Coexistence of (Partial) Immune Defects and Risk of Recurrent Respiratory Infections

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Background: Respiratory infections are major causes of morbidity and mortality, but determinants of susceptibility are poorly defined. We studied whether and to what extent immunologic and genetic factors are associated with increased susceptibility to respiratory infections.

Methods: We evaluated the prevalence of IgA, IgM, IgG, and IgG subclass deficiencies, impairment in the antibody response against pneumococcal polysaccharides, G2m(n) allotypes, FcyRIIa polymorphisms, partial C2 and partial C4 deficiency, promoter polymorphisms in MBL2, and lymphocyte subset deficiencies in a control population and in consecutive children with recurrent respiratory infections.

Results: IgA and/or IgG subclass deficiency was found in 27 of 55 patients (49%) and 6 of 43 controls (14%) (P = 0.0006). An impaired antibody response to polysaccharides was found in 7 patients (19%) and in 0 of 37 controls (P = 0.002). The Gm(n) marker was absent in 25 of 55 patients (45%) and 6 of 42 controls (14%) (P = 0.009). The MBL2 variants O/O, A/O, and A/A occurred in 9, 14, and 32 of the 55 patients, respectively, and in 1, 0, and 23 of the 43 controls, respectively (P = 0.05). There was no increase in the prevalence of partial C4 deficiency, C2 deficiency, lymphocyte subset deficiency, or FcyRIIa polymorphism in the patients compared to the controls. A combination of at least 2 immune defects was found in 31 of 55 patients (56%) and in 4 of 42 controls (11.6%) (P < 0.0001).

Conclusion: Specific antipolysaccharide antibody deficiency, IgA and/or IgG subclass deficiency, Gm(n) allotype, and MBL2 genotype are susceptibility factors for recurrent respiratory infections, and coexistence of several immune defects is the strongest risk factor in this study.

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Streptococcus pneumoniae and Haemophilus influenzae are important bacteria in recurrent respiratory infections (1). The immunological response against them is based on the synthesis of specific immunoglobulins, the generation of complement factors, and phagocytosis.

Antibodies to polysaccharide antigens of S. pneumoniae and H. influenzae are important in protection against these microorganisms (2, 3). The IgG2 subclass is the main antipolysaccharide antibody and patients with IgG2 subclass deficiency are more prone to develop pneumonia, sinusitis, and invasive pneumococcal disease (4). The G2m(n) allotype of IgG2 has been associated with increased susceptibility to infections with encapsulated bacteria (5). The link between IgG2 and phagocytosis of encapsulated bacteria is constituted by Fcy receptor (R) IIa, the only Fcy receptors that bind IgG2 (6). FcyRIIa exhibits allelic polymorphism with different capacities for binding IgG2 and phagocytosis. A single nucleotide difference at base 494 (A or G) results in a histidine (H) or an arginine (R) at amino acid 131 of the protein. FcyRIIa-H131 has a high affinity for IgG2, whereas FcyRIIa-R131 has a low affinity for IgG2 (7).

The complement system facilitates phagocytosis through C3b. The generation of C3b by the classical pathway depends on C1, C2 and C4. Heterozygous C2 deficiency occurs in 1%–1.5% of the general population (8). Homozygous deficiency of either C4A or C4B occurs in 3% of the population (9). Mannose-binding lectin (MBL2) binds to carbohydrates present on the surface of a variety of microorganisms. On binding, the protein activates the complement system, by MBL2-associated serine proteases. Besides, MBL2 facilitates phagocytosis by interacting with receptors on phagocytes (10). Variants of MBL2 in exon 1 (codon 52, 54, 57) result in very low serum levels of the protein and are associated with an increased risk for infections (11, 12). Concentrations of MBL2 in serum are ~20% of normal in heterozygotes for any...
codon variant, and are very low (<2% or absent) in homozygotes or compound heterozygotes. The serum MBL concentration is further dependent on polymorphisms in the promoter (13).

