Mannan-Binding Lectin (MBL) Deficient Individuals with the O/O Genotype are Highly Susceptible to Gastrointestinal Diseases

Helga Bjarnadottir1*, Valgerdur Thorsteinsdottir2, Gudmundur Haukur Jorgensen2, Margret Arnardottir2 and Bjorn Runar Ludviksson1,2

1Department of Immunology, Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland
2Faculty of Medicine, University of Iceland, Reykjavik, Iceland

Abstract

Background: Mannan-binding lectin (MBL) and ficolin-3 are initiators of the lectin pathway that is important for clearance of pathogens and apoptotic cells through complement activation. MBL deficiency (MBLD) has been associated with infectious complications but its clinical relevance in adults is unclear. Definition of MBLD is commonly linked to its low serum levels, but is mainly due to functional polymorphisms in the MBL2 gene leading to dysfunctional MBL forms. Homozygotes for dysfunctional alleles (O/O) have the lowest serum levels (<50 ng/ml) with a defect in opsonisation and complement activation. Ficolin-3 deficiency due to homozygosity of a frameshift mutation (1637delC) in the FCN3 gene was recently shown to be associated with pyogenic infections mainly in the lungs.

Objective: The aim of the study was to thoroughly evaluate the clinical findings of previously defined MBL deficient cohort in relation to their MBL2 and FCN3 genotypes.

Methods: A total of 120 adult individuals previously defined as MBL deficient (≤500 ng/ml) were genotyped for variants in the MBL2 gene and for the 1637delC allele in the FCN3 gene. They answered detailed questionnaire focused on pulmonary and gastrointestinal infections. For comparison, 63 adult individuals were randomly selected from the general population and served as control subjects.

Results: In the MBLD cohort the prevalence of genotypes A/A, A/O and O/O was 14.2%, 71.2%, and 14.2%, respectively. Thus, 85% carried the dysfunctional allele O. The MBLD cohort was significantly more prone to a variety of recurrent and severe infections than the control cohort. O/O individuals were more susceptible to oesophagitis and gastritis and had undergone gastroscopy significantly more often than A/A or A/O. Prevalence of heterozygosity (C/-) for the 1637delC allele was 4.2%.

Conclusion: MBL deficient individuals suffer from recurrent and severe forms of infections. The O allele might predispose to gastrointestinal diseases.

Keywords: Mannan-binding lectin; Primary immunodeficiency; Lectin-pathway; Complement; MBL2 genotypes; Ficolin-3; Gastritis

Introduction

Complement activation is a first-line innate defence against invading pathogens. Activation of complements via the lectin pathway (LP) is mediated by five separate soluble serum pattern recognition proteins (PRP). These are mannan-binding lectin (MBL), collectin-11 (CL-11), ficolin-1 (M-ficolin), ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin) [1-5]. The LP-PRPs circulate in serum complexed with specific proteases named MASPs (MBL associated serine proteases; MASP-1, MASP-2 and MASP-3), and upon binding to pathogen associated sugar patterns the MASPs become activated and initiate the complement cascade leading to membranolytic attack and opsonophagocytosis [6].

The physiological role of MBL has been well studied whereas the role of ficolins and CL-11 is less well investigated. MBL has a significant role in a number of pathogenetic and homeostatic processes. It binds and eliminates (through complement activation) various microorganisms and altered self-components, including dying host cells (apoptotic/necrotic), circulating immune complexes (CICs) and immunoglobulins (agactosylated IgG and certain forms of IgM and IgA) [7-10]. The MBL molecule itself can act as a TLR-2/6 co-receptor within the cell and direct intracellular signalling, thus, mediating functions outside complement activation [11]. Additionally, MBL modulates pro-inflammatory cytokine production and clearance of endotoxins via Kupffer cells [12,13].

The concentration of MBL in serum can vary 10,000-fold between individuals and this can be explained by combinations of single nuclear polymorphisms (SNPs) in exon 1 and in the promoter region of the MBL2 gene [14]. The SNPs in exon 1 have been named variant allele B,C and D (collectively called O) whereas the normal allele is referred to as A [14]. Individuals carrying the O allele have dysfunctional MBL forms unable to bind their ligands [15-17]. SNPs at positions -50 (H/Y) and -221 (X/Y) in the MBL2 promoter and at position +4 (P/Q) in the 5′-untranslated portion of exon 1 are associated with different MBL levels [18-20]. The X variant has the strongest down-regulating effect. Genotypes XX/O and OO are generally referred to as MBL deficiency genotypes [21]. Approximately 60% of Caucasians have been found to be wild-type (A/A), 36% heterozygous for the O allele (A/O) and 4% homozygous (O/O) [14].

