screened for anti-HBc to demonstrate previous exposure, and the positive individuals were tested for HBsAg to identify those with persistent infection.[17] Adults with smear-positive pulmonary TB were identified from TB/ leprosy clinics in The Gambia. Blood samples were not collected from patients known to be infected with the human immunodeficiency virus (HIV) and all patients who gave their consent ($>95\%$) were screened for HIV antibodies. Random unrelated blood donor controls were collected from the Gambian blood transfusion service and were from the same geographical area and ethnic groups as the TB cases. Less than 2% of the general population and less than 10% of TB cases in The Gambia are HIV-positive (own unpublished data).

MBP genotype analysis

Venous blood (10 ml) was collected into potassium/EDTA tubes and DNA was extracted using Nucleon II (Scotlab). A 339 bp fragment of the MBP gene was amplified by the polymerase chain reaction (PCR) using the primers 5′-dGCACCCAGATTGTAGGACAGAG–3′ and 5′-dCAGGCAGTTTCCTCTGGAAGG–3′. The PCR product was transferred to a nylon membrane by slot-blotting and the filter was probed with digoxigenin-labelled sequence-specific oligonucleotides, and signal was detected using an anti-digoxigenin antibody chemiluminescence system (Boehringer-Mannheim). The codon 54 wild (w) and mutant (m) variants were detected by the oligonucleotides 5′-dCGTGATGGCACCAAGGA–3′ and 5′-dCGTGATGACACCAAGGA–3′, respectively, codon 57 w and m variants by 5′-dCACCAAGGGAGAAAAGGG–3′ and 5′-dCACCAAGGAAGAAAAGGG–3′, and codon 52 w and m by 5′-dAAGATGGGCGTGATGG–3′ and 5′-dAAGATGGGTGTGATGG–3′. Statistical analysis used χ^2-tests on 3×2 and 2×2 contingency tables to compare overall genotype frequencies and specific genotype frequencies, respectively. Data was also analysed by logistic regression analysis to examine the effects of potential confounding variables.

Results

Ethnic groups

In total, 2041 Gambians were screened for MBP mutations, and the overall genotype frequencies are shown in Table 1. Forty-six per cent (944/2041) were homozygous for the wild-type MBP allele, 45% (922/2041) were carriers of a single variant allele (898, codon 57 variant; 24, codon 54 variant) and 8.6% (175/2041) possessed two mutant alleles (FMHs) (171, codon 57 homozygotes; 4, codon 54/57 heterozygotes). The codon 52 variant (CGT to TGT) was not present in any of those studied. There were no significant differences in variant allele frequencies between the different ethnic groups present in The Gambia (Table 1).

Malaria

Five hundred and four patients with severe malaria (including 368 cerebral malaria cases and 185 patients with severe malarial anaemia), 292 mild malaria controls and 426 non-malaria controls were screened for MBP variant alleles. Neither MBP FMHs nor heterozygotes were over-represented among the severe malaria patients, and genotypes did not differ from those expected under Hardy-Weinberg equilibrium (Table 2). Although many of the control children had

Table 1 Ethnic group and MBP genotype

Ethnic group... Genotype	Mandinka	Wollof	Fula	Jola	Manjago	Serrahule	Other	Unknown	Total
w/w	47	45	51	42	56	38	46	50	944
w/54	1.1	1.6	1.3	0.9	1.1	1.1	0.7	0	24
w/57	42	44	42	48	35	53	47	40	898
54/57	0.1	0	0.9	0.3	0	0	0	0	4
57/57	8.9	9.5	5.1	9.6	8.0	7.6	6.0	10	171
Total	843	305	235	334	88	92	134	10	2041
χ^2	0.14	0.99	4.7	3.6	2.2	1.3	0.57	0.14	
p	NS	NS	NS	NS	NS	NS	NS	NS	

Data are percentage of individuals with each genotype, including patients and controls from each study. Totals are actual numbers. W, wild-type allele, the codon 54 variant (GGC to GAC) and the codon 57 variant (GGA to GAA) are denoted 54 and 57, respectively. A 3×2 χ^2, 2 degrees of freedom (df) was used to compare the allele frequencies found in each ethnic group with the other ethnic groups combined. The number of FMHs did not differ from that expected under Hardy-Weinberg equilibrium. Overall allele frequencies were: allele w, 0.69; variant allele 54, 0.007; variant allele 57, 0.30; variant allele 52, 0.00.

