

# Evaluation of the relevance of humoral immunodeficiencies in a pediatric population affected by recurrent infections

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Recurrent infections are a common cause of morbidity in childhood. Several reports have associated this condition to low levels of IgA and IgG subclasses and/or lack of specific antipolysaccharide antibody response, although the relevance of these defects in terms of prognosis and therapeutic approach is still unclear. The aim of our study was to determine the frequency and the clinical relevance of humoral immunodeficiency (HID) other than hypogammaglobulinemia in children affected by recurrent infections. We recruited 67 pediatric patients affected by recurrent infections. Serum IgG, IgA, IgM, IgG2, IgG3, and specific anti-*Haemophilus influenzae* (anti-Hib) antibodies were determined. Thirty-seven out of 67 patients showed antibody defects (55%). IgA deficiency was observed in 21 out of 67 patients (31%), followed by IgG2 (18%), IgG3 (15%) and IgM (6%) defects. Anti-Hib deficiency was present in three out of 44 patients (7%). A tendency for a higher occurrence of pneumonia and otitis, although not statistically significant ( $p > 0.05$ ), was observed in HID patients compared to children with normal humoral function. No statistical difference as to the frequency of mild infections (URI) was found between HID and non-HID patients. We therefore suggest that the therapeutic program is based on the clinical status of the patients. Long-term follow-up with repeated determinations of antibody levels is crucial, however, to detect those defects that might evolve into more complex immunodeficiencies.

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Recurrent infections in children are a substantial cause of morbidity and hospitalization worldwide. A variety of risk factors have been associated with recurrent infections. Several studies have demonstrated that a remarkably high proportion of children with recurrent infections, whose symptoms are significant enough to warrant referral to a clinical immunologist, have abnormalities in the humoral immune system.

The spectrum of antibody deficiencies ranges from severe deficiencies of all immunoglobulin isotypes (agammaglobulinemia) to milder but

clinically relevant deficiencies of specific antibodies in patients with normal immunoglobulin concentrations (1).

When the total immunoglobulin concentrations are normal and the child's history indicates that further evaluation is required, additional determination of serum IgG subclasses and specific antibodies against common pathogens should be performed (2–4).

IgA deficiency (IgAD) is considered to be the most common antibody deficiency in humans. Although often asymptomatic, it has been associated with increased susceptibility to allergy, infections, auto-immunity and malignancies. The concomitant association of one or more IgG

subclass deficiencies seems to predispose patients to more severe and frequent infections (5, 6).

Sporadically, IgM deficiency (IgMD) has been reported in patients affected by recurrent infections, atopy, asplenia and auto-immune diseases (5, 7, 8).

Many reports have shown a high frequency of respiratory tract infections in patients with IgG subclass deficiency (IgGScD). IgG2 antibodies are mainly directed against polysaccharide antigens whereas IgG3 are mostly constituted of antibodies directed to viral antigens (9, 10).

Interestingly, a reduced synthesis of IFN- $\gamma$  has been observed in peripheral blood mononuclear cells (PBMC) of IgG2 deficient patients and IgG2 levels were restored upon addition of IFN- $\gamma$ , although IFN- $\gamma$  gene analysis showed no mutation (11, 12).

Undetectable IgG4 subclass levels are a common finding in normal individuals and an accurate detection of very low levels of IgG4 is technically difficult to achieve, therefore the clinical relevance of IgG4 evaluation is still unclear (13).

The molecular basis of IgG subclass deficiencies has been associated with the deletion of Ig H constant region in some patients, and more recently, for the first time, a mutation in the C $\gamma$ 2 gene in two Japanese siblings has been reported (14, 15).

The aim of our study was to determine the frequency and the clinical relevance of humoral immune defects (HID) such as IgA, IgM and IgG2 and IgG3 subclass deficiencies in children with recurrent infections and normal IgG values.

### Patients and methods

We retrospectively analyzed 67 children between the ages of 7 months and 13 years, referred to our Immunology Clinic with a history of recurrent infections.

Recurrent infections were defined as more than six episodes of upper respiratory tract infections and/or more than three lower respiratory tract infections in the previous year. Some patients had other associated infectious episodes such as septicemia, meningitis, endocarditis, gastroenteritis and urinary tract infections.

All patients had already been seen by an allergologist before they were referred to our Clinic.

Only children with normal levels of IgG were included in this study. Patients with IgG1 subclass deficiency were not considered because they are hypogammaglobulinemic. Patients affected by combined or acquired immunodeficiency syndrome were also excluded from this study.

All patients were analyzed for serum concentrations of IgG, IgA, IgM, IgG2 and IgG3. Total IgG, IgA and IgM levels were determined by nephelometry. IgG2 and IgG3 subclasses were assessed by radial immunodiffusion (The Binding Site, UK). A deficiency was defined as 2 SD below the geometric mean of the age-matched control levels for both immunoglobulin and IgG subclasses (16, 17). Total IgG anti-Haemophilus Influenzae antibodies (anti-Hib) were detected by EIA (The Binding Site, UK) in 44 out of 67 patients with recurrent infections before and after vaccination with Act-Hib (Pasteur Merieux, France). Out of these 44 patients, 25 were HID and 19 non-HID patients. Protective titres of anti-Hib antibodies have been defined as  $> 1 \mu\text{g/ml}$ .