*Corresponding author: Helga Bjarnadottir, Department of Immunology, Landspitali - The National University Hospital of Iceland, Hringbraut (building 14 at Eiríksgata), 101 Reykjavik, Iceland, Tel: (+354) 543 5800; Fax: (+354) 543 4828; E-mail: hjbjar@landspitali.is

Received October 29, 2013; Accepted January 07, 2014; Published January 17, 2014


Copyright: © 2014 Bjarnadottir H, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
MBL deficiency (MBLD) is now classified as primary immunodeficiency (PID) by the International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies [22]. MBL cutoff serum value has been defined as ≤ 500 ng/ml in a large cohort (N=1642) from four separate studies and has been suggested to be used in studies of MBL disease associations [21].

MBL-MASPs mediated defence is innate and therefore believed to be critical when the adaptive immune response is either immature and/or compromised [23]. Numerous clinical studies have linked MBLD with increased susceptibility to a variety of infectious diseases [23-28]. In addition, studies indicate that MBLD can aggravate certain autoimmune disorders, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [25,29-32].

Ficolin-3 is the most abundant of the five LP-PRPs in serum (20 μg/ml) [33] and the FCN3 gene is the most highly expressed gene in the liver and lungs among the five LP-PRPs [34]. Three cases of total ficolin-3 deficiency due to the 1637delC mutation have recently been reported [35-37]. One case was an adult with recurrent severe pulmonary infections, another case a neonate with necrotizing enterocolitis (NEC) and the third case was a premature newborn with Streptococcus agalactiae infections. The clinical relevance of having low levels or deficiency of both MBL and ficolin-3 is unknown and the role of ficolin-3 is still unclear. Ficolin-3 deficiency is classified as PID by the International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies [22].

To date, numerous disease specific cohorts have been screened for MBL deficiency [38]. However, to our knowledge, no data is available were the clinical phenotype of an adult MBLD cohort is compared to randomly selected cohort from the general population. Thus, we collected a relatively large population of adult MBLD cohort is compared to randomly selected cohort from the general population. Thus, we collected a relatively large population of adult MBLD cohort is compared to randomly selected cohort from the general population. Thus, we collected a relatively large population of adult MBLD cohort is compared to randomly selected cohort from the general population. Thus, we collected a relatively large population of adult MBLD cohort is compared to randomly selected cohort from the general population.

Materials and Methods

Subjects and samples

From the diagnostic laboratory database at the department of immunology 228 individuals (≥ 18 years old) were found to have MBL levels ≤ 500 ng/ml measured between 2006-2009. A total of 205 individuals were contacted and 163 of them agreed to participate in the study. Forty-three of these 163 were not able to attend the clinic within the timeframe settings for blood and data collection. Data was gathered from 120 individuals, 90 women (75%) and 30 men (25%). The age range was 18-76 years with mean age 44.7 years. Approximately 70% of the samples sent to our diagnostic laboratory are from ambulatory- or general practice settings in Iceland, sent for various medical reasons unknown to our laboratory. The study was approved by the National Bioethics Committee of Iceland and the Data Protection Committee of Iceland. Informed consent was obtained from all participants in the study. All participants answered detailed questionnaire focusing on health in general including infections. The questionnaire has been previously used in our earlier studies on IgA deficiency [39-42]. The control group consisted of 63 individuals (age 27 to 76 years) who were randomly selected from the Icelandic National Registry [39,40,42]. Serum and EDTA blood were collected from all participants. Serum was frozen at -80°C until used in ELISA (enzyme-linked immunosorbent assay) and EDTA blood was kept at -20°C until DNA was isolated. Genomic DNA was extracted from EDTA blood samples using NucleoSpin® Blood QuickPure kit (Machery-Nagel, catno: 740569.50) according to the manufacturer’s instructions.