The role and relative frequency of the above-mentioned (partial) immunodeficiencies and polymorphisms of the immune system in increased susceptibility to respiratory infections is poorly understood. Neither is it known whether the combined presence of several (partial) immune defects contributes to increased susceptibility to infection. In this study, a comprehensive immunological exploration was undertaken to identify the nature and the incidence of the immune defects that underlie increased susceptibility to recurrent respiratory infections.

Materials, Methods, and Patients
Patient and Control Populations
Recurrent infection of the upper respiratory tract was defined as at least 5 episodes in a 1-year period of upper respiratory tract infections complicated by otitis media or chronic (>3 weeks) draining ears. Recurrent infection of the lower respiratory tract was defined as at least 3 lower respiratory tract infections in a 1-year period, with radiographic evidence of pneumonia in at least 2 episodes. Patients with recurrent infections caused by anatomic abnormalities and cystic fibrosis were excluded. All children were >4 years of age to exclude immaturity in the specific antibody response to polysaccharides (3). Two patients were allergic to *Dermatophagoides pteronyssimus* and 1 patient to grass pollens. Consecutive patients were recruited between October 2000 and September 2003.

We studied 55 children of whom 15 had recurrent infections of the upper airways, 27 had recurrent infections of the lower airways and 13 had recurrent upper and lower airway infections. The age distribution was: 36, 13, and 6 for 4–6 years, 7–10 years, and 11–14 years (29 boys, 26 girls). Eighteen patients were receiving steroids at the time of blood sampling.

The control population consisted of 43 healthy children (31 boys, 12 girls), ages 4–14 years from the same geographical area. The age distribution was 15, 10, and 18 for 4–6 years, 7–10 years, and 11–14 years. The control children were older than the patients. They were either healthy sons or daughters of hospital personnel or under- went a pediatric urological surgery procedure.

Genotyping of MBL2, FcγRIIa, and C2
Detection of polymorphisms of the MBL2 gene at codons 52, 54, and 57 (designated D, B, and C variants, respectively; wild type allele is designated A) and of single nucleotide polymorphisms in the promoter at position −221 (Y genotype having high and X genotype having low MBL2-expressing activity) was done as described (19). The 6 MBL2 genotypes were: the A/A group: 2 normal structural alleles with high-expression promoter activity in position −221 (YA/YA) or 1 high expression promoter and 1 low-expression promoter (YA/XA) or 2 low-expression promoters (XA/XA); the A/O group, 1 variant structural allele and 1 normal structural allele combined with a high expression promoter (YA/O) or a low-expression promoter (XA/O); and the O/O group, 2 defective structural alleles.

The distribution of the MBL2 genotypes in the control population (n = 43) was comparable to the distribution in a larger control population (n = 162) including children and adults from the same geographical area (data not shown).

Genotyping of FcγRIIa was as described (20). The distribution of the FcγRIIa genotypes in the control pop-
ulation (n = 43) was comparable to the distribution in a larger control population (20).

The 28-bp deletion in the C2 gene was detected by an allelic discrimination assay using GGG AAG GAG ACA GAG AGA GAT AGT GA (forward primer), TCG CAG AGT GTG TCG GAA AA (reverse primer), TGA TTC CTG GAG AGA GAT AGT GA (forward primer), TCG CAG allelic discrimination assay using GGG AAG GAG ACA GAG AGA GAT AGT GA (forward primer), and CCC TCA TGC CTG CAG (VIC-labeled probe).

ANALYSIS OF LYMPHOCYTE POPULATIONS BY FLOW CYTOMETRY
Flow-cytometric analysis was performed using a FACS Calibur instrument (BD Biosciences). Antibodies used included Multitest™ CD3(FITC)/CD16 + 56(PE)/CD45(PerCP)/CD19(APC) (four color analysis) and CD3(FITC)/CD8(PE)/CD45(PerCP)/CD41(APC) (four color analysis) from BD Biosciences and CD21 (FITC) from Beckman-Coulter. The age-dependent reference intervals were from Comens-Bitter et al. (21).

