

# Childhood Levels of Immunoglobulins and Mannan-Binding Lectin in Relation to Infections and Allergy

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## Abstract

Respiratory tract infections, allergies and otitis media are common problems in early childhood. Our aim was to evaluate in a longitudinal community-based cohort study the association between maturation of immunoglobulin (Ig) and mannan-binding lectin (MBL) responses and disease manifestations in the first 4 years of life. Sustained low levels of IgA proved the strongest single indicator of susceptibility for recurrent otitis media ( $P=0.008$ ) and respiratory tract infections ( $P=0.02$ ), and this condition was also associated with low production of IgG subclasses. About 7% of the cohort had sustained low levels of MBL ( $<0.4$  mg/l). Low MBL did not predispose to any ailments studied, but children with low IgA and recurrent otitis media had relatively low MBL at birth, which failed to increase during the study period and was significantly reduced at the age of 4 years ( $P=0.04$ ). MBL levels increased from birth to 2 years ( $P<0.0001$ ) and were higher in children than in adults ( $P=0.001$ ). The increase was 1.9-fold in children with no recorded clinical events and 1.7-fold in children with asthma or infections, but significantly lower, 1.2-fold, in children with recurrent otitis media. Low levels of IgA within the normal range may reveal disease susceptibility not detected by conventional criteria. Slow maturation of Ig appears to be the main factor of susceptibility during childhood, but a strong corollary role for MBL is indicated by the high levels produced during childhood as well as the precipitation of disease in children with low levels of MBL and Ig.

## Introduction

Respiratory tract infections, allergies and otitis media are common problems in early childhood [1–4]. This is thought to reflect delayed maturation of the immune system, especially the ability to produce antibodies. In support of this, frequencies of allergic manifestations and otitis media are increased in young children with relatively low serum immunoglobulin A (IgA) levels [2]. Many authors believe that serum levels of mannan-binding lectin (MBL) are important for pathogen resistance during this period, and associations between low MBL and abnormal infections or allergy in young children have been reported [5–10]. The increase in MBL observed after birth [11–14] and the observation that MBL may be higher in children than adults [15] are consistent with this notion. Importantly, however, the latter possibility still needs confirmation. For full evaluation of the relative importance of Ig and MBL during childhood, detailed

studies on quantitative and qualitative aspects of these factors as well as their interplay to diseases are in demand.

MBL is a calcium-dependent serum lectin with structural and functional resemblance to complement C1q [16–18]. Its interaction with associated serine proteases after binding to N-acetyl-glucosamine, mannose or fucose on the glycocalyx of pathogens leads to activation of complement C4 and C2 and thus promotes phagocytic elimination of pathogens that express sufficient amounts of the appropriate sugars [10, 19]. MBL is produced by the liver and levels rise mildly during the acute phase of the immune response [19–21]. In man, the gene coding for MBL is on the long arm of chromosome 10 and is composed of four exons as well as large introns which are involved in regulating the expression or synthesis of MBL [22, 23]. Levels of MBL vary quite widely within populations, reflecting promoter polymorphism or mutations in the first exon of the gene [24–26] and other less well-defined

genetic defects [27]. The median MBL serum level has been determined as 1.23 mg/l in homozygous wild-type Caucasians and below 0.05 mg/l in those who are homozygous for an exon 1 mutation, with heterozygous individuals ranging from less than 0.05 mg/l to more than 1 mg/l [26, 28]. Severe MBL deficiency associated with promoter polymorphism or exon 1 mutations is found in 5–7% of the population [29, 30]. The cut-off level for a severe deficiency is commonly set at 0.1 mg/l but even less severe defects may affect pathogen resistance; recent studies indicate that MBL concentrations of less than 0.4 mg/l cannot induce complement activation in the presence of microorganisms carrying the appropriate sugars [31] and are associated with serious infections during treatment for hematological malignancies [32]. Failure to produce MBL above this level appears to be well tolerated at the adult stage because it affects 20–25% of adults [28]. However, one observation suggests that a much lower proportion of children have MBL below this level [15] and this interesting but largely overlooked possibility requires confirmation.

