Clin Exp Immunol 2003; **132**:92–95

Mannan-binding lectin and hepatitis C infection

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(Accepted for publication 24 January 2003)

SUMMARY

Japanese patients with chronic hepatitis C infection unresponsive to treatment with interferon possessed genotypes disproportionately conferring low mannan-binding lectin (MBL) concentrations. Our aims were to confirm or refute this finding in European patients at the MBL protein level, and to investigate whether a low circulating concentration of MBL might influence susceptibility to, or disease progression from, hepatitis C viral infection. Serum samples obtained from 180 hepatitis C patients and 566 blood donors were assayed for MBL. MBL concentrations were related to disease characteristics retrieved from patients' records. MBL concentrations were higher in hepatitis C patients (median 2·5 μg/ml *versus* 1·3; $P < 0{\cdot}0001$) and the proportion of patients with very low (MBL-deficient) concentrations was similar to that of the healthy controls. There were no significant associations between patients with low serum MBL and the disease features studied, including response to antiviral therapy. Therefore, low circulating MBL does not increase susceptibility to hepatitis C infection, and MBL concentration does not have a major influence on the course of the disease or the response to antiviral therapy. MBL replacement therapy would therefore not be indicated for chronic hepatitis C patients who failed to respond fully to treatment with interferon and ribavirin.

Keywords hepatitis C interferon mannan-binding lectin ribavirin

INTRODUCTION

Mannan-binding lectin (MBL) is a plasma collectin thought to have an important role in the innate immune system [1,2]. MBL contributes to the complement-dependent opsonization of baker's yeast by phagocytes, and its relative deficiency corresponds to a common opsonic defect found in some infants with recurrent infection and also 5–10% of apparently healthy adults [3]. The concentration of MBL in human blood plasma is influenced strongly by the inheritance of haplotypes that may differ at a series of allelic dimorphisms affecting both the structural gene and its promoter region [1–4]. Plasma MBL is also affected by other factors, including growth hormone [5], and is known to be an acute phase reactant, increasing up to threefold after infection or surgical trauma [6]. The MBL genotype of an individual provides only a rough guide to plasma concentration, and any combination of haplotypes is associated with a wide concentration range [2,4]. Consequently, the genotype is a good predictor of average circulating MBL concentrations in groups or populations, but provides a less reliable prediction of plasma MBL for individuals [2].

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Matsushita *et al*. [7], describing Japanese patients, found that hepatitis C-infected patients with MBL haplotypes known to confer low MBL concentrations were significantly less likely to respond to therapy with interferon than similar patients genetically able to produce higher amounts of MBL. This was a potentially important finding, as the expense, adverse side-effects and long duration of antiviral therapy make screening for detection of potential non-responders desirable. Moreover, the obvious implication that MBL contributes to the elimination of the virus raises the possibility that hepatitis C carriers with low MBL concentrations might benefit from MBL replacement therapy.

However, it was curious that as many as 53% of Matsushita *et al*.'s patients and 57% of their healthy controls had 'low' MBL genotypes as defined in their study, and perhaps surprising that the proportion of sustained interferon responders was as high as 33%. Nevertheless, an independent investigation of Japanese patients [8] found a possible association between possession of the codon-52 mutation (implying low MBL) and disease progression, and chronic infection with hepatitis C infection was associated with lower MBL levels in a Chinese series [9]. Both these studies are consistent with a role for MBL in the elimination of the virus, although other explanations are possible.

We therefore aimed to investigate the relationship between MBL concentration and hepatitis C infection at the protein level

in European patients. We report that MBL concentrations were generally high in patients compared to healthy controls, but we found no support for the possibility that MBL influences disease susceptibility or progression.

PATIENTS AND METHODS

Subjects

Sera were collected from 180 patients (131 male, 49 female; median age at first blood sampling, 42 years, range 21–78) attending the hepatitis C out-patient clinic at the Royal Infirmary of Edinburgh from 1992 onwards. All had serological evidence of hepatitis C infection and most were polmerase chain reaction (PCR)-positive on at least one occasion. In 111 patients, the HCV genotypes were known: the most common genotypes were 3a (42%), 1a (37%) and 1b (12%). Biopsies were carried out on 176 patients and in many of them the time from infection to biopsy could be estimated reliably. Patients with cirrhosis or bridging fibrosis after less than 20 years of infection were deemed to be fast progressers, while patients with no fibrosis or only pericellular or portal spurring after > 25 years were deemed to be slow progressers. A single patient with a circulating hyaluronic acid level of 146 ng/ml at less than 9 years after being infected was also deemed to be a fast progresser. The degree of inflammation (hepatitis) could often be classified as mild, moderate or severe, on the basis of histological information provided in the biopsy reports.

