Immunodeficiency Among Children with Recurrent Invasive Pneumococcal Disease

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Background: Recurrent invasive pneumococcal disease (rIPD) occurs mostly in children with an underlying disease, but some cases remain unexplained. Immunodeficiency has been described in children with rIPD, but the prevalence is unknown. We used a nationwide registry of all laboratory-confirmed cases of rIPD to identify cases of unexplained rIPD and examine them for immunodeficiency.

Methods: Cases of rIPD in children 0–15 years of age from 1980 to 2008 were identified. Children without an obvious underlying disease were screened for complement function, T-cell, B-cell, natural killer--cell counts and concentration of immunoglobulins. B-cell function was evaluated by measuring antibody response to polysaccharide-based pneumococcal vaccination and the extent of fraction of somatic hypermutation. Toll-Like receptor (TLR) signaling function and mutations in key TLR-signaling molecules were examined.

Results: In total, rIPD were observed in 54 children (68 cases of rIPD of 2192 IPD cases). Children with classical risk factors for IPD were excluded, and among the remaining 22 children, 15 were eligible for analysis. Of these 6 (40%) were complement C2-deficient. Impaired vaccination response was found in 6 children of whom 3 were C2 deficient. One patient had a severe TLR signaling dysfunction. No mutations in *IRAK4*, *IKBKG* or *MYD88* were found.

Conclusion: Of an unselected cohort of children with rIPD at least 11% were C2 deficient. Data suggest that screening for complement deficiencies and deficient antibody response to pneumococcal vaccines in patients with more than 1 episode of IPD is warranted.

Key Words: *Streptococcus pneumoniae*, complement deficiency, toll-like receptor, recurrent, immunodeficiency

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Streptococcus pneumoniae is a leading cause of septicemia, pneumonia and meningitis in young children worldwide. Some children are prone to repeated episodes of invasive pneumococcal disease (IPD),[1] but recurrent IPD (rIPD) is a rarity, and published reports on rIPD are limited by having few children included, selected groups of patients or short follow-up periods.[2–6] Predispositions to rIPD such as acquired immunodeficiency (eg, HIV or immunodeficiency following cancer) or anatomical abnormalities (eg, cerebrospinal fluid leak, asplenia) have been reported in 40–92% of the children.[2–6] Nevertheless, in some children with rIPD, the enhanced susceptibility to pneumococci is unexplained and therefore gives suspicion of underlying primary immunodeficiency.

The risk of IPD is reported to be especially high in patients with B-cell dysfunction,[7–9] complement deficiencies[9,10] and defects in molecules involved in the Toll-like receptor signaling pathway (TLR signaling).[11–13] Complement deficiencies are rare. Individuals with complement deficiency have increased susceptibility to invasive, recurrent infections with encapsulated bacteria, such as *S. pneumoniae, Neisseria meningitidis* and *Haemophilus influenza*.[9,10] Although many studies of persons with deficiencies of the classical components of complement (C1, C2 and C4) report a predisposition to infections with *S. pneumonia*, only few clinically defined groups of patients experiencing pneumococcal disease have been systematically examined for the frequency of complement deficiencies.[14]

In addition to deficiencies in the classical complement pathway, it has been shown that single gene mutations in key molecules (IKBKG, IRAK4 and MYD88) of the TLR signaling pathways play an important role as predisposing factor in children with rIPD.[11–13] TLR deficient children may present with otherwise normal basic immunological parameters (such as immunoglobulins, T-cell, B-cell and natural killer (NK)-cell count and complement), and consequently, they can be a diagnostic challenge.

In Denmark, a nationwide study of pediatric rIPD documented that one-third of the children did not have any obvious clinical features explaining their recurrent episode.[15] Here, we present results from a follow-up of this cohort. We evaluated immunological parameters including complement function, B-cell-function and TLR signaling of all eligible children.

MATERIALS AND METHODS

Study Design

A retrospective analysis was conducted using The National *Streptococcus pneumoniae* Register, which contains nationwide laboratory-confirmed data from IPD cases in Denmark since 1938. Bacterial isolates are submitted routinely from all departments of clinical microbiology to the *Neisseria* and *Streptococcus* Reference Center at Statens Serum Institut. Children aged 0–15 years with a laboratory-confirmed case of IPD during January 1980–December 2008 were identified as previously described.[15] Recurrent episodes of IPD were defined as isolation of *S. pneumoniae* from any

normally sterile site ≥30 days after initial positive culture or ≤30 days if the recurrent infection was with a new pneumococcal serotype. In Denmark, pneumococcal conjugate vaccination was introduced in the infant immunization program in October 2007.[15] One of the included children (patient 12) received this vaccination as part of the vaccine program before the first rIPD episode.