Table 2 MBP genotype and clinical malaria

Genotype		Cerebral malaria	Severe malarial anaemia	Mild malaria	Controls	Total
Normal	w/w	167 (45)	87 (47)	130 (45)	205 (48)	566 (46)
Carriers	w/54	2 (0.5)	0 (0)	4 (1.4)	4 (0.9)	10 (0.8)
	w/57	162 (44)	89 (49)	141 (48)	175 (41)	544 (45)
Functional	57/57	35 (9.5)	8 (4.3)	17 (5.8)	42 (10)	100 (8.2)
Mutant Homozygotes	54/57	2 (0.5)	1 (0.5)	0 (0)	0 (0)	2 (0.2)
Total		368	185	292	426	1222

Number of patients and controls (%) with each genotype. Forty-nine patients had both cerebral symptoms and severe malarial anaemia. There were no significant differences between patients and controls. In particular, the percentage of FMHs in each patient group did not differ significantly from the control population.

infections, the genotype frequencies were similar in those with and without infections, and matched those of the adult healthy blood donors and the expected frequencies under Hardy-Weinberg equilibrium.

HBV carriage

Of 990 patients tested, 337 were positive for anti-HBc, and of these, 180 had persistent infection demonstrated by positive HBsAg serology. MBP genotype was not associated with positive anti-HBc serology: 9/157 (5.7%) patients who had cleared the infection (anti-HBc positive, HBsAg negative) were FMHs compared with 16/180 (8.9%) who had persistent infection (Table 3). This non-significant difference ($p = 0.37$) is unlikely to represent a true biological difference, as there was no over-representation of FMHs in the HBsAg positive group compared to that expected under Hardy-Weinberg equilibrium. Heterozygotes were also not at increased risk of persistent HBV infection.

Tuberculosis

Among the 397 smear-positive TB cases screened for MBP variant alleles, there were 33 (8.3%) FMHs, which did not differ from the control group (10.0%) nor from the number expected under Hardy-Weinberg equilibrium (Table 4). However, the overall frequency of the MBP codon 57 mutation was lower among the TB patients than the controls (0.28 and 0.33 respectively, $\chi^2 = 4.36$, 2df, $p = 0.037$), suggesting this mutation may protect against TB in Africans. Only six TB patients were HIV-positive, and excluding them from the analysis did not substantially affect the results.

MBP variants

Stratification of patients by ethnic group did not alter the conclusions from any of the studies (data not shown). Logistic regression analysis was used to examine for any potential confounding interactions

Table 3 MBP genotype and chronic HBV infection

Genotype		Anti-HBc negative	HBsAg negative/anti-HBc positive	HBsAg positive	Total
Normal	w/w	310 (47)	73 (46)	80 (44)	463 (47)
Heterozygote carriers	w/54	2 (0.3)	2 (1.2)	3 (1.7)	7 (0.7)
	w/57	289 (44)	73 (46)	81 (45)	443 (45)
Functional mutant	57/57	50 (7.7)	9 (5.7)	16 (8.9)	75 (7.6)
homozygotes	54/57	2 (3.1)	0 (0)	0 (0)	2 (0.2)
Total		653	157	180	990

Previous exposure to HBV was demonstrated by positive anti-HBc serology. Among the anti-HBc-positive patients, those with negative HBsAg have cleared the infection and those with positive HBsAg serology have persistent infection. Neither risk of infection nor inability to clear infection was significantly associated with MBP genotype.

Table 4 MBP genotype and TB

Genotype		TB cases	Blood donors	Total
Normal	w/w	198 (50)	183 (43)	381 (47)
Heterozygote	w/54	7 (1.8)	5 (1.2)	12 (1.5)
carriers	w/57	159 (40)	192 (45)	351 (43)
Functional	57/57	29 (7.3)	42 (10)	71 (8.7)
mutant	54/57	4 (1.0)	0 (0)	4 (0.5)
homozygotes				
Total		397	422	819

The frequency of the codon 57 mutation was significantly lower among the TB cases (221/794) than the controls (276/844) ($\chi^2 = 4.36$, Yates' correction, 1df, $p = 0.037$, OR = 0.79, 95%CI 0.64–0.99). The number of FMHs did not differ significantly between the cases and the controls, and followed the numbers expected under Hardy-Weinberg equilibrium.

between HBV and malaria status, and for any confounding effects of other polymorphisms which have been typed on these samples. MBP genotype was not a significant predictor of disease status (data not shown). The codon 52 mutation was not present in The Gambia and the codon 54 mutation was very rare. Although our results could be said to only apply to the codon 57 mutation, heterozygotes for the codon 57 and codon 54 mutations have similar levels of serum MBP, and the codon 52 mutation produces less reduction in circulating MBP.[21] Therefore the results are probably applicable to all three mutations.

