Impact of immunoglobulin G3 subclass deficiency on chronic lung disease in children

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Abstract

In this paper molecular and functional characteristics of immunoglobulin G3 (IgG3) subclass are described as well as clinical relevance of IgG3 deficiency in children with a review of the current literature. Special emphasis is put on the impact of this immune defect on chronic lung disease as presented with examples of pediatric patients. Finally, indications for treatment in IgG3 deficient children and therapeutic options are discussed.

Key words: *immunodeficiency, IgG subclass, lungs, infection, children.*

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Introduction

Each of four immunoglobulin G (IgG) subclasses exhibits a specific profile of effector functions relevant to the clearance and elimination of infecting microorganisms. The IgG subclass distribution in specific antibody responses has been found to vary with the structure of the antigen, the number and nature of the epitopes, its physicochemical properties, its dose, route of entry, as well as the genetic constitution of the host. Whereas antibodies against T-cell dependent bacterial and viral protein antigens, such as tetanus toxoid and outer membrane components can be detected in all four IgG subclasses, IgG1 is the most prevailing isotype, along with IgG3. Polysaccharide antigens stimulate a more selective antibody response, almost exclusively confined to the IgG2 subclass [1].

Differences between IgG subclasses are discernible with the respect to their two major effector functions – the activation of the complement and the induction of phagocytosis due to opsonisation are related to differences in their structure. The length and flexibility of the hinge regions within the IgG subclass molecule determine the variability of the angle between Fab (Fragment antigen binding) arms and the position between Fab-arms in relation to the Fc (Fragment crystallisable) region. The IgG3 subclass is characterized by a unique extended hinge region and the distance between Fab and Fc fragments is relatively long, allowing for greater molecule flexibility. Therefore, the IgG3 subclass has the highest activity in triggering cellular effector functions due to interactions with Fc receptors for IgG (FcγR) and is the most potent activator of the classical complement pathway subsequent to C1q binding to C_H2 domain [2, 3].

Three classes of FcγR are widely distributed in hematopoietic cell lineages. Immunoglobulin G3 is bound with high affinity to FcγRI (CD64), and with low affinity to FcγRIIa (CD32) and to both isoforms a and b of FcγRIII (CD16). These receptors include at least 12 different isoforms, many of which are polymorphic. Receptor polymorphism, as well as ligand heterogeneity and kinetics of ligand binding, govern the FcγR-mediated cellular effector functions [4].

Relevant evidence must be taken into account during the evaluation of IgG subclasses concentration in serum. Total serum IgG levels may vary considerably between healthy individuals, but the proportion of particular subclasses is maintained within a narrow range, but IgG3 proportion vary between 7 and 10% of total IgG. However, the proportions of each subclass after antigenic stimulation may differ as a result of B cell subsets stimulation in a local environment with IgG3-bearing lymphocytes predominating within tonsil cell subsets. Another parameter of critical importance is the child's age. At birth, concentrations of all subclasses reflect the maternal level as a result of placental transfer along with the active transport of IgG1 subclass resulting in IgG1 fetal/maternal ratio > 1 and con-

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centrations of IgG3 being the same in cord blood and in maternal serum. Each IgG subclass has its individual maturation pattern in childhood with IgG1 and IgG3 subclasses reaching adult levels at an earlier age than IgG2 and IgG4. The serum concentrations of IgG1 and IgG3 reach about 50% of adult serum concentrations at the age of one year and about 75% at the age of five, with large intersubject variability [5]. A further parameter of importance is the polymorphism of immunoglobulin genes. The different allelic forms of the immunoglobulin genes for the heavy chains of IgG1, IgG2 and IgG3 subclasses and for the γ light chains give rise to Gm1, Gm2 and Gm3 allotypes. The alleles of the heavy chain genes are inherited as haplotypes because these genes are located close to each other on chromosome 14 [6]. Individuals homozygous for Gm(b) allele have higher levels of IgG3 subclass than individuals homozygous for Gm(g) allele and heterozygous individuals have intermediate levels of IgG3 subclasses [5]. Since the distribution of these alleles vary between populations, the IgG3 subclass concentrations and specific antibody responses may similarly vary [7, 8].

The association between IgG3 subclass level and infectious problems is not clear cut, although recurrent infections of the lower airways is a frequent health problem of individuals with this immune defect. In patients without coexisting serious comorbidities, IgG3 subclass deficiency may be asymptomatic, but in patients with chronic lung diseases this immune deficiency may have a severe impact on their clinical course, as is presented in the following case study.