Follow-Up

Clinical data were obtained from hospital records and from The National Register of Patients (Landspatientregistret). The following data were collected: sex, age, background disease, localization of infection, data on vaccination and outcome. Children without a background disease known to predispose to IPD (such as acquired immunodeficiency or anatomical abnormalities) were invited to participate in an investigation study by having a blood sample taken and screened for abnormalities in basic immunological parameters and B-cell and TLR signaling function (Fig. 1). In cases with a family history of infections, all close relatives (parents, siblings and grandparents) were invited to be tested for abnormalities in basic immunological parameters.

Blood Samples

Blood samples were taken from children with rIPD, healthy adult blood donors and healthy children (aged 0.25–8.0 years, sex distribution: 7 girls and 5 boys) after informed consent was obtained.
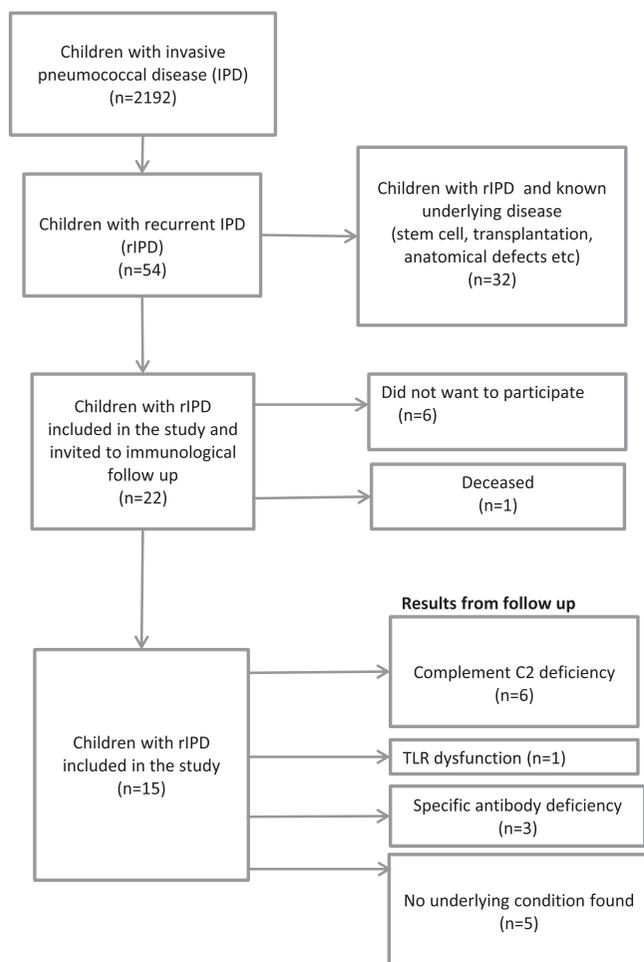
FIGURE 1. Inclusion of children with recurrent invasive pneumococcal disease and results of follow-up.

All samples, except 1 (Table 2, patient 13) were collected during an infection-free interval. The blood was analyzed as soon as possible and always within 16 hours. The healthy adult blood donors and healthy children were used as controls in the TLR signaling assay.

Standard Immunological Parameters

Plasma concentrations of immunoglobulin classes and subclasses were determined by an automated immunonephelometric assay (BN ProSpec, Siemens, München, Germany). T-cell, B-cell and NK-cell counts were performed in a single-platform assay on ethylenediaminetetraacetic acid anticoagulated blood using anti-CD19, anti-CD20, anti-CD3, anti-CD4, anti-CD8, anti-CD56 and anti-CD16 and TRUCount beads (BD Biosciences, Beckman Coulter, Miami, Florida) and analyzed on a Beckman Coulter-FC500 flow cytometer.

Complement Analysis

Screening of the classical, alternative and lectin complement pathways was performed using a commercially available solid-phase assay (Wielisa) as recommended by the manufacturer (Wieslab, Malmö, Sweden). Detection of individual complement components were performed in patients with reduced complement activation, using rocket immunoelectrophoresis as previously described.[16,17] Cases with suspected complement C2 deficiency were genetically confirmed by investigation for the 28 base pair genomic deletion.[18] Mannan binding lectin genotypes were determined.[19]

B-Cell Function

Pneumococcal Antibody Determination

In patients vaccinated with the 23-valent pneumococcal polysaccharide vaccine (pneumovax 23 Merck; PPV23), data on the serotype-specific IgG response were accessed retrospectively. In a standardized enzyme-linked immunosorbent assay, concentrations of antibodies against 6 serotypes: 1, 4, 7F, 14, 18C and 19F, and a geometric mean for all 6 serotypes combined were determined as described in detail previously.[20,21] Children with an impaired response to half or more of the 6 tested serotypes were defined as having an impaired response.[22–24]

Somatic Hypermutation

To examine the level of affinity maturation of antibodies, somatic hypermutation (SHM) in κ-light-chain transcripts was assessed using a VκA27-specific restriction enzyme-based hot-spot mutation assay as previously described.[25]

Functional Assay for TLR Signaling

Every analysis of a patient sample was compared with 2 simultaneously analyzed healthy controls, and the result considered low if below 50% of the lowest control.