### Statistical analysis

Data were analyzed using  $\chi^2$ -test (EPIINFO 6 software).

### Results

As reported in Table 1, among the 67 patients studied, 37 (55%) showed HID. The mean age of the HID patients was  $4.8 \pm 3$  years (range: 0.83–13.6 years). The mean age of the remaining 30 patients without HID was  $4 \pm 3$  years (range: 0.66–13.4 years).

As shown in Table 2 the most frequent defect was IgA deficiency, present in 21 patients (31%). In 15 patients IgAD was isolated, in six cases it was associated with IgGScD.

IgG subclass deficiency was observed in 20 patients (30%). Eleven of them had an isolated defect, seven of them an IgG2 deficiency and four an IgG3 deficiency. In nine patients the defect was associated with other isotypes or subclass defects. In detail, three children had an IgG2 deficiency associated with IgAD, two children had an IgG3 deficiency associated with IgAD, one child had a combined IgG2, IgG3 and IgA deficiency, two children had IgG3 deficiency in association with IgMD, and one child showed a combined IgG2–IgG3 deficiency.

IgM deficiency was detected in four (6%) patients. Isolated IgM defect was present in two patients, and two children had an associated IgG3 subclass deficiency.

In order to evaluate the specific functional antibody response, 44 out of 67 patients with recurrent infections were studied for postvaccination antibody titers to Haemophilus Influenzae. Forty-one out of 44 patients had a normal functional response. Three out of 44 (7%) showed undetectable postvaccination titers of

Table 1. Immunoglobulin class and subclass deficiencies in children with recurrent infections

|                          | IgG | IgA | IgM | IgG1 | IgG2 | IgG3 | IgG4 | Total |
|--------------------------|-----|-----|-----|------|------|------|------|-------|
| n = 67                   | —   | 31* | 6   | —    | 18   | 15   | —    | 55    |
| Review of the literature |     |     |     |      |      |      |      |       |
| Smith (1984)             | 38  | 30  | —   | 22   | 24   | 3    | 38   | 59    |
| n = 37                   |     |     |     |      |      |      |      |       |
| Stanley (1984)           | 5   | 10  | 7   | 1    | 11   | 1    | 12   | 25    |
| n = 47                   |     |     |     |      |      |      |      |       |
| Smith (1985)             | 26  | 5   | —   | 25   | 44   | 19   | 37   | 63    |
| n = 16                   |     |     |     |      |      |      |      |       |
| Shakelford (1986)        | 10  | 27  | —   | 3    | 23   | —    | 23   | 37    |
| n = 30                   |     |     |     |      |      |      |      |       |
| Noyes (1986)             | —   | —   | —   | 3    | 17   | 21   | —    | 41    |
| n = 75                   |     |     |     |      |      |      |      |       |
| Loftus (1988)            | 15  | 6   | —   | 4    | 32   | 2    | —    | 34    |
| n = 82                   |     |     |     |      |      |      |      |       |
| De Baets (1992)          | 15  | 17  | —   | —    | 17   | 17   | 38   | 57    |
| n = 53                   |     |     |     |      |      |      |      |       |
| Shapiro (1991)           | 10  | 2   | —   | 2    | 3    | 11   | 3    | 28    |
| n = 61                   |     |     |     |      |      |      |      |       |
| Gross (1992)             | 25  | 33  | 3   | 8    | 13   | 7    | 11   | 58    |
| n = 267                  |     |     |     |      |      |      |      |       |

\*Values are expressed in percentage of patients in the study.

Table 2. Clinical phenotype of patients with humoral deficiencies

| Immune defect     | n = 37 | Pneumonia | Otitis | Uri | Other                           |
|-------------------|--------|-----------|--------|-----|---------------------------------|
| IgA               | 15     | 5         | 5      | 12  | SSSS, Mastoiditis, Pericarditis |
| IgM               | 2      | 1         | —      | 1   |                                 |
| IgG2              | 7      | 1         | 1      | 3   | Sepsis, endocarditis            |
| IgG3              | 4      | 4         | 2      | 2   |                                 |
| IgG2 + IgG3       | 1      | —         | —      | 1   | CMV infection                   |
| IgA + IgG2        | 3      | —         | —      | 2   |                                 |
| IgA + IgG3        | 2      | 1         | 1      | —   | Upper urinary infection         |
| IgA + IgG2 + IgG3 | 1      | —         | 1      | 1   |                                 |
| IgM+IgG3          | 2      | 1         | —      | 2   |                                 |

anti-Hib antibodies. All of them had IgG3 subclass deficiency, isolated in two children and associated to IgG2 deficiency in one child.

A tendency for a higher occurrence of pneumonia and otitis in patients affected by HID than in children with normal humoral function was observed, although this was not statistically significant ( $p > 0.05$ ). The frequency of mild infections (URI) was, however, the same in both groups (Fig. 1).