Case presentation

Case 1

A 12-year-old girl, under observation at the pneumonology department of a university hospital since the age of 18 months, when she suffered for the first time a lower airways infection with symptoms of respiratory insufficiency and manifested disseminated interstitial infiltrations on a chest radiogram and on HRCT (high-resolution computed tomography) scans. Her personal history concerning the fetal and perinatal period was not contributory; until the age of 18 months she had not suffered either from airway infections or presented alarming respiratory symptoms such as dyspnea, cough or exercise intolerance. The physical examination did not reveal any abnormalities. Capillary blood gas analysis as well as pulsoximetry showed normal hemoglobin saturation during rest and exertion. To establish a definitive diagnosis, detailed differential diagnostic procedures were carried out. Chronic infection induced by bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria* was assessed with a microbiologic examination of airway discharge samples taken during endoscopy at the beginning of a period of observation. Infections with respiratory viruses – *Respiratory syncytial virus* (RSV), *Parainfluenza* and *Adenovirus*, other viruses such as *Hepatitis B*, *C* (HBV and HCV, respectively) and *Cytomegalovirus* (CMV), atypical bacteria – *Mycoplasma*, *Chlamydophila pneumoniae* as well as mycotic infections with *Aspergillus* and *Pneumocystis jiroveci* were excluded. Immunological diagnostic tests revealed an immune defect in the form of IgG3 subclass and C4 complement component deficiency. Total IgG concentration as well as of other isotypes were within the limits of normal values for the age range and the level of postvaccination specific anti-HBs antibodies was protective. Cytometric immunophenotyping of the main immune cells subsets, activation markers and adhesion molecules, as well as the evaluation of phagocytic cells function in an oxidative burst test did not show any abnormalities. Diagnostic tests toward autoimmune connective tissue diseases revealed transient elevation of anti-alveolar and glomerular basement membrane autoantibodies, characteristic for Goodpasture's syndrome and elevated levels of anti-nuclear antibodies. Autoantibodies typical for systemic lupus, such as anti-Sm, anti-nDNA, against histones and ribosomal P protein, anti-SSA and anti-SSB (occurring also in Sjögren's syndrome), lupus anticoagulant and antiphospholipid antibodies were not detected. Both anti-Scl70 and anti-centromere autoantibodies typical for scleroderma, anti-Jo antibodies occurring in dermatomyositis or autoantibodies directed against neutrophil cytoplasmic enzymes – cANCA, pANCA, characteristic of vasculitides (Wegener's syndrome and Churg-Strauss syndrome, respectively) were not present.

An open lung biopsy and histological examination carried out at the age of eight years allowed for the establishing of the final diagnosis of chronic pneumonitis of infancy (CPI) with cholesterol pneumonia, typical for surfactant protein C deficiency (Fig. 1). At the age of ten years, significant progression of the interstitial lung disease was assessed radiologically and on HRCT (high-resolution computed tomography) examination, which revealed the progression of nodular changes, accompanied by a thickening of intralobular septa. The radiological pattern correlated with the progressive deterioration of ventilatory parameters assessed in a lung function test [5% decrease of vital capacity (VC) over 6 months], as well as clinical symptoms, such as exercise intolerance, ineffective cough, and clubbing fingers and toes. Taking into consideration clinical exacerbation and radiological progression of chronic intersitial lung disease, systemic corticosteroid therapy was introduced. The role of the chronic bacterial infection resulting from coexisting immune deficiency and being an important factor superimposing on pneumopathy was an indication of immunoglobulin therapy. Taking into consideration poor clinical outcome and the further progression of the chronic interstitial lung disease lung transplantation in the future may be a therapeutic option.