TLR Signaling Monitored by Analysis of CD62L Shedding

Whole blood was stimulated as previously described,[26] with little modification in relation to the used agonists. For each stimulation, 100 μL heparinized blood was treated with Polymyxin B, 10 μg/mL for 20 minutes (except the sample stimulated with lipopolysaccharide) and hereafter, incubated at 37°C in a 5% CO_2 atmosphere for 1 hour with the individual agonists Pam3csk48 (Invivogen, San Francisco, CA; TLR1/2), Pam2csk4 (Invivogen; TLR2/6), lipopolysaccharide (Invivogen; TLR4), flagellin-ST (Invivogen; TLR5), CL097 (Invivogen; TLR7/8), ssRNA40 (LyoVec, Invivogen; TLR8) and with polymyxin A (Sigma-Aldrich, St. Louis, MO) or tumor necrosis factors a (TNFa; R&DSystems, Minneapolis, MN) as positive controls. After activation, the erythrocytes were lysed in EasyLyse (Dako) and washed twice in phosphate-buffered saline. The cells were incubated with anti-human CD62L or isotype control (BD Bioscience Pharmingen 555544 resp. 555749) for 20 minutes in 4°C.[26]

TABLE 1. Clinical Profile of Children Experiencing Recurrent Invasive Pneumococcal Disease

Patient	Episode of IPD	Pneumococcal Serotype	Age at episode of IPD	Other Clinical Features	Family History of Infections	Age at Sampling (years)	Age at Diagnosis (years)	Diagnoses
1	Meningitis Bacteremia	38 6B	5 mo 7 yrs 5 mo	Age 0–6 yrs: Otitis, failure to thrive; Age 7–14 yrs: Frequent URT; Sequelae: unilateral deafness	Yes	27	4	Complement C2 deficiency
2	Meningitis Meningitis	4, 14	2 yrs 2 mo 2 yrs 11 mo	Frequent URTI	Yes	10	3	Complement C2 deficiency
3	Bacteremia Bacteremia	9V	11 mo 12 mo	12 days old: febrile episode with high CRP	Yes	4	3	Complement C2 deficiency
4	Bacteremia Bacteremia Bacteremia	6B 16F 4	2 mo 2 yrs 5 mo 2 yrs 10 mo	15 days old: febrile episode with high CRP; Joint symptoms, unspecific	Yes	4	3	Complement C2 deficiency
5	Bacteremia Bacteremia	12F, 4	6 yrs 6 yrs 1 mo	Age 0–6: sinusitis, tonsillitis; Age 6: tonsillectomy; Age 6+: no increased morbidity	No	17	17	Complement C2 deficiency
6	Bacteremia Bacteremia	6B, 34	11 mo 6 yrs	Age 0–6: common febrile episodes of unknown origin; Age 7–14: prone to URTI, sinusitis	No	12	12	Complement C2 deficiency
7	Bacteremia Bacteremia	6B, 6B	11 mo 13 mo	Prone to otitis	No	2		No underlying condition found
8	Bacteremia Bacteremia	23F, 9N	8 mo 11 mo	Prone to otitis	No	4	4	SAD toward pneumococcal polysaccharides
9	Bacteremia Bacteremia	12F, 4	1 wk 11 mo		No			No underlying condition found
10	Meningitis Meningitis	11A 6A	3 yrs 6 yrs	Meningitis × 7–8 (various agents)	No	7		No underlying condition found
11	Meningitis Bacteremia	18C 11A	2 yrs 3 yrs	Pneumococcal endocarditis with cerebral embolia	No	17		No underlying condition found
12	Meningitis Bacteremia	19F 1	10 wks 5 mo		No	1		No underlying condition found
13	Bacteremia Bacteremia Bacteremia Bacteremia	6B 6B 9V 6B	7 mo 18 mo 19 mo 32 mo	Ectodermal dysplasia Invasive *Escherichia coli* × 1, GBS × 2 Bacteremia Bacteremia	No	2	2	Toll-like receptor defect
14	Meningitis Bacteremia	18C 9V	6 mo 8 mo	Frequent URTI	Yes	6	6	SAD toward; pneumococcal polysaccharides
15	Bacteremia Bacteremia	6B 6B	13 mo 15 mo	Prone to otitis, conjunctivitis *Kingella kingae* × 1 (Bact.)	Yes	4	4	SAD toward Pneumococcal polysaccharides

URTI indicates upper respiratory tract infections, CRP, C- reactive protein, GBS, group B *Streptococcus,*