Levels of MBL increase after birth [11–14] but it is not clear when or why. We have noted an opsonic defect in a few children, including three siblings, which persisted through childhood but became corrected later in life (unpublished), suggesting that in addition to promoter polymorphisms and exon 1 mutations, MBL responses may in certain cases also suffer from a maturational defect, e.g. slow maturation of cytokine responses or lack of transcription factors. Using samples and data from a previous population-based cohort study on immunological maturation in children 0–4 years of age [2–4], we now investigate the maturation of MBL production and the relative importance of and possible synergy between Ig and MBL in preventing infections and allergy; the interesting possibility that levels of MBL are increased during childhood [15] was checked by comparison with 228 healthy adults.

## Materials and methods

**Subjects.** MBL levels were measured in 228 healthy blood donors and in samples from a previous study on allergy and recurrent infections [2–4] involving 180 children (93 boys and 87 girls) randomly selected and evaluated clinically at the age of 2 years (18–23 months) and again at 4 years of age (42–48 months) at which time 160 complied; a sample of cord blood had been prospectively obtained from the children at birth, and blood samples were again drawn at both visits. Informed consent was obtained from all subjects/parents, and approval was gained from all relevant ethical committees.

**Clinical and diagnostic evaluation.** Clinical data were recorded separately for each of the two periods, that is 0–2 years and 2–4 years. Initially, the emphasis was mainly

on allergic manifestations and otitis media, but after the age of 2, information was also collected on respiratory tract infections. A structured questionnaire was used to obtain detailed clinical information on eczema, allergic rhinitis, episodes of wheezing, otitis media, respiratory infections including pneumonia, diarrhoea and other gastrointestinal disturbances and medications [2, 3]. Episodes of otitis media, wheezing or pneumonia were only recorded if they had been diagnosed by a physician. For each evaluation period, recurrent otitis media was defined as three or more episodes. Children with three or more episodes of wheezing were recorded as having asthma. Confirmed respiratory tract infections with episodes of wheezing that were not diagnosed as asthma or pneumonia were recorded as bronchiolitis. Allergic rhinitis was defined as episodes of watery nasal discharge with or without conjunctivitis not accompanied by fever or other symptoms of respiratory tract infections. Diagnosis of allergy was based on commonly used criteria [33, 34], including skin prick tests to common allergens [2, 3]. All recorded observations were evaluated by a pediatric immunologist.

**Clinical classification of the study cohort.** The children who attended at 4 years were divided into five groups according to their clinical manifestations between the age of 2 and 4 years; 34 children had recurrent otitis media, nine asthma, three allergic rhinitis, 13 lower respiratory tract infections and 40 had a combination of these disorders. The remaining 61 children did not have any complications relevant to the present study and formed a control group.

**Materials.** Rabbit anti-human  $\alpha$ -chain (code A262), alkaline phosphatase (AP)-conjugated rabbit anti-human IgA (code D338), F(ab)2 rabbit anti-human IgE (code A094), AP-conjugated rabbit anti-mouse IgG (code D314) and AP-conjugated avidin were from Dako (Copenhagen, Denmark), AP substrate (p-nitrophenyl phosphate) from Sigma (St. Louis, MO, USA), mouse anti-human IgE (I-339-03/04) from Medix (London ON, Canada), IgA standard from Beckman-Coulter (Fullerton, CA, USA), IgE standard from Pharmacia Diagnostics (Uppsala, Sweden) and IgG subclass-specific sheep antibodies and IgG subclass standards from Sanquin (Amsterdam, Netherlands). Human MBL (0.2 mg/ml) was from Statens Serum Institut, Copenhagen, Denmark (kind gift from Claus Koch) [35]. Microtiter plates were from Nunc (Maxisorp; Nunc, Roskilde, Denmark). In MBL measurements, coating antibodies were diluted in carbonate buffer (pH 9.6) and phosphate-buffered saline (PBS), pH 7.4 (10 mM PBS containing 140 mM NaCl) used for other dilutions except as indicated.

**Ig measurements.** Subclasses of IgG were measured by single radial immunodiffusion and IgA and IgE by enzyme-linked immunosorbent assay (ELISA) as previously described [2–4]. The results were expressed as g/l by comparison to known standards.

IgG subclasses were measured by radial immunodiffusion using subclass-specific sheep antibodies and a standard calibrated against WHO Immunoglobulin Reference preparations 67/97 [4]. The within-assay variability was 10%, and the between-assay variability did not exceed 15%. The detection limit was 0.28 g/l for IgG1, 0.15 g/l for IgG2, 0.04 g/l for IgG3 and 0.06 g/l for IgG4.