## Discussion

Our study shows that 55% of our pediatric population affected by recurrent infections had HID, such as IgA, IgM, IgG2 and IgG3 deficiencies.

In Table 1 our results are compared with data previously reported in the literature.

As expected, the most represented defect was IgA deficiency. The prevalence of immunoglob-

ulin isotype and IgG subclass deficiency was comparable with data reported by several authors, although significant differences result from other studies (2, 13, 18–24). Improvement in the techniques used to determine IgG subclass levels in the last decade and the racial differences in the cohorts studied may account for the observed discrepancies.

From a clinical point of view, HID patients showed a higher frequency of pneumonia and otitis than non-HID patients, although not statistically significant, while upper respiratory tract infections were equally represented in both groups.

More severe infections such as sepsis, pericarditis and upper urinary infections were occasionally observed in our population with recurrent infections and were mostly associated with HID. This observation supports the pathogenic role of HID in increasing the risk of contracting moderate to severe infections with obvious

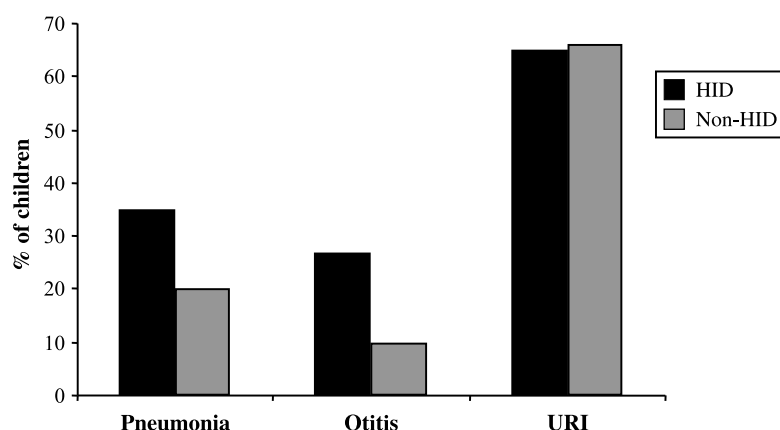


Fig. 1. Percentage of HID (black bars) and non-HID children (grey bars) with pneumonia, otitis and upper respiratory tract infections.

consequences in terms of social cost and familial and psychological involvement.

The relevance of IgM deficiency is still debated; rare reports describe an association of this defect with recurrent infections. Surprisingly, in our cohort of children affected by recurrent respiratory infections, four patients had an IgM deficiency: isolated in two and associated in two patients. A more complete evaluation by iso-hemoagglutinin determination might be helpful to better define the functionality of this immunoglobulin class.

Regarding specific anti-Hib antibody response, in our cohort of non-HID children none had abnormalities. Conversely, all three children with undetectable postvaccination antibody titers had an IgG3 defect either isolated or combined with IgG2 deficiency.

Transient and persistent IgA and/or IgG2 deficiencies have been reported in a small percentage of asymptomatic children (25) this finding suggests that IgA and IgG subclass are not absolutely required as such for a normal immune response, but deficiency may predispose to recurrent infections.

IgG isotype and IgG subclass deficiency could be the expression of an immunoregulatory disorder. IgA and IgG2 deficiency has been indeed reported in a patient who later developed a common variable immunodeficiency with panhypogammaglobulinemia (1). Español *et al.* described three patients with IgA deficiency who developed a Common Variable Immunodeficiency during follow-up and improved their clinical symptoms after I.V. IgG treatment. This finding suggests that IgA deficiency might be considered as a presenting sign of CVID (26–30). The concomitant presence of IgAD and CVD has been frequently observed in the same families suggesting a genetic linkage between these diseases.

Our study confirms that humoral deficiency is strongly associated with recurrent respiratory infections in childhood and is likely to have a pathogenetic role.

Repeated determination of IgA, IgG subclasses and specific functional antibody evaluation should be performed in order to discriminate between a transient antibody deficiency due to a delayed maturation of the immune system and a primary humoral immunodeficiency.

The management of patients with humoral immunodeficiencies with normal IgG concentrations is still being discussed. The causative bacteria in the majority of mild to moderate respiratory tract infections are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* (31). Bacterial infections of the respiratory tract are often treated empirically; several protocols of antibiotic treatment have been proposed such as antibiotic prophylaxis and/or periodic antibiotic therapy. However, the recent increase in antimicrobial resistance rates has prompted experts to re-evaluate the therapeutic approaches to these infections. Surgical intervention, in selected patients with recurrent otitis and sinusitis, could be an alternative effective prevention therapy (3).

IgG replacement therapy is usually considered when the patient shows evidence of chronic infections and immunodeficiency and fails to respond to other therapies. However, in isolated IgA deficiency, IgG replacement therapy might be inappropriate because of the low content of IgA in the commercial preparations of I.V. IgG and the potential risk of development of anti-IgA antibody (1, 32).

Prolonged follow-up of these patients is mandatory to promptly detect the number of defects that could evolve in more severe immunodeficiencies, although we believe that the treatment of these defects should be driven by the clinical

status of the patient more than merely by antibody determinations.

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