TLR Signaling Monitored by Analysis of Cytokine Production

Human peripheral blood mononuclear cells were isolated by Lymphoprep density gradient centrifugation according to the manufacturer's instructions (Nycom A/S, Nycom, Oslo, Norway). Peripheral blood mononuclear cells were stimulated in duplicates with TLR 1–2 and 4–8 agonists as described above and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. After incubation, the supernatant was analyzed for the presence of IL-6 and TNFa using a Multiplex Bead-based Luminex assay.[27]

TLR Genetics

All boys were screened for mutations in *IKBKG* [NF–kappa B Essiential Modulator (NEMO)] and all included patients for mutations in *IRAK4* and *MYD88*. Sequencing was done with BigDye technology (Big Dye Terminator sequencing kit 3.1; PE Applied Biosystems, Foster City, CA) and included the coding exons and consensus splice sites.

Statistics

Cytokine production between groups was compared using Student's *t*-test or 1 way analysis of variance, a *P*-value less than 0.05 was considered significant.

Ethical Approval

The study was approved by the Danish Data Protection Agency (j.nr. 2007-41-1407) and the local Ethics Committee (j.nr HB-2008–094).

RESULTS

Identification of Children with rIPD

Over the 29-year study period, 2192 children were diagnosed with IPD. Among the 2128 children surviving, their first episode of IPD recurrent infection was reported in 54 children. Clinical data from hospital journals were reviewed in these 54 children, and in 32 children, a clinical feature (stem cell transplantation, anatomical

TABLE 2. Immunological Profile of Children Experiencing Recurrent Invasive Pneumococcal Disease

Patient	Complement Pathway	MBL Genotype	Somatic Hypermutation	Specific IgG Response to Pneumococcal Polysaccharides	TLR Functional Assay	TLR Cytokine Response
1	C2 def	XA/XA†	Normal	Normal	Normal	Normal
2	C2 def	YA/YA*	Normal	Normal	Preactivated	Normal
3	C2 def	XA/YA*	Normal	Normal	Not done	Normal
4	C2 def	D/D§	Normal	Impaired	Not done	Normal
5	C2 def	B/D§	Reduced¶	Impaired	Normal	Normal
6	C2 def	YA/YA*	Subnormal‖	Impaired	Normal	Normal
7	Normal	YA/YA*	Normal	Normal	Not done	Reduced
8	Normal	YA/XA*	—	Impaired	Preactivated	Normal
9	Normal	YA/B†	—	Not vaccinated	Normal	Normal
10	Normal	XA/XA†	Normal	Normal	Normal	Normal
11	Normal	YA/YA*	Normal	Not vaccinated	Normal	Normal
12	Normal	YA/YA*	Normal	Normal	Not done	Normal
13	Normal	XA/D‡	Normal	Impaired**	Impaired	Low††
14	Normal	YA/D†	normal	Impaired	Normal	Normal
15	Normal	YA/YA*	Normal	Impaired	Normal	Normal