IgA was measured by a sandwich ELISA [2, 3] using rabbit anti-human  $\alpha$ -chain for coating and AP-conjugated rabbit anti-human IgA for developing. Samples were diluted 1/3200 in PBS-Tween. The absorbance of each sample was read against dilution curves of a standard with known concentration of IgA. The within-assay variability was 10%, and the between-assay variability did not exceed 15%. The detection limit was 0.023 g/l.

IgE was measured by ELISA [2, 3], using F(ab)2 rabbit anti-human IgE for coating and mouse anti-human IgE followed by AP-conjugated rabbit anti-mouse IgG for developing. Samples were compared to serial dilutions of an IgE standard. The detection limit of the ELISA was 0.23 kilounits (KU)/l. The within-assay variability was 10%, and between-assay variability did not exceed 20%.

**Anti-MBL antibodies.** Two rabbits were immunized with MBL in complete Freund's adjuvant followed by repeated immunizations in incomplete Freund's adjuvant. The IgG fraction was obtained by affinity chromatography of rabbit plasma on a column with staphylococcal protein A bound to a Sepharose matrix (Pharmacia). They were used in the native state (rabbit 1) or after biotinylation (rabbit 2).

**MBL reference serum.** MBL measurements were initially calibrated with a MBL preparation that had been subjected to quantitative amino analysis, and this method used for confirming total absence of measurable MBL in sera previously determined to be devoid of *Saccharomyces cerevisiae* opsonin activity. For improved accuracy, we then used one of these sera as reference after adding purified MBL to a final concentration of 1.0 mg/l; the serum was aliquoted and stored at  $-70^{\circ}\text{C}$ .

**MBL measurements.** For MBL measurement, samples were available from 118, 177 and 158 of the children at each of the three sampling points, respectively. Measurements were performed on aliquots stored at  $-70^{\circ}\text{C}$  throughout. MBL was measured by a sandwich ELISA, using anti-MBL antibodies for coating (native) and developing (biotinylated). Serum samples were incubated for 3 h at room temperature in the presence of 0.05% Tween 20 and 5 mM EDTA. Two reference sera were included on each plate for estimation of intra- and interassay variation. The assay was visualized by AP-conjugated avidin followed by AP-substrate in 10% diethanolamine buffer (pH 9.8). The absorbance was read in an ELISA reader at 405 nm. Absorbance values from each sample were calculated from a standard curve made from serial twofold dilutions of the previously calibrated

reference serum. All reactants were added in 100  $\mu\text{l}$  volumes, and the plates were washed three times in PBS-Tween between steps.

The detection limit of the MBL assay was 0.025 mg/l. The within-assay variability was 8%, and the between-assay variability 17% ( $<8\%$  in samples lower than 2.8 mg/l). The concentration required for normal opsonization of yeast (*S. cerevisiae*) was studied by a radiometric opsonization assay [36] using MBL-deficient serum spiked with different concentrations of purified MBL, and determined to be 0.4 mg/l, which is in keeping with published accounts [31, 32].

**Calibration of the MBL ELISA.** The MBL ELISA was extensively calibrated by exchange of antibodies, reference sera and purified MBL between collaborating labs in Reykjavík and Aarhus; exchange of reagents between the latter and other collaborators ensured comparable results in Aarhus, London, Oxford, Copenhagen and Reykjavík [21, 28]. Towards the end of our project, we also implemented an ELISA based on mouse monoclonal antibody 131-1 supplied by the Aarhus lab or Cymbus (Southampton, UK) and calibrated this method in the same fashion [35]. This ELISA yielded values which were 10% lower than the ELISA described above, and to facilitate comparison with results based on the 131-1 ELISA [12, 14, 26, 27, 32, 35, 37-40], we used 0.9 as conversion factor; after this correction, the two methods showed excellent agreement ( $r=0.832$ ,  $P<0.0005$ ) as tested on samples from 132 children, aged 14 months (Th. Vikingsdóttir, unpublished).

**Statistical analysis.** MBL and Ig values were compared between groups with Mann-Whitney or Fisher exact statistics and correlated with clinical data using Spearman Rank statistics. The student's  $t$ -test was used for subgroup comparisons. Statistical power was calculated when possible (parametric methods only). Probability ( $P$ ) values of  $<0.05$  were considered significant.