MBL2 and FCN3 genotyping

The MBL2 variants in exon 1; Arg52Cys (D) (rs5030737), Gly54Asp (B) (rs1800450) and Gly57Glu (C) (rs1800451) were analysed with real-time polymerase chain reaction (PCR) using fluorescent hybridization probes with subsequent melting temperature (Tm) curve analysis on a LightCycler® instrument (Roche Diagnostics. Mannheim. Germany) as described previously [43]. To screen for the frameshift mutation (rs28357092, 1637delC) in the FCN3 gene we used restriction fragment length polymorphism PCR (RFLP-PCR) as previously described [35]. Briefly, a 650 bp PCR product spanning the putative mutation site is amplified and digested with ApaI restriction enzyme. When run on agarose gel the wild-type genotype (C/C) represents 2 bands (384 bp and 266 bp), heterozygotes (-/C) represent 3 bands (650 bp, 384 bp and 266 bp) and homozygotes represent undigested product (650 bp). Mutation analysis was confirmed with Sanger sequencing (sequencing service of company Microsynth AG, Switzerland).

MBL serum levels

MBL serum levels were measured using a sandwich ELISA system previously described (29).

Figure 1: (A) Distribution of MBL levels in control subjects (n=63) and MBLD subjects (n=120). (B) Distribution of MBL levels of the MBL2 genotypes within the MBLD cohort.
Statistical analysis

The two cohorts were compared with the Mann-Whitney U test in case of continuous variables and Kruskal-Wallis H test was applied when comparing the three genotype subgroups of the MBLD cohort. Categorical data was compared with χ² test or Fisher’s exact test. The level of significance was set at 0.05 and the program package SPSS 11.0 (SPSS, Inc, Chicago, Ill) was used for processing the data.

Results

MBL serum levels in the cohorts and among the MBL2 genotypes

Distribution of MBL serum levels of the control (n=63) and MBLD (n=120) subjects is illustrated in Figure 1A. Mean MBL levels for controls and MBLD were 1564 and 175 ng/ml, respectively. The
prevalence of the MBL2 genotypes A/A, A/O and O/O was 14.2% (17/120), 71.7% (86/120), and 14.2% (17/120), respectively. Thus, 85% of the MBLD cohort carried the dysfunctional allele O. In addition, 17/50 (34%) of MBLD individuals with MBL levels ≤ 50 ng/ml were of the O/O genotype. Mean MBL levels for genotype A/A, A/O and O/O were 320 ng/ml (range 220-500), 177 ng/ml (range 50-410 ng/ml) and 50 ng/ml (no range), respectively (Figure 1B).

Clinical manifestations of the MBLD cohort

**Respiratory tract infections:** The MBLD cohort reported a higher 12-month incidence of common cold (MBLD 28.3% versus control 7.9%, \( p=0.0014 \)) (Figure 2A). In addition, otitis media, sinusitis, pharyngitis, tracheitis, bronchitis and pneumonia were significantly more often reported by the MBLD cohort the preceding 5 years compared to controls (Figure 2A). The difference between the two cohorts was highly significant (\( p<0.0001 \)) in the case of sinusitis (MBLD 64% versus control 25%), tracheitis (MBLD 37.5% versus control 9.5%) and pneumonia (MBLD 35% versus control 1.6%). In contrast, there was no statistical difference in the incidence of pleurisy between the two cohorts (Figure 2A). Thus, as could be expected, MBLD individuals had significantly more often undergone tonsillectomy (MBLD 47% versus control 24%, \( p<0.0001 \)). Interestingly, four individuals had been diagnosed with *Campylobacter* infections and they all belonged to the MBLD cohort. The two cohorts did not differ in occurrence of ulcer (MBLD 8.5% versus control 1.6%, \( p=0.0642 \)) or *Salmonella* infections (MBLD 3.3% versus control 1.6%, \( p=0.48957 \)).

**Gastrointestinal infections:** Surveying the gastrointestinal symptoms profile of the two groups revealed that the occurrence of oesophagitis and gastritis in the preceding 5 years was high among MBL deficient individuals and significantly higher than found in the control cohort (Figure 2B). In addition, about 8% of the MBLD individuals had more than five episodes of gastritis over the last two years, whereas none of the control cohort had (\( p<0.0001 \)). Furthermore, the MBLD cohort had significantly more often been subjected to gastroscopy (MBLD 61% versus control 24%, \( p<0.0001 \)). Interestingly, four individuals had been diagnosed with *Campylobacter* infections and they all belonged to the MBLD cohort. The two cohorts did not differ in occurrence of ulcer (MBLD 8.5% versus control 1.6%, \( p=0.0642 \)) or *Salmonella* infections (MBLD 3.3% versus control 1.6%, \( p=0.48957 \)).