Functional test for somatic hypermutation in the rearranged immunoglobulin genes of B-cells (27), Em-dash indicates information not available because of lack of material.
*MBL genotype associated with high MBL level.
†MBL genotype associated with reduced MBL level.
‡MBL genotype associated with much reduced MBL level.
§MBL genotype associated with no functional MBL level
¶SHM of 6.9%, age at blood sampling: 17 years.
‖SHM of 14.8%, age at blood sampling: 12 years
**This patient was vaccinated with the pneumococcal conjugate vaccine (PCV7) and not the polysaccharide based vaccine (PPV23).
††The blood sample for the TLR cytokine assay could not be obtained during an infection free interval because of chronic mycobacterial infection. This patient had generally a low TNF response but responded with IL-6, however, reduced (more details in Fig. 2).
MBL indicates mannan binding lectin.
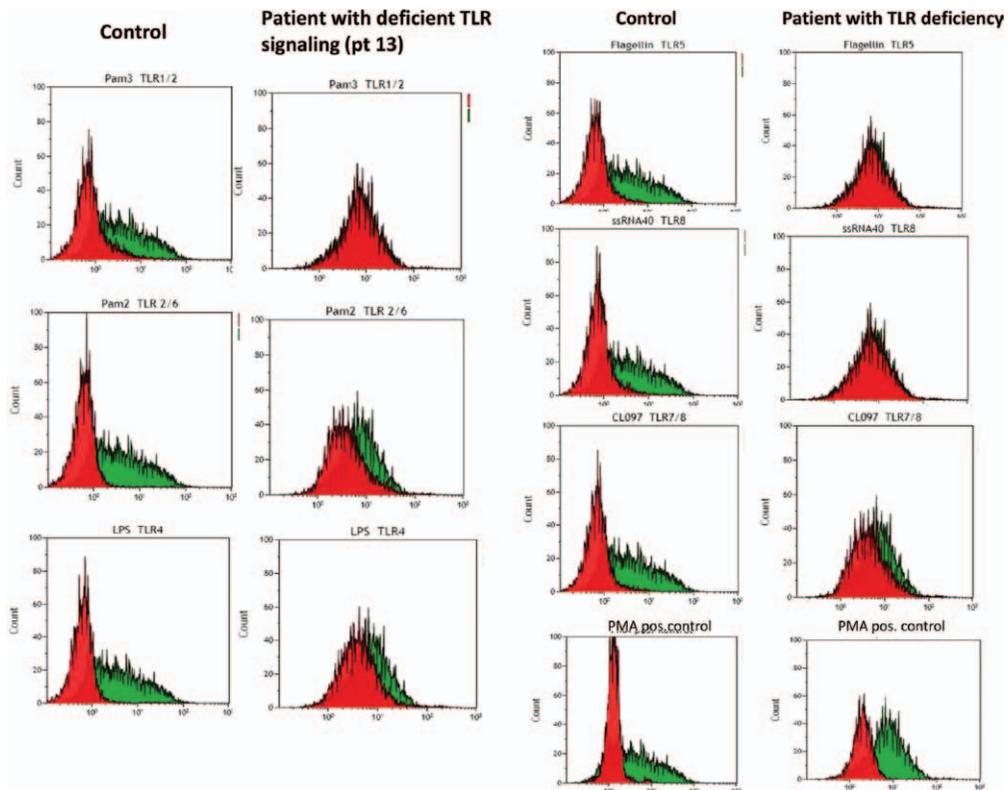
FIGURE 2. CD62L shedding upon TLR stimulation with 6 agonists (and the polymyxin A positive control) in 2 persons: a healthy control and a TLR signaling deficient patient. TLR stimulation was analyzed by flow cytometry. The receptor expression on granulocytes before (red) and after (green) is presented. The results are presented as histograms of events gated on granulocytes in a sideward scatter/forward scatter diagram.

defects, renal disease, etc.) predisposing to rIPD was documented.[15] The remaining 22 children were invited to participate in a further immunologic follow-up. Among the 22 children with no obvious underlying diseases, 1 child died before follow-up, and 6 children did not wish to participate in the study. The remaining 15 patients and their families (if positive family history) were included in the immunological follow-up investigation (Fig. 1).

The average age of study subjects when blood was assessed was 12.7 years (median: 6.2, range 2.1—36 years), sex distribution: 7 girls and 8 boys, and the mean time between the first episode of rIPD and the immunological evaluation was 6 years (range 0.5–20 years).

Complement Function and Standard Immunological Parameters

All children had T-cell, B-cell and NK-cell counts within normal ranges. Furthermore, immunglobulin levels including IgG-subclasses were within normal ranges as defined by age-matched controls.

We found 6 cases of C2 deficiency among the 15 children (40%) (Fig. 1, Table 1). Four patients were diagnosed with C2 deficiency at the initial clinical investigation following rIPD, and 2 were diagnosed in our investigational follow-up because a functional screen of the complement pathway was not included in the

initial clinical evaluation. All patients with reduced complement activation were identified as C2 deficient and confirmed genetically. No other complement deficiencies were found. Among the C2 deficient patients, 2 also had a reduced activation of the lectin pathway (Table 2).

B-Cell Function

Of the 15 children, 12 were vaccinated with the PPV23, and of these, 6 (50%) responded insufficiently to vaccination despite normal ranges of immunoglobulins. The nonresponders comprised 3 children with C2 deficiency and 3 children with no other immunological abnormalities (Tables 1 and 2).

Two of the 3 C2 deficient patients with impaired vaccination response had a reduced fraction of SHM in their B cells indicating a fundamental B cell dysfunction (Table 2).