## Results

### Association of Ig/MBL to infections

The frequency of clinical events in the children that could be followed between the age of 2 and 4 years was 40% for recurrent otitis media, 20% for bronchitis or bronchiolitis, 11% for pneumonia, 21% for asthma, 27% for eczema and 10% for diarrhoea. No associations were seen between MBL levels at birth or 2 years of age and any of these disorders or combinations of disorders. We have previously reported an association between low IgA and manifestations of allergy in this cohort [2-4], and in the current study, levels of Ig were also found to be significantly associated with respiratory infections. Children in the lowest quartile of IgG3 at 2 years ( $<0.19$  g/l) thus had an increased prevalence of respiratory disorders (asthma and infections)

**Table 1** Median levels of immunoglobulins (Ig) (g/l) in children with sustained low or high IgA\*

|           | 2 years |         |           |           | 4 years |         |         |         |
|-----------|---------|---------|-----------|-----------|---------|---------|---------|---------|
|           | IgG1    | IgG2    | IgG3      | IgG4      | IgG1    | IgG2    | IgG3    | IgG4    |
| Low IgA   | 5.40    | 0.78    | 0.21      | 0.03      | 5.49    | 0.95    | 0.19    | <0.06   |
| Quartiles | 4.0–6.0 | 0.4–0.9 | 0.15–0.27 | 0.0–0.1   | 4.8–6.8 | 0.7–1.6 | 0.1–0.3 | 0.0–0.1 |
| High IgA  | 6.30    | 0.90    | 0.29      | 0.12      | 7.71    | 1.15    | 0.30    | 0.15    |
| Quartiles | 5.5–7.0 | 0.7–1.4 | 0.2–0.4   | 0.05–0.25 | 6.2–9.3 | 0.8–1.6 | 0.2–0.4 | 0.1–0.5 |
| P         | <0.001  | 0.03    | 0.01      | 0.01      | 0.002   | NS      | 0.02    | 0.002   |

\*Children in the lowest or in the higher three quartiles at both 2 and 4 years.

in the next 2 years ( $P=0.02$ ), and this was mainly due to an increased number of infections ( $P=0.05$ ). Both respiratory infections ( $P=0.01$ ) and otitis media ( $P=0.03$ ) were increased in children in the lowest quartile of IgA at 2 years ( $<0.47$  g/l), and in fact also at 4 years ( $<0.60$  g/l) ( $P=0.05$  and  $0.03$ , respectively) suggesting that low IgA responses might be an inherent feature in a subgroup of infection-prone children. We tested this hypothesis by comparing children belonging to the lowest quartile of IgA at both time points ( $n=16$ ) to the rest of the cohort, and found a marked prevalence in this subgroup of both recurrent otitis media ( $P=0.008$ ) and respiratory infections ( $P=0.02$ ). Besides low IgA, this group had significantly lower levels of IgG subclasses at 2 and 4 years than children in the upper three quartiles of IgA at 2 and 4 years (Table 1). The importance of IgA is also indicated by the fact that children who continued to suffer attacks of otitis media between age 2 and 4 ( $n=22$ ) had lower serum IgA levels at age 2 than those who recovered ( $n=26$ ); thus, children who suffered more than four attacks between age 2 and 4 had a median serum IgA concentration of  $0.56$  g/l compared with  $0.67$  g/l in those who had become asymptomatic at age 4 ( $P=0.03$ ) and this also applied to IgG4 ( $P=0.03$ ).

#### Age distribution of MBL values

Figure 1 shows the distribution of serum MBL levels in the adults as well as the study cohort at three different ages. MBL levels were significantly higher in the children at all sampling points than in the adults. The median MBL concentration increased significantly during the first 2 years after birth ( $P>0.0001$ ) but then remained similar at 4 years, indicating that maturation of MBL production takes place before the age of 2 years. The distribution was bimodal at birth, but by 2 years of age, the proportion of children with low MBL levels had decreased substantially ( $P=0.006$ , power 0.8). Thus, 21 (18%) of the neonates had MBL levels under  $0.4$  mg/l (Fig. 1, open circles) whereas by 2 years of age, a moderate increase in MBL had occurred in nine of these children, leaving only 12 (7%) with persistently low MBL. The proportional increase in children with low ( $<0.4$ ) and high ( $\geq 0.4$  mg/l) MBL at

birth was similar, as shown in Fig. 2, but it is clear that children with low MBL at birth tend to remain low in MBL. This was confirmed by correlation statistics, MBL levels being highly correlated between birth and 2 or 4 years of age ( $r=0.72$  and  $0.73$  respectively,  $P<0.01$  for both), and between 2 and 4 years of age ( $r=0.82$ ,  $P<0.01$ ).