STATISTICAL ANALYSIS
Levels of significance of the differences between groups were determined by χ²-test tables [Analyze-it™ for Microsoft Excel (vsn 1.62)].

Results
IMMUNOGLOBULIN DEFICIENCY
IGA DEFICIENCY
We defined IgA deficiency as a value <2.5th percentile of the reference population. Among 55 children with recurrent respiratory infections, 7 (13%) had IgA deficiency as did 1 of 43 (2.3%) control children (χ² = 2.23; P = 0.13). Of the 7 patients with IgA deficiency, 1 had a total IgA deficiency, 3 had a partial IgA deficiency, 1 had a partial IgA deficiency combined with IgG3 deficiency, 1 had a partial IgA deficiency combined with IgG2 and IgG3 deficiency, and 1 had a partial IgA deficiency combined with IgG1 and IgG2 deficiency. In 4 of 7 IgA-deficient patients, subsequent determinations were available and all confirmed the deficiency.

IGG SUBCLASS DEFICIENCY
Among the 55 children with recurrent respiratory infections, 2 (9%) had decreased IgG1 values (<2.5th percentile of reference population) as did 1 of 48 (2%) control children (χ² = 0.92; P = 0.19). In these patients, total IgG was low as well. In 2 patients, low IgG1 values were accompanied by low IgG2 concentrations. IgG2 below the 2.5th percentile of a reference population was observed in 10 of 55 patients. This was a significantly (χ² = 4.6; P = 0.03) higher prevalence than in the control group (n = 48) in which we found 1 child with a low IgG2 value. In 2 patients IgG2 deficiency was combined with IgG1 deficiency, and in 1 patient it was combined with IgA and IgG3 deficiency. Low IgG3 values were found in 11 of 55 patients and in 3 of 43 controls. In 1 patient, this was combined with an IgA and IgG2 deficiency. In 2, 7, and 7 of the 5 IgG1, 10 IgG2, and 11 IgG3-deficient patients, respectively, subsequent determinations were available. All confirmed the deficiency. There was no association between an immunoglobulin deficiency and administration of steroids (data not shown). The nephelometric technique used was unable to determine if IgG4 deficiency was present in children <9 years because of the low concentration expected for this IgG subclass. Therefore, IgG4 deficiency was not considered in this study.

Overall, an IgA and/or an IgG subclass deficiency was found in 27 of 55 (49%) patients. This was a significantly higher prevalence (χ² = 11.81; P = 0.0006; odds ratio 5.9 [95% confidence interval (CI) 2.16–16.3]) than in the control group, in which it was found in 6 (14%) of the 43 children.

DEFICIENT ANTIBODY RESPONSE TO PNEUMOCOCCAL POLYSACCHARIDES
After vaccination with Pneumovax, 7 children with recurrent respiratory infections (19%) had a defective antibody response to at least 4 of the 6 serotypes tested (3, 4, 6B, 9N, 18C, and 19F). A defective antibody response to 6, 5, and 4 serotypes was seen in 1, 4, and 2 children, respectively. A defective antipolysaccharide immune response to ≥4 of the 6 serotypes tested was not found in any of the 37 healthy children. In the patients, a defective response to 3, 1 or 2, and 0 serotypes was found in 6, 23, and 19 patients, respectively. In the control group, the values were, respectively, 2, 6, and 29. The differences observed between the patient group and the control group are significant (χ² = 14.83; P = 0.002).