The last 3 children, all boys, with an impaired PPV23 vaccination response fulfilled the criteria of the diagnosis: specific antibody deficiency (SAD) toward pneumococcal polysaccharides.[23,28] Two of the children with SAD had a family history of infections, 1 (Tables 1 and 2, patient 14) had 2 brothers with repetitive episodes of upper respiratory tract infection and 1 healthy sister. All 3 brothers had an impaired response to PPV23 vaccination for all 6 tested pneumococcal serotypes. Family members, including parents and grandparents on both sides were tested for abnormalities in standard immunological
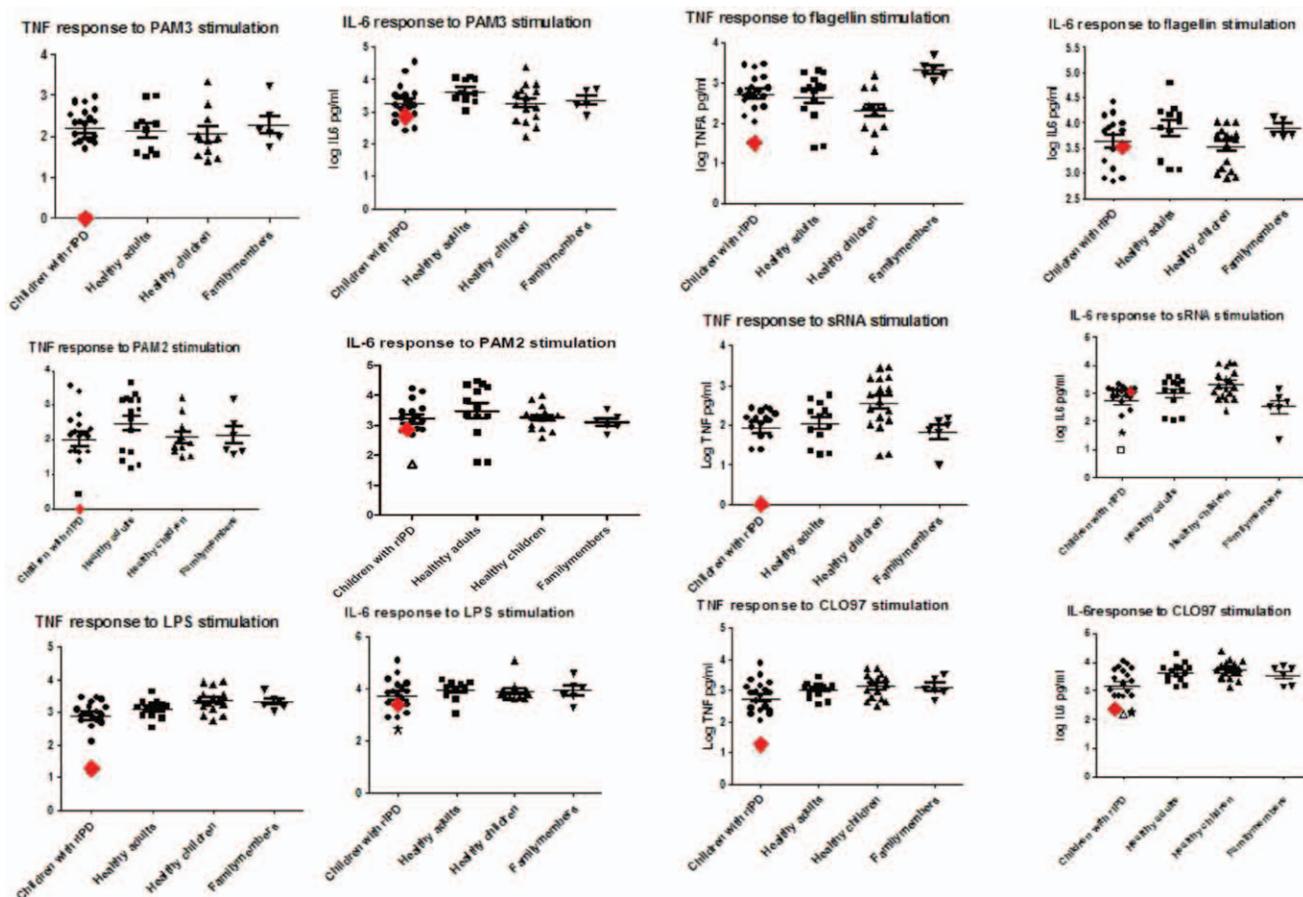
FIGURE 3 Cytokine production upon TLR stimulation with 6 agonists in (1) children with recurrent IPD, (2) healthy adults (controls), (3) healthy children (controls) and (4) family members to children with recurrent IPD. Diamond formed dot: child with a defective response in the CD62L shedding assay (patient 13). Star-shaped dot indicates child with specific antibody deficiency to pneumococcal polysaccharides (patient 15). Triangle dot indicates child with Complement C2 def. (patient 6). Squared dot indicates child with complement C2 def. (patient 5).

parameters, and as in the index child, none were found. Another child (Tables 1 and 2, patient 15) had a brother and a mother with repetitive pneumonic episodes in the first 2 years of life (>8 episodes/Year). Family members including parents and grandparents were tested and found negative for abnormalities in standard immunological parameters. Of note, it was not possible to test the specific IgG response to PPV23 vaccination of family members in this family.