The post-natal increase in MBL levels was not simply due to acute phase responses as it was similar in children with and without clinical events; levels increased on average 1.7-fold in children with clinical events ( $P<0.001$ ) but 1.9-fold in children with no clinical events

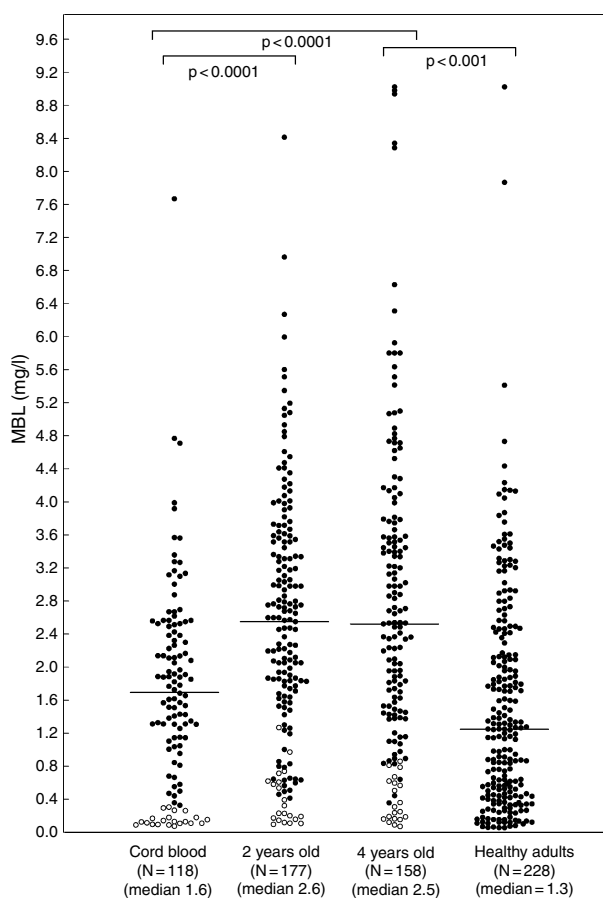


**Figure 1** Distribution of mannian-binding lectin (MBL) levels at birth, 2 and 4 years of age and in adults. The medians are drawn as horizontal lines. Open circles denote children with low ( $<0.4$  mg/l) MBL levels at birth.

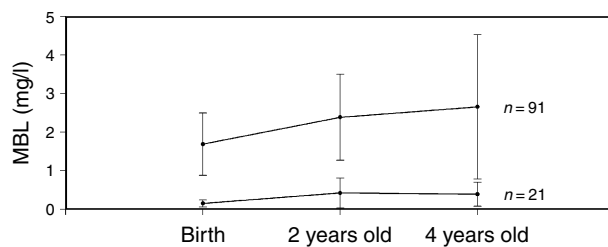


**Figure 2** Changes in average mannan-binding lectin (MBL) levels in children with birth levels of MBL either  $<0.4$  mg/l or  $\geq 0.4$  mg/l.

( $P < 0.001$ ). When the children with clinical events were analyzed separately, the post-natal increase in MBL was observed only in children with asthma (1.7-fold,  $P < 0.001$ ) and respiratory infections (1.9-fold,  $P = 0.02$ ) while in marked contrast to these groups as well as the children with no clinical history, children with recurrent otitis media showed no appreciable increase (Fig. 3). The possibility that this was due to a defect in post-natal MBL maturation in a subgroup of children was tested by calculating the lower normal limit for MBL increase and comparing the proportion of children who fell short of this figure between the different clinical groups. This approach identified 18 children with less than 0.9-fold increase in MBL (non-responders), and a Fisher exact test confirmed that the group with recurrent otitis media contained an increased proportion of these, or 8/21 (38%) compared with 10/82 (14%) ( $P = 0.01$ ).