**Mucosa, cutaneous and blood infections:** The MBLD individuals reported a significantly higher incidence of stomatitis, conjunctivitis and onychia than the control group (Figure 2C). In addition, gingivitis tended to be higher amongst MBLD individuals (MBLD 41.5% versus control 28.6%, \( p=0.0868 \)). No difference was detected between the groups regarding prevalence of external otitis and bacterial skin infections the preceding 5 years (MBLD 12.6% versus control 7.9%, \( p=0.3408 \) and MBLD 17.8% versus control 19.0%, \( p=0.8377 \), respectively). Eleven MBLD individuals reported 1-4 episodes of sepsis the preceding 5 years, whereas no one in the control cohort did (Figure 2C). No difference was found between the two cohorts in the recurrence (≥ 5 times last 12 months) of herpes labialis (MBLD 6% versus control 2%, \( p=0.840 \)).

**Urogenital infections:** About 25% (8/32) of the men in the MBLD cohort reported prostatitis ≥ 1 times in the 5 preceding years, whereas only 5.9% (2/34) of the men in the control cohort did (Figure 2D). Episodes (1-4 times the last 12 months) of common infections such as cystitis, urethritis and bacterial infections of the vagina were significantly more frequent in the MBLD group (Figure 2D). The cohorts neither differed in reported frequencies of vaginal yeast infections nor nephritis (MBLD 47.8% versus control 37.9%, \( p=0.3582 \) and MBLD 6.7% versus control 3.2%, \( p=0.3208 \), respectively).

**Antibiotic treatment:** Antibiotic treatment during the last 12
months was significantly more common in the MBLD cohort compared to controls (Figure 3A). In addition, the MBLD individuals needed at some time in their life prophylactic antibiotic therapy more often than the control group (MBLD 34% versus control 14%, \( p = 0.0001 \)).

**Recurrent and severe infections:** The infections were classified into three classes according to severity and frequency (Figure 3B). This classification is based on a study-specific classification system previously used in our studies on clinical manifestations of selective IgA deficiency [42]. The infections subjected to this analysis were common cold, pharyngitis, sinusitis, tracheitis, bronchitis, pneumonia, urethritis, prostatitis and conjunctivitis. Approximately 43% of the MBL deficiency individuals suffered from recurrent and severe episodes of infections (class 3), whereas only 16% of the control group did (\( p = 0.0003 \)) (Figure 3B).

**MBL2 and FCN3 genotypes and clinical findings**

Recurrent common cold was relatively more often reported by the O/O genotype subgroup than in the A/A genotype subgroup (Figure 4A). However, no association was detected between genotype and various upper and lower respiratory infections (Figure 4A). Interestingly, among the individuals in the MBLD cohort that reported pleurisy, two were O/O (2/17), one A/O (1/86) and no one was A/A (0/17) (Figure 4A). The O/O genotypes reported 4-7 episodes of pleurisy the last two years whereas the A/O genotype reported 1-2 times the last two years.

Significantly higher occurrence of gastritis was found among individuals with the O/O genotype and they also tended to have higher frequency of oesophagitis than A/O and A/A and genotypes (Figure 4B). In the case of gastritis, the effect of the O allele tends to be gene dose dependent. In addition, O/O genotypes had significantly more often been subjected to gastroscopy than A/O and A/A genotypes (Figure 4B). However, no association was detected between genotypes and Salmonella/Campylobacter infections or ulcer (data not shown).

The O/O subgroup tended to have higher frequency (1-4x per year) of labial herpes (41.2% (O/O), 23.8% (A/O) and 11.8% (A/A), \( p = 0.1337 \)). In contrast, gingivitis appeared to be rare amongst O/O individuals (17.6% (O/O), 44.7% (A/O) and 50% (A/A), \( p = 0.0917 \)). Stomatitis, conjunctivitis, cutaneous infections, urogenital infections and sepsis were not associated with genotype (data not shown).