TLR Signaling Function

TLR signaling was determined for the 15 children with rIPD and 6 family members. Because of sample transportation times in excess of 6 hours 4 blood samples were not eligible for the CD62L shedding assay. One child, a boy, who experienced 4 episodes of pneumococcal bacteremia in his first 3 years of life, had a severely reduced response to all 6 used TLR agonists in the CD62L shedding

assay, with an adequate response to the positive control (polymyxin A; Fig. 2). When his cytokine responses to the 6 used TLR agonists were evaluated, his TNFa response was below unstimulated controls (Figs. 3 and 4). All his IL6 responses were reduced but not absent. No mutations in *IRAK4*, *IKBKG* (NEMO) or *MYD88* were found.

In addition, 2 other patients were suspected for reduced TNFa and IL6 responses upon TLR stimulation, and the analyses were repeated on a fresh blood sample. In one of these patients reduced TNFa and IL6 responses to TLR stimulation were confirmed (when compared with 2 simultaneously studied healthy controls, reduced TNFa response to 4 of 6 agonists and reduced IL6 response to 5 of 6 agonists was found; Fig. 4). The remaining cohort of patients with rIPD had normal TLR signaling function as evaluated by the CD62L shedding and cytokine assays (Fig. 3). We screened all boys for mutations in IKBKG (NEMO), and we
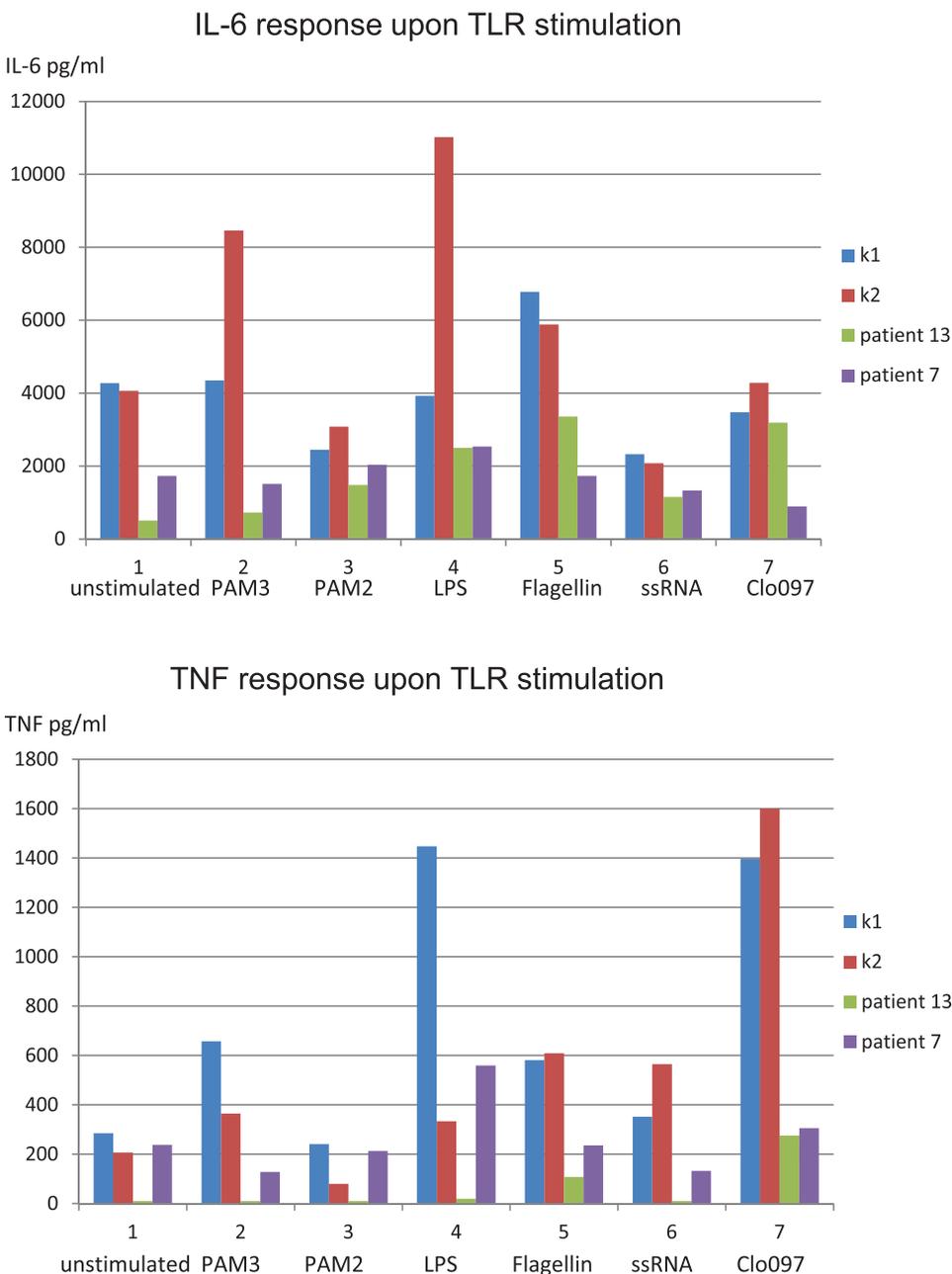
FIGURE 4 Cytokine production upon TLR stimulation with 6 agonists. Two patients presented responses with reduced/absent levels of cytokines upon TLR stimulation.

examined all patients for mutations in IRAK4 and MYD88. None of these mutations were found.