To further elucidate the relationship of MBL levels and maturation to medical complications in childhood, we next looked at MBL levels in the children with and without clinical complaints between 2 and 4 years of age. Children with asthma had significantly higher MBL levels than children without medical complications (Table 2); the fact that increased MBL levels were observed in this group even at birth indicates that this association is due to intrinsic factors rather than acute phase responses. High MBL levels were also recorded in children with respiratory infections and allergic rhinitis, although only significant after birth. Allergic rhinitis was not diagnosed until after 2 years, and the increased MBL at 4 years may thus represent acute phase reactions in active disease. High MBL values in children with infections between 2 and 4 years were not solely due to acute phase responses, as they were observed even at 2 years, and children who were symptomatic at sampling had similar MBL values as the rest of the group. Children with recurrent otitis media did not differ from controls. Children with a combination of disorders had intermediate values of MBL (data not shown).

#### MBL as cofactor in disease predisposition

Although we did not establish a major role for MBL as an independent susceptibility factor in microbial defence or allergy, the possibility remained that it could be a cofactor

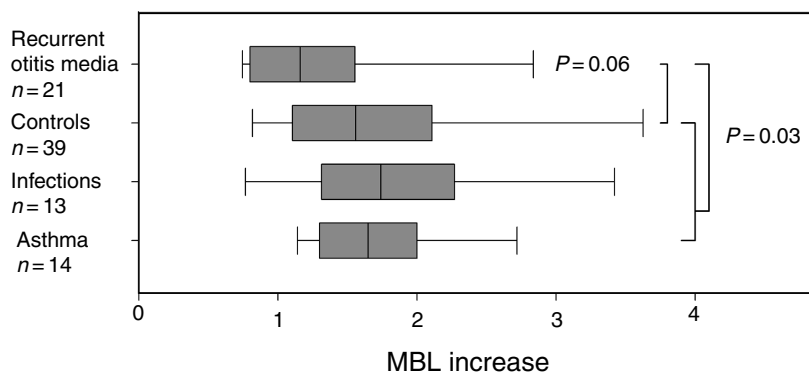
in disease predisposition. In particular, the results connecting low IgA to infections and otitis media and MBL non-responders to otitis media appeared pertinent. We therefore analysed clinical parameters and MBL levels in children with low IgA (lowest quartile at 4 years,  $<0.06$  g/l). In this subgroup, it was found that children with recurrent otitis media between the age of 2 and 4 years tended to have lower levels of MBL at birth than those with few or no episodes of otitis media ( $P = 0.09$ ); furthermore, in striking contrast to the cohort as a whole, MBL levels in these children did not increase during the study period (Fig. 4) and were in fact significantly lower at the age of 4 years than the MBL levels in the corresponding subgroup of children who did not suffer from recurrent otitis media ( $P = 0.004$ ; power 0.82). Analogous results were obtained when children with low IgA at 2 years were analysed (results not shown). The group with low IgA at both 2 and 4 years could not be analysed meaningfully as it contained only one child with no medical complications.

Thus, recurrent otitis media is associated with sustained low levels of both IgA and MBL as well as low production of IgG (Table 1). Recurrent otitis media (more than two episodes) was observed in 36 of the 53 children who had tonsillectomy (22 had more than four episodes) but only 30 of the remaining 108 children (13 had more than four episodes) ( $P < 0.001$  for both comparisons); no association was seen between MBL or IgA values and tonsillectomy.

#### Discussion

Respiratory infections and otitis media are an important problem of childhood not only due to their high overall prevalence but also because infections are the most common cause of asthmatic attacks in children with this condition. This study identifies sustained low IgA as the strongest single indicator of susceptibility in children between 2 and 4 years of age; conspicuously, only one of 16 children in this group was free of clinical complications. In our cohort, only one child had a complete IgA deficiency as determined by commonly accepted criteria, and our results thus show that even subtle IgA variation within the normal range may affect resistance to respiratory pathogens. Once identified by their sustained low IgA within the normal range, the children turned out to also have sustained low production of MBL and IgG. These results are particularly interesting in the light of our findings of low IgA (0.37 g/l) and apparent cure of symptoms in the 2-year-old MBL-deficient girl who became the first child treated with affinity-purified MBL [35]. The possibility that simultaneous defects in both MBL and Ig are needed for disease to precipitate has been noted in previous studies [7, 37, 38], and our findings are in good agreement with a recent study showing that common variable immunodeficiency patients with aberrations in their capability to hypermutate Ig genes are extremely

**Figure 3** The increase in mannan-binding lectin (MBL) levels (MBL at 2 years/MBL at birth) in three groups of children according to their medical history. Only children who could be followed from birth to 2 years of age were analysed ( $n=103$ ). The medians, 10th and 25th percentiles, and numbers of children are shown.



vulnerable to lung infections if they at the same time carry MBL variant alleles [41].