Discussion

MBP and resistance to mycobacteria

Variant MBP alleles are extremely common throughout most world populations, suggesting they confer some advantage to heterozygotes. It has been suggested that MBP could act as a binding protein for mycobacteria and other intracellular pathogens, enabling them to enter host macrophages.[22] Low levels of MBP may therefore assist the host in resisting infection due to these organisms. We observed a significantly lower frequency of the MBP codon 57 variant in the TB cases than in the controls, suggesting that carriage of this allele may protect against TB. This supports the suggestion of Garred *et al.*[22] that the high frequency of MBP variant alleles in many populations might be due to enhanced resistance to mycobacteria. However, this finding could be due to chance and will require replication in other studies.

MBP and immune deficiency

MBP deficiency due to homozygosity for variant MBP alleles has been described as the commonest immunodeficiency and estimated to account for up to 25% of cases where children have frequent unexplained infections.[1] This susceptibility has been described for a variety of different pathogens and affected organs.[5,7–11] However, we found that MBP deficiency in children was not associated with clinical malaria nor progression to severe malaria, nor was it associated with inability to clear HBV infection. In adults, MBP deficiency is not associated with smear-positive pulmonary tuberculosis. Other work in our laboratory has found no association between MBP deficiency and susceptibility to HIV infection (S. Ali, unpublished data), leprosy (S. Roy, unpublished data) nor severe dengue infection (H. Loke, unpublished data). Previous smaller case-control studies have found no association between low MBP concentrations and severe infection with group B or C meningococci[23] nor with otitis media,[24] and MBP levels have been shown to correlate with neither the course of HIV infection nor progression to AIDS.[25] An association between persistent HBV infection and carriage of the codon 52 allele in Caucasians but not Asians has recently been described.[26] The lack of an association with the codon 57 variant in

Africans supports the view that the codon 52 allele is in linkage disequilibrium with a HBV susceptibility gene, rather than MBP deficiency itself being the susceptibility factor.[26]

Reports linking MBP deficiency (or the resultant phenotype of failure to opsonize baker's yeast) with susceptibility to infections have, with a single exception,[11] consisted of small case series. The one large study found that among 229 patients screened for immunodeficiencies 8.3% were FMHs, a frequency not significantly above that usually found in northern European populations[12–14] and not higher than expected under Hardy-Weinberg equilibrium. A study of 96 HIV-infected men also found that only 8% were MBP FMHs.[27]

Although MBP deficiency may predispose to recurrent infections, the evidence supporting this is inconclusive, particularly in adults. Further studies of large series of patients with other infectious diseases are required to establish whether or not MBP deficiency is an important risk factor for these, and assessment of pyogenic infections would be of particular interest. Until such rigorous studies are performed, recommendations to routinely screen children and adults with recurrent infections for MBP deficiency[11] are not justified.

Acknowledgements

The patients deserve special thanks for giving their consent to enter these studies. Ethical approval was provided by the joint Gambian Government/Medical Research Council Ethical Committee. We are very grateful for the help we received from the Gambian National TB Control Programme Director, Dr V. Bouchier and all of her staff, especially M. Saidykhan, K. Bayang, E. Mendy, M. Jallow, M. Sanyang and S. Gassama. We are also endebted to the field workers Y. Sowe and M. Jawo, and to many other MRC staff, especially S. Sabally, S. Obarro, K. Joof, E. Harding, V. Thomas, P. Langfield and P. Njie. This study was funded by the Wellcome Trust (grant ref. 044418/Z/95/Z/139).