DISCUSSION

In this nationwide follow-up study of pediatric rIPD, we found a surprisingly high prevalence (≥11%) of C2 deficient children, and among 15 children experiencing rIPD without obvious underlying disease, 40% were C2 deficient. In the background population, the incidence of C2 deficiency is about 1:20.000.[29] An additional important finding was the identification of 3 children with a selective antibody deficiency toward pneumococcal polysaccharides and 1 child with a severe TLR signaling dysfunction.

Delayed diagnosis of complement deficiency can be associated with increased morbidity and mortality; therefore, it is of clinical importance that persons with C2 deficiency and recurrent infections are identified. Two of the C2 deficient patients in our cohort were diagnosed late, despite having experienced 2 invasive bacterial infections and frequent episodes of upper respiratory tract infections. This finding agrees with a recent study of 22 complement-deficient patients, which reported a median delay of 56 months (range 0–136) from the initial symptoms until diagnosis.[30]

The high incidence of C2 deficiency in our cohort highlights the importance of an intact complement function in the host defense against *S. pneumonia* and underlines the relevance of complement screening individuals with rIPD. It is important to perform a functional testing of the complement pathways to detect C2 deficiency. C3 and C4 measurements alone cannot exclude complement deficiency.

Among the 6 C2 deficient children, 3 responded insufficiently to the 23-valent pneumococcal vaccine. Reduced vaccination response in some, but not all,[31] complement deficient persons is previously reported.[32,33] Complement is involved in antigen-driven immune activation presentation[34–36] explaining why a deficiency may lead to impaired vaccination response. Two of the C2 deficient children with impaired vaccination response had a reduced fraction of SHM in their B cells. This reduced capability to produce high-avidity immunoglobulins could be a part of the explanation why some C2 deficient persons lack adequate vaccine responses and are more prone to infections than others.

We diagnosed 3 children with a selective antibody deficiency toward pneumococcal polysaccharides (SAD). Apart from being a normal phenomenon in children under 2 years, SAD is commonly reported in certain groups of children with repetitive respiratory infections including acute otitis media.[23,29] Children that do not respond to PPV23 may reach sufficient antibody levels when given pneumococcal conjugate vaccine [37,38] indicating normal T-cell-dependent B-cell immunity.

Children with defective TLR signaling (IRAK-4 and MyD88 deficiencies) present with a narrow phenotype with recurrent invasive pyogenic bacterial infection. Mutations in the X-linked NEMO gene cause impaired NF-κB-mediated cellular responses to multiple receptors and therefore confer a broader predisposition to infections.[11–13,39] Together, these disorders have now been diagnosed in around 150 patients worldwide[39] but always in highly selected groups. We investigated TLR signaling in a population-based child cohort and found 1 child with severely impaired functional TLR stimulation deficiency in the CD62L shedding assay, absent TNFa response and reduced IL-6 response. Specific hypomorphic variants of NEMO are associated with distinct phenotypes,[40] and certain patients with NEMO deficiency exhibit varying degrees of impaired TLR signaling often thought to lead to the susceptibility of these patients to mycobacteria and other pathogens.[41,42] It may, therefore, be speculated that our patient with impaired TLR function, despite the fact that we have not detected specific mutations or deletions in the NEMO gene, might suffer from an indirectly impaired but clinical important NEMO dysfunction. The more moderately reduced but not absent TNFa and IL6 response to TLR stimulation observed in another patient may be caused by modifying polymorphisms in the TLRs or in molecules involved in their signaling pathways.

To our knowledge, a study of prevalence of TLR defects in a larger population of unselected children with a clinical narrow phenotype has not been carried out. Our study confirms that TLR signaling defects exist as a predisposing factor to rIPD in children but underline that other primary immune deficiencies are more frequent in a Danish setting. Taking into consideration the high proportion of rIPD children with recognized anatomical etiology one may speculate whether some of the unexplained rIPD cases may in fact have an underlying unrecognized, eg, functional hyposplenism, which notoriously is very difficult to diagnose in due time.[43,44] Furthermore, as the narrow spectrum of the infection susceptibility resembles the pattern of pathogen specific immunodeficiencies resulting from mutations in the innate immune system, one may speculate if hitherto unknown mechanisms may be suffering.

Our findings confirm the notion that 2 invasive infectious episodes,[45] in the absence of background disease knowing to predispose to IPD, is a major pointer toward the presence of primary immune deficiencies.

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