Our study confirms a previous observation [15] of MBL levels being raised during childhood; this phenomenon has now been noted in two completely remote populations indicating a special role for MBL during childhood. A closer inspection of published reports of MBL levels in children and adults adds further support to this notion, as medians of 1.0–1.3 appear to be typical of adult populations while medians of 1.7–2.7 are commonly reported for children [12, 13, 17, 27, 28, 39]. Our results are consistent with a median of 2.54 obtained in a study on 135 children 14 months of age (Vikingsdóttir, unpublished). The recent discovery of an association between levels of growth hormone and MBL [40] offers a logical explanation to the high levels of MBL measured during childhood. In fact, our findings and those of Terai *et al.* [15] may help to explain why a growth-hormone responsive element has been included in the regulatory sequence of the MBL gene during evolution. It would appear that increased levels of MBL in juveniles have turned out to be advantageous to the species, and it would be interesting to examine the possibility that this advantage is also used by lower vertebrates. The high proportion of adults (21%) compared with children (7%) with MBL levels below the limit found in this study to allow for effective opsonization

probably relates to the high redundancy of MBL in microbial defence when the immune system has fully matured.

The median level of MBL at birth in our children (1.6) is in good agreement with previously published figures from Denmark (1.3–1.7) and China (1.6–1.7) but slightly higher than those (1.1) recorded in Japan [6, 11–13]. In agreement with a previous report [13], MBL concentrations showed an overall increase of about 50% during the first 2 years after birth, while an overall increase in MBL levels was not observed between the age of 2 and 4. Previous reports have indicated that a post-natal increase in MBL may occur already in the first 5 days after birth [11], but it has not yet been determined whether this increase is sustained, reflecting maturation, or represents a transient acute phase response to inhaled stimulants or the stress of labour and delivery. The persistence of increased MBL levels at 3 months and thereafter suggests that a true post-natal maturation takes place in the first 3 months, leading to a sustained MBL increase lasting through childhood (cf. correlation statistics in the present study) [13]. Previous studies have not assessed the contribution of acute phase responses to the observed increase, but in the present study, several lines of evidence suggest that this effect is minimal; (i) the increase was similar in controls as in children with clinical episodes, (ii) the increase at 2 years was still apparent at 4 years, and MBL

**Table 2** Mannan-binding lectin (MBL) levels (mg/l) in children with different clinical manifestations between the age of 2 and 4 years\*

|                               |     | Birth | P    | 2 years | P     | 4 years | P     |
|-------------------------------|-----|-------|------|---------|-------|---------|-------|
| Asthma (n=9)                  | AVG | 3.45  | 0.01 | 4.61    | 0.001 | 5.16    | 0.001 |
|                               | STD | 2.56  |      | 1.03    |       | 2.24    |       |
| Allergic rhinitis (n=3)       | AVG | 2.58  | NS   | 4.05    | NS    | 5.64    | 0.007 |
|                               | STD | 0.46  |      | 1.98    |       | 1.82    |       |
| Infections (n=13)             | AVG | 2.37  | NS   | 3.52    | 0.07  | 3.98    | 0.01  |
|                               | STD | 1.30  |      | 1.74    |       | 1.81    |       |
| Recurrent otitis media (n=34) | AVG | 2.09  | NS   | 3.08    | NS    | 3.05    | NS    |
|                               | STD | 1.15  |      | 1.85    |       | 2.18    |       |
| Controls (n=61)               | AVG | 1.76  |      | 2.61    |       | 2.59    |       |
|                               | STD | 1.38  |      | 1.74    |       | 1.83    |       |

\*The averages (AVG), standard deviation (STD) and differences from controls are indicated. NS, not significant. Power: 0.26 for  $P=0.07$ , 0.65 for  $P=0.01$ , 0.75 for  $P=0.007$ , >0.9 for  $P=0.001$ .