Approximately 100 patients were given a course of antiviral therapy consisting either of interferon-alpha alone or interferon plus ribavirin (combination therapy). Those who were PCR-negative for HCV 6 months after a single course of interferon alone ($n = 5$) or combination therapy ($n = 32$) were deemed to be sustained responders. Those who failed to respond to combination therapy, including those who had previously been treated unsuccessfully with interferon alone, plus a few non-responders to interferon only ($n = 4$), were deemed to be non-responders. Others who had partial but incomplete responses, those lost to follow-up, those still undergoing treatment and a few who were unresponsive to interferon alone but responsive to combination therapy, were not classified as responders or non-responders and were excluded from analysis.

Informed consent was obtained from each patient and the project was approved by the local medical ethical committee.

Blood donors ($n = 566$) were used as healthy controls.

Methods

Mannan-binding lectin (MBL) was measured in serum by enzyme-linked immunosorbent assay (ELISA), as described previously [10].

Statistics

Statistical analyses were performed using Prism for Windows software from Graph Pad (San Diego, CA, USA).

RESULTS

Hepatitis patients versus *healthy controls*

MBL concentrations were generally higher in hepatitis C patients (Table 1), with the median value for patients approximately twice that of healthy controls (2·5 *versus* 1·3 μg/ml; $P < 0{\cdot}0001$). The proportion of individuals with very low concentrations was slightly greater in blood donors; this was true irrespective of the cut-off level chosen, not just the useful 0·1 μg/ml level given in Table 1.

Twenty-seven patients had blood samples taken on at least two occasions, with intervals ranging from 6 months to 7 years. Most showed very little change in MBL concentration irrespective of time, and there was no correlation with change in PCR status. All seven pairs of sera where each patient had converted from PCR-positive to PCR-negative showed no appreciable change in MBL concentration.

Patient characteristics in relation to MBL concentration

The patients were divided into four groups on the basis of MBL concentration (Table 2). Cirrhosis was present in a slightly lower proportion of groups 1 and 2 (low MBL) patients, but this trend was not statistically significant. As expected, frequency of cirrhosis correlated with duration of infection, so a more meaningful comparison would be between patients with slow disease progression and those with fast disease progression. We were able to identify 10 slow progressers and 16 fast progressers (as defined in the Methods section). No relationship was apparent between disease progression and MBL concentration.

There was a weak and non-significant ($P = 0{\cdot}4$, χ^2 test for trend) trend towards a greater degree of hepatitis with increasing MBL concentration; although there were higher proportions of patients in groups 3 and 4 with moderate or severe hepatitis, comparing the two high (normal) MBL groups with the two low MBL groups did not achieve statistical significance ($P = 0{\cdot}27$, Fisher's exact test).

MBL concentration and treatment outcome

The distributions of antiviral therapy responders and non-responders as a function of MBL concentration range (Table 2) were not significantly different ($P = 0{\cdot}17$, χ^2 test for trend); however, the ratio of responders to non-responders was actually higher in the low MBL groups 1 and 2 compared to the corresponding ratio for groups 3 and 4 ($P = 0{\cdot}08$, Fisher's exact test). Conversely, the distributions of MBL concentrations found in the non-responder (median, 2·8 μg/ml; range, 0·4–5·9) and sustained responder groups (median, 2·5 μg/ml; range, 0–6·19) were similar.

DISCUSSION

This is the largest reported series of hepatitis C patients in which MBL has been measured. These patients had, in general,

Table 1. MBL concentrations in hepatitis C patients and blood donors

	[MBL]	
	Patients ($n = 180$)	Blood donors ($n = 566$)
Range (ug/ml):	0–6·7	0–8·4
25th percentile (ug/ml):	1·15	0·5
Median (ug/ml):	2·5*	1·3
75th percentile (ug/ml):	3·75	2·1
≤ 0·1 μg/ml (%):	6	10

*$P < 0{\cdot}0001$ (Mann–Whitney test).

Table 2. Patient characteristics according to MBL concentrations

	Group 1 ($n = 11$) (0 = [MBL] = 0·1)	Group 2 ($n = 32$) (0·1 < [MBL] < 1·15)	Group 3 ($n = 44$) (1·15 = [MBL] < 2·5)	Group 4 ($n = 93$) ([MBL] ≥ 2·5)
Cirrhosis	1/11 (9%)	4/31 (13%)	9/42 (21%)	15/92 (16%)
Slow : fast progressers	1 : 0	1 : 2	3 : 5	5 : 9
Mild : more severe hepatitis[a]	6 : 1	18 : 6	22 : 14	47 : 22
SR[b] : NR[c]	2 : 0	8 : 4	8 : 14	19 : 22

Patients were first divided into four groups defined by MBL concentration ranges (the number of patients in each group is given). Clinical information retrieved from medical records was then used to relate patients' clinical features to MBL concentration range. The presence or absence of cirrhosis was established in 176 patients and is expressed as a proportion for each concentration group. Only 26 patients could be classified reliably as slow progressers or fast progressers; 136 patients were scored for degree of inflammation; and 77 patients could be classified as either sustained responders or non-responders to antiviral therapy. For those last three categories, the actual numbers involved are given in the form of ratios.