References

1. Thompson C. Protein proves to be a key link in innate immunity. *Science* 1995; **269**:301–2.
2. Lu J, Thiel S, Wiedemann H, Timpl R, Reid KBM. Binding of the pentamer/ hexamer forms of mannan-binding protein to zymosanactivates the proenzyme $C1r_2C1s_2$ complex of the classical pathway of complement without involvement of C1q. *J Immunol* 1990; **144**:2287–94.
3. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Lancet* 1989; **ii**:1236–9.
4. Madsen HO, Garred P, Kurtzhals JAL, *et al*. A new frequent allele is the missing link in the strucural polymorphism of the human mannan-binding protein. *Immunogenetics* 1994; **40**:37–44.
5. Sumiya M, Super M, Tabona P, *et al*. Molecular basis of opsonic defect in immunodeficient children. *Lancet* 1991; **337**:1569–70.
6. Lipscombe RJ, Sumiya M, Hill AVS, *et al*. High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. *Hum Mol Genet* 1992; **1**:709–15.
7. Soothill JF, Harvey BAM. Defective opsonization. A common immune deficiency. *Arch Dis Child* 1976; **51**:91–9.
8. Richardson VF, Larcher VF, Price JF. A common congenital immunodeficiency predisposing to infection and atopy in infancy. *Arch Dis Child* 1984; **58**:799–802.
9. Candy DCA, Larcher VF, Tripp JH, Harries JT, Harvey BAM, Soothill JF. Yeast opsonisation in children with chronic diarrhoeal states. *Arch Dis Child* 1980; **55**:189–93.
10. Summerfield JA, Ryder S, Sumiya M, *et al*. Mannose binding protein gene mutations associated with unusual and severe infections in adults. *Lancet* 1995; **345**:886–9.
11. Garred P, Madsen HO, Hofmann B, Svejgaard A. Increased frequency of homozygosity of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency. *Lancet* 1995; **346**:941–3.
12. Davies JE, Snowden N, Hillarby MC, *et al*. Mannose-binding protein gene polymorphism in systemic lupus erythematosus. *Arthritis Rheum* 1995; **38**:110–14.
13. Snowden N, Stanworth S, Donn R, Davies E, Ollier B. Mannose-binding protein genotypes and recurrent infection. *Lancet* 1995; **346**:1629–30 (letter).
14. Super M, Gillies SD, Foley S, *et al*. Distinct and overlapping functions of allelic forms of human mannose binding protein. *Nature Genet* 1992; **2**:50–5.
15. Hill AVS, Allsopp CEM, Kwiatkowski D, *et al*. Common West African HLA antigens are associated with protection from severe malaria. *Nature* 1991; **352**:595–600.
16. McGuire W, Hill AVS, Allsopp CEM, Greenwood BM, Kwiatkowski D. Variation in the TNFalpha promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; **371**:508–11.
17. Thursz MR, Kwiatkowski D, Allsopp CEM, Greenwood BM, Thomas HC, Hill AVS. Association between an MHC class II allele and clearance of hepatitis B virus in The Gambia. *N Engl J Med* 1995; **332**:1065–9.
18. Hill AVS. Genetics of infectious disease resistance. *Curr Opin Genet Develop* 1996; **6**:271–4.
19. Schlesinger LS. Macrophage resistance of virulent but not attenuated starins of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. *J Immunol* 1993; **150**:2920–30.
20. Jungery M, Pasvol G, Newbold CI & Weatherall DJ. A lectin-like receptor is involved in invasion of erythrocytes by Plasmodium falciparum. *Proc Natl Acad Sci USA* 1983; **80**:1018–22.
21. Madsen HO, Garred P, Thiel S, *et al*. Interplay between promoter and structural gene variants control basl serum level of mannan-binding protein. *J Immunol* 1995; **155**:3013–20.
22. Garred P, Harboe M, Oettinger T, Koch C, Svejgaard A. Dual role of mannan-binding protein in infections: another case of heterosis? *Eur J Immunogenet* 1994; **21**:125–31.

23. Garred P, Michaelson TE, Bjune G, Thiel S, Svejgaard A. A low serum concentration of mannan-binding protein is not associated with serogroup B or C meningococcal disease. *Scand J Immunol* 1993; **37**:468–70.

24. Garred P, Brygge K, Sorensen CH, Madsen HO, Thiel S, Svejgaard A. Mannan-binding protein levels in plasma and upper-airways secretions and frequency of genotypes in children with recurrence of otitis media. *Clin Exp Immunol* 1993; **94**:99–104.

25. Nielsen SL, Andersen PL, Koch C, Jensenius JC, Thiel S. The level of the serum opsonin, mannan-binding protein in HIV–1 antibody-positive patients. *Clin Exp Immunol* 1995; 100:219–22.

26. Thomas HC, Foster GR, Sumiya M, *et al.* Mutation of gene for mannose-binding protein associated with chronic hepatitis B viral infection. *Lancet* 1996*;* 348:1417–19.

27. Garred P, Madsen HO, Balslev U, *et al.* Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. *Lancet* 1997; **349**:236–40.