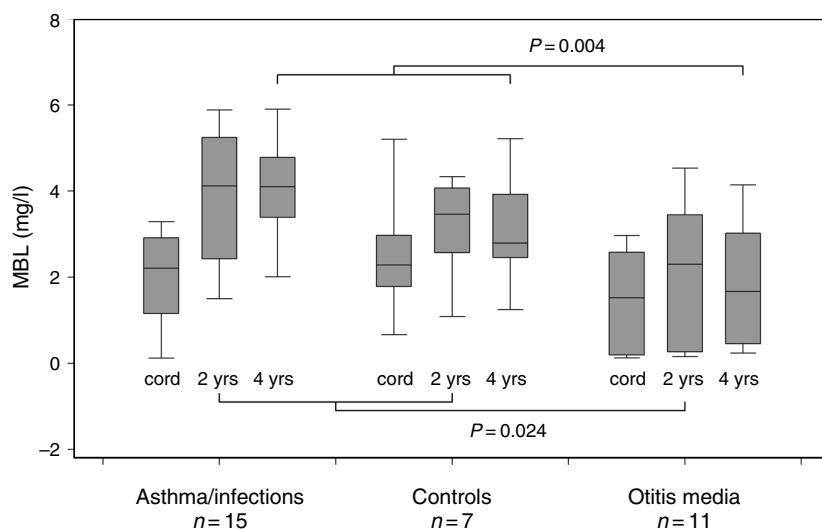


**Figure 4** Mannan-binding lectin (MBL) levels (medians, 10th and 25th percentiles) in children with relatively low IgA (<0.06 g/l) and asthma or respiratory infections, recurrent otitis media or no medical complaints between the age of 2 and 4 years.

levels were highly intercorrelated, (iii) levels of MBL were increased from birth in the asthmatic children, suggesting intrinsic MBL-producing capacity rather than acute phase responses, and (iv) in the remaining children, the complications at 2 or 4 years were almost entirely historical, with the great majority of children being asymptomatic at sampling [4]; acute phase increases in MBL, when they occur, are rather modest (1.5–2-fold) and transient, with maxima typically occurring in only one out of a series of measurements [20, 21]. Again, a sustained MBL increase in childhood is consistent with the finding that the levels are affected by growth hormone [40].

Our findings indicate that MBL deficiency is not a major independent risk factor for allergy or respiratory tract infections in children between 2 and 4 years of age, and this is consistent with results from several recent studies [39, 42–46]. However, the high levels of MBL in the children and low frequency (7%) of values below the concentration required for effective opsonization suggests that for this age group, even larger cohort sizes are required for full assessment of the association of low MBL to ailments. Moreover, our study involved a random sample in which both major and minor ailments were prospectively followed. Studies on large groups of critically affected children may yield added power, and a relationship between low MBL and infections requiring hospitalization is indeed indicated by two such studies [5, 6]. In addition, our study was informative mainly for the age group of 2–4, while it has been suggested that the importance of MBL may be greatest at 6–18 months [30], and this notion is supported by a recent study [47] as well as our observations of a peak of MBL levels in childhood and an additive effect of low MBL and Ig in disease predisposition.

We did not perform genotyping on samples from our cohort, but the presence or absence of low-MBL producing haplotypes can usually be inferred with acceptable precision

from protein concentration [17]. Twelve children (6.8%) had persistently low serum levels of MBL (median 0.16 mg/l), consistent with the presence of a variant haplotype (B, C, D or LXA) on both chromosomes [26].

Our study offers no explanation to the molecular identity of the factor(s) responsible for the post-natal increase in MBL, but the possibility that this may be connected with levels of pro-inflammatory cytokines or growth hormone [38] should be considered in future studies. This factor could in fact also explain the high MBL values in children with asthma even at birth, as well as the association between recurrent otitis media and lack of post-natal MBL maturation (especially prominent in children with low IgA). An association between susceptibility to otitis media and low MBL levels has been reported previously [8].

In conclusion, a sustained partial defect in IgA (as defined by the lower 25% cut-off) appears to be a helpful indicator of disease susceptibility in young children; low levels of MBL were not identified in this study as a major cause of morbidity in 2–4 years children but appeared to precipitate disease in children with low Ig, and levels of MBL peak in childhood. Our results are consistent with the notion of a supportive role for MBL in subjects with an intact immune system and a more prominent role in immunocompromised subjects, in infants at the nadir of Ig levels between 6 and 18 months of age, and in infants with slow maturation of IgA and IgG.

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