[a]Hepatitis graded as moderate was combined with the few cases of severe hepatitis. [b]SR = sustained responders. [c]NR = non-responders (to antiviral therapy).

significantly higher MBL concentrations than blood donor controls, and the proportion with very low levels (corresponding to the 'opsonic defect' of the early literature) was slightly lower. The latter finding does not support the possibility that very low MBL concentrations increase susceptibility to hepatitis C infection. The higher levels compared to healthy controls is not in itself surprising, because MBL is an acute phase reactant [6], but is in contradiction to previously published data [9].

For our analysis, the patients were divided into four MBL concentration groups. Group 1 [([MBL) ≤ 0·1 μg/ml, although all values were actually ≤ 0·05 μg/ml] corresponds to traditional MBL deficiency, possessed by the 5–10% of the population with the opsonic defect. Group 2 [0·1 < [MBL] < 1·15 μg/ml], comprising the remaining subjects with MBL concentrations within the lowest 25th percentile, almost certainly (based on the literature) consists mainly or exclusively of wild-type/mutant haplotype heterozygotes. Group 3 [1·15 ≤ (MBL) < 2·5 μg/ml] presumably also includes a preponderance of such heterozygotes. Group 4 [(MBL) ≥ 2·5 μg/ml], representing median values and above, is presumed to consist of genotypes homozygous for the wild-type structural alleles (and promoter variants other than the low-producing LX).

No relationship was apparent between MBL concentration and disease progression or response to treatment. If anything, the low MBL patients (groups 1 and 2) had a slightly higher proportion of slow progressers, a lower proportion of moderate to severe hepatitis and a higher proportion of sustained responders to antiviral therapy. Combining groups 1, 2 and 3 and comparing the combined data to that of group 4 provides the closest comparison with the genotyping data of Matsushita and colleagues [7]: the ratio of sustained responders to non-responders was 18 : 18 in the combined group and 19 : 22 in group 4.

These results do not seem to be compatible with those of Matsushita *et al*. However, a direct comparison is not possible. It may be relevant that a large group of our patients were partial responders and therefore excluded from the analysis, whereas no mention of partial/temporary responders was made by Matsushita *et al*. Certainly, Matsushita *et al*. demonstrated that healthy individuals with genotypes containing either haplotype LXPA or LYPB had a mean MBL concentration more than threefold lower than those possessing only 'YA type' (wild-type) haplotypes. The corresponding data at the protein level were not given, and analysis was carried out using only genotypes (not protein concentrations), which showed that LXPA and LYPB were found less frequently in patients responsive to interferon therapy. The converse relationships, MBL genotype and phenotype according to whether patients were interferon responsive or unresponsive, was not reported as such, but it was stated that interferon responders had 'on average' (average not defined) a comparable concentration (1·28 μg/ml) to non-responders (1·38 μg/ml). This is in perfect agreement with our findings reported here, but there appears to be an internal inconsistency in the Japanese data. In their Discussion, Matsushita *et al*. suggest interferon therapy itself may up-regulate MBL synthesis, yet it is stated in their Methods section that serum samples were obtained just before interferon therapy commenced. It could be inferred that an excess proportion of haplotypes LXPA and LYPB in interferon-resistant hepatitis C patients (unlike in healthy controls) would not be detected by measuring serum MBL concentrations alone, but that was not actually demonstrated and must be considered unproven [7].

Although the association between low MBL and mild rather than more severe hepatitis was not statistically significant, it may still be real. This observation could be another reflection of MBL as an acute phase reactant and is consistent with the observed increase in MBL in hepatitis C patients compared to blood donor controls which was highly significant.

While this report was in preparation, Dumestre-Perard and colleagues [11] published a paper claiming that complement component C4 activity could be used to predict success in interferon ± ribavirin treatment. Those authors also measured MBL (in 66 patients) but did not report any relationship to interferon therapy outcome. (It was implied that MBL had no predictive value for antiviral therapy, but the question was not addressed directly.)

The results reported here, while essentially negative, are none the less valuable, as the obvious corollary is that MBL replacement therapy would not be indicated in hepatitis C patients with low MBL and an incomplete response to interferon/ribavirin therapy.

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