G2M(N) ALLOTYPES
Children with recurrent respiratory infections were significantly more likely to lack the G2m(n) marker than controls [25 (45%) of 53 patients vs 6 (14%) of 42 controls; χ² = 9.65; P = 0.009; odds ratio 5.13 (95% CI 1.9–14.8)]. Individuals negative for G2m(n) had significantly lower IgG2 values than individuals positive for G2m(n) (P = 0.0082, Mann–Whitney U-test). The mean (SD) IgG2 concentration was 1.17 (0.48) g/L (n = 24) in G2m(n) negative individuals compared to 1.71 (0.74) g/L (n = 27) in G2m(n) positive individuals.

FCγRIIA POLYMORPHISM
The distribution of the FcγRIIA genotypes was similar for the patients and the controls (χ² = 0.21, P = 0.9). Seventeen (31%) of 55 patients and 14 (32%) of 43 controls were homozygous for FcγRIIA-H131, 10 (18%) patients and 9 (21%) controls were homozygous for FcγRIIA-R131, and 28 (51%) patients and 20 (47%) controls were heterozygous.

COMPLEMENT FACTORS AND C4 ALLOTYPES
Total complement activity was normal in both patients and controls. Heterozygous deletion of C2 was found in 1 of 54 patients and in 1 of 43 controls. C3 was normal in
significantly increased (Caucasians C4A or C4B deficiency has been reported to occur in 3% of and in 1 and 0 controls (2.5%), respectively. Complete deficiency was found in 2 and 1 patient(s) (6%), respectively, and in 40 controls. A homozygous C4A and C4B deficiency, IgA and/or IgG subclass deficiency, Gm(n) allo-
type, and MBL2 genotype as significant factors that contribute to increased susceptibility to recurrent respiratory infections. Besides, children with recurrent infections tended to have more partial C4 deficiency. FcγRIIIa-R131/ R131 was not associated with increased susceptibility to respiratory infections. An inappropriate antibody response to at least 4 of the 6 serotypes tested was consid-
ered a specific antipolysaccharide antibody deficiency, a condition not found in control individuals.

Lymphocyte subsets

Table 2. Prevalence of coexisting (partial) immune defects.

<table>
<thead>
<tr>
<th>Number of (partial) immune defects</th>
<th>Patients (n = 55)</th>
<th>Controls (n = 43)</th>
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<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>27</td>
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<tr>
<td>1</td>
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As partial immune defects, we considered Ig deficiency (IgA and/or IgG subclass deficiency), Gm(n) negative allo-
type, MBL2 O/O, MBL2 XA/XX, MBL2 XA/YO (these genotypes are associated with low MBL2 concentrations), partial C4 deficiency, partial C2 deficiency, and lymphocyte subset deficiency (de-
creased B lymphocytes, decreased CD4 T lymphocytes, decreased NK cells). An inappropriate antibody response to at least 4 of the 6 serotypes tested was considered a specific anti-polysaccharide antibody deficiency. Such condition was not found in control individuals.
A remarkable finding in our study was that many children (19%) with recurrent respiratory infections had abnormalities in their ability to produce specific antibodies to pneumococcal capsular polysaccharide antigens. They suffer from specific antipolysaccharide antibody deficiency. A defective antibody response to polysaccharides can be found despite normal serum concentrations of Ig, including IgG subclasses (2, 22, 23). It is also found in infants at least 18–24 months, after bone marrow transplantation, in patients with Wiskott-Aldrich syndrome and AIDS (24), and in patients with defects in the NFκB pathway (25). One study estimated that 5–10% of the children referred for evaluation of recurrent infection suffer from this syndrome (23). However, Javier et al. (26) found this disorder in 23% of patients who underwent immunologic evaluation for recurrent infection. Our findings are comparable to the observations of Javier et al. (26). It has been reported that abnormal antipolysaccharide antibody responses are often found in patients with other humoral immunodeficiency diseases, for example IgA or IgG2 deficiency (24, 27, 28). In our study an IgA and/or an IgG subclass deficiency was found in 3 of the 7 patients with an specific antibody deficiency.