Five individuals in the MBLD cohort (4.2%) were heterozygous (C/-) for the 1637delC allele in the FCN3 gene. This is higher heterozygote frequency than we have observed previously in 500 Icelandic blood donors (2%) (unpublished data). The MBL serum levels of the C/- individuals ranged from 350 to 500 ng/ml and the MBL2 genotypes were all A/O (three A/B, one A/C and one A/D). The MBLD individuals heterozygous for the 1637delC allele were not more susceptible to infections than the wild-type individuals (C/C), regardless of MBL2 genotype.

**Discussion**

In this case-control study, we found that adult MBL deficient
individuals have increased proneness to various respiratory, gastrointestinal, urogenital, mucosal, skin and blood infections compared to a randomly selected control group. In addition, we found that the infections were recurrent and severe in the MBLD cohort. Furthermore, we showed that MBLD individuals with the O/O genotype were significantly more likely to suffer from esophagitis and gastritis as well having undergone gastroscopy than MBLD individuals with the A/O and A/A genotypes.

Our results on high occurrence of respiratory tract infections in adult MBL deficient individuals support previous findings [44]. However, our MBLD cohort was more frequently diagnosed with sinusitis than previously reported [44]. The high incidence of tonsilllectomy and adenoidectomy among MBL deficient individuals has also been previously observed among MBLD individuals [45]. In addition, we found that tonsilllectomy tended to be linked to the O allele which also supports previous results [45]. It has been shown that pneumonia caused by Streptococcus pneumoniae is linked to the O allele and low MBL levels increases the death due to pneumococcal infection [21,46]. Since our questionnaire was retrospective it is not possible to identify the underlying cause of pneumonia of our study cohort.

Previous studies have also reported significantly increased frequency of severe MBL deficiency (MBL levels ≤ 50 ng/ml) in adult patients with a history of recurrent/and or severe infections, including pneumonia and bronchitis [44]. In that study, it was ascertained that the patients neither had concomitant immunodeficiencies nor had received immunosuppressive therapy [44]. Our results (Figure 3B) support their findings, however we can not rule out that MBL deficiency was the only cause to increased infection susceptibility because concomitant immunodeficiencies were not investigated in our study.

Interestingly, 9.3% of the MBL deficient individuals have had more than one episode of sepsis during the last 5 years, whereas none of the control group had (p=0.013). These results are in concordance with previous reports which indicate that high serum MBL levels may be protective against sepsis [47,48]. The MBL2 genotypes did not differ with respect to occurrence of sepsis in our study which contrasts other findings suggesting that the O allele predisposes to sepsis [49,50].

What is perhaps the most outstanding finding in our study is the significant association we found between O/O genotypes and gastrointestinal inflammatory symptoms. Supporting this association is the observation that ficolin-2 has previously been reported in O/O patients [57]. An observation that suggests that ficolin-2 might be compensating for MBL deficiency. This may also apply for ficolin-3. Ficolin-2 and ficolin-3 are both expressed in liver, have high structure homology, share ligand binding affinity, circulate in serum and both initiate the LP [58]. Ficolins may substitute for MBL deficiency and this may explain why the frequency of the O/O genotype is relatively high in Caucasians (4%) and why a subset of O/O individuals are healthy [24,59]. Our results warrant further studies involving screening for both deficiency alleles (i.e. O and J637delC) in larger and different cohorts and investigate the advantages and/or disadvantages for the individual.

The results of our study indicate that more clinical attention should be made to adult patients with MBL deficiency. The MBLD cohort was highly susceptible to infections, therefore the MBL serum levels of patients with recurrent and severe infections should be routinely determined. The O allele was only associated with esophagitis, gastritis and gastroscopy but not with the various respiratory, urogenital, skin, mucosal and blood infections in the MBLD cohort. Thus, more attention needs to be paid towards MBL2 genotyping patients with gastrointestinal complications. Genotyping of a larger study cohort which includes patients with gastrointestinal inflammation symptoms is warranted to better understand the significance of MBL in gastrointestinal immunity and/or homeostasis.

Acknowledgements
The authors would like to thank Prof. Jon Johannes Jonsson, Department of Genetics and Molecular Medicine, Landspitali University Hospital, for the use of the LightCycler™ instrument. This work was supported by the Landspitali University Hospital Research Fund.

References