We found IgG2 subclass deficiency in a substantial number (20%) of children with recurrent respiratory infections. In 8 of the 10 patients with IgG2 deficiency, another immune defect coexisted. Our data, therefore, strongly indicate that recurrent infection is more common when an individual with IgG2 deficiency also manifests another immune deficiency. Patients with increased infection susceptibility were more likely to lack the G2m(n) allotype, and there was a correlation between G2m(n) immunoglobulin allotype and IgG2 concentration.

The lack of association of FcγRIIa-R/R131 genotype with increased susceptibility to respiratory infections is consistent with previous observations in invasive pneumococcal disease (20).

Genetically determined C2 deficiency is associated with increased susceptibility to infections (8, 29). In our patient group there was only 1 case of (homozygous) C2 deletion. An increase in homozygous C4B deficiency was reported in 46 children with meningitis (9) and in 50 patients with bacteremic infection with encapsulated bacteria (30). Another study involving 257 patients of all ages, however, found no increase in C4B deficiency among patients with bacteremia or meningitis caused by encapsulated bacteria (31). We found a slightly increased prevalence of partial C4 deficiency in children with recurrent respiratory infections.

It is possible that some potential confounding factors may be present. Some patients received steroids, which can reduce serum immunoglobulin concentrations. Some changes (e.g., of complement levels) might be secondary to inflammation and infection, rather than the cause of infection. Other factors such as passive smoking and atopy might also have an effect on recurrent respiratory symptoms. In our patient population, the prevalence of atopy was not higher than in the control population, and intake of steroids was not associated with immunoglobulin deficiency.

MBL2 variant alleles have been associated with increased risk for infections (hospital-based) (11, 12, 32). A population-based study suggested that MBL2 plays an important role in acute respiratory infection during the vulnerable period of childhood from age 6–17 months (33). A second population-based study in adults found no evidence for significant differences in infectious disease or mortality in MBL2-deficient individuals (34). In our hospital-based study in children aged >4 years MBL2 O/O genotype was more prevalent in the patient group compared to the control group. The association of homozygosity for MBL2 structural variants with infections was first observed by Garred et al. (35). Variant MBL2 has a lower molecular weight and is dysfunctional compared with normal MBL2 (36). Mutations in the collagenous region of MBL2 compromise assembly of higher order oligomers, resulting in reduced ligand binding capacity and reduced capacity to activate complement (37).

Remarkably, in 10 of the 25 patients with either O/O, XA/O, or XA/XA MBL2 genotype there was at least 1 coexisting minor immunodeficiency. These data suggest that MBL2 plays a role in susceptibility to infection in children older than 4 years when coexisting immune defects are present. In most circumstances, other components of the immune system can compensate for the lack of MBL2 (38).

Finally, and most strikingly, we found that coexisting immune defects predispose to clinical illness. Many patients with increased susceptibility to respiratory infections had a combination of several immune defects. Such a combination of (partial) immune defects was much less prevalent in the control population. Many factors contribute to an adequate immune response and it is probably unlikely for a single (partial) immune defect to be solely responsible for clinical illness. A single immune defect was seen in 37% of the controls. By contrast, coexistence of 2 or more partial immune defects was strongly associated with recurrent infections. The concept of coexistence of partial immunodeficiencies as a cause of clinical symptoms in childhood has previously been hypothesized in the context of MBL2 deficiencies (10). Although our study is of rather limited size, it substantiates this concept and suggests that one needs to move away from the approach of looking for a single etiologic factor. Additional studies are needed to further corroborate this idea. A recent study (39) on the phenotypic variation in patients with C2 deficiency reached many of the same conclusions as our study. In patients with C2 deficiency, homozygosity of the G2m(n) allele was protective against severe infections. The combination of C2 deficiency and MBL2 deficiency was a minor susceptibility factor, and low concentrations of IgG2 and factor B sometimes contributed to susceptibility to infection.
In conclusion, we identified several factors that are associated with increased susceptibility to recurrent infection, the strongest risk factor being coexistence of several (partial) immune defects.

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References