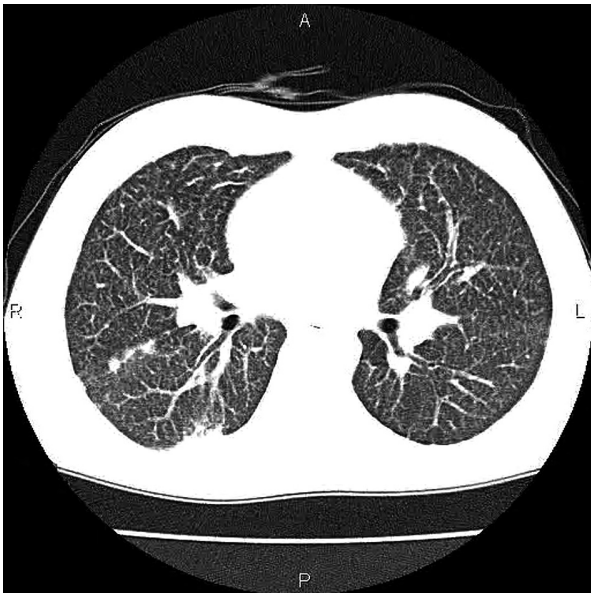

Fig. 1. HRCT imaging of the IgG3 deficient patient with chronic interstitial lung disease and surfactant protein C deficiency

Case 2

A 3-month-old female infant was referred to the pneumonology department because of recurrent lung infections. By the age of three months the patient already required hospitalization due to a lung infection with an obstructive lower airways reaction. Since the neonatal period, persistent stridor and hypersecretion of mucus in the airways were observed. These symptoms suggested a congenital anomaly of the airways and endoscopic examination was performed. Bronchoscopy was carried out at the age of five months and revealed severe laryngotracheomalatia. Further differential diagnostic investigations showed a coexisting gastroesophageal reflux and a congenital heart defect – ASDII (atrial septal defect). Since early infancy, repeated microbiologic examinations of the lower airway secretion showed numerous colonies of pathological bacterial flora – *Proteus mirabilis*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. Diagnostic tests toward viral infections with RSV, *Adenovirus*, *Parainfluenza virus*, as well as *Cytomegalovirus* were negative. Immunological tests revealed a decreased level of IgG3 subclass with normal concentrations of IgG and other immunoglobulin classes, a protective level of specific post-vaccination anti-HBs antibodies and normal complement components. Detailed B and T lymph cells subsets assessed in the flow cytometry did not show abnormalities. In the light of the presence of multiple predisposing factors for the severe course of recurrent bronchopulmonary infections, intravenous immunoglobulin therapy was started at the age of 12 months. However, despite monthly antibody infusions, currently at the age of 2 years, persistent respiratory symptoms and lung infections are still occurring, requiring repeated hospitalization and intensive antibiotic as well as systemic corticosteroid therapy.

Case 3

In this male patient, a diagnosis of tracheoesophageal fistula with esophageal atresia was established after birth on the contrast radiological examination of the gastrointestinal tract and on the second day of the child's life, corrective surgery was performed. Since the age of three month, the patient had been suffering from severe bronchial and lung infections with an obstructive reaction. Endoscopic examination of the lower airways revealed severe tracheomalatia and infection with Gram negative bacterial flora – *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* ESBL (extended-spectrum β-lactamase) positive. Viral infections with RSV, *Adenovirus*, *Parainfluenza virus*, CMV and *Epstein-Barr virus* (EBV) were excluded. Differential diagnosis of pulmonary disease included echocardiography, which showed a patent foramen ovale with bidirectional shunt. Immunological tests revealed total IgG concentration below the reference value adjusted for the age, along with IgG3 subclass deficiency. The concentration of other isotypes, specific anti-HBs post-vaccination antibodies, complement components, immune cell subsets and activation markers assessed in cytometric immunophenotyping were normal. With regard to immune deficiency associated with multiple comorbidities which may have important impact on the severity of respiratory tract disease, intravenous immunoglobulin therapy was introduced from the age of 18 months for the period of two consecutive years until normalization of total IgG concentration. Nevertheless, currently at the age of five years, even if significant alleviation of lung disease is observed, ambulatory treatment with antibiotics, systemic corticosteroids and inhaled bronchodilators is frequently necessary.

Discussion

Immunoglobulin G subclass deficiencies are frequently diagnosed as immune defects and serve for the explanation of diverse health problems, not confined solely to immunodeficiency, but comprising allergic and autoimmune diseases as well. In children, determination of IgG subclasses concentration is usually an element of differential diagnostic procedures of recurrent airway infections and, indeed, these infections predominate as clinical manifestations in patients in whom IgG subclass deficiencies have been assessed. In the three patients described here, recurrent severe lower respiratory tract infections were the indication for broad differential diagnostic procedures which disclosed not only selective IgG3 deficiency, but coexisting chronic lung disease in case 1 or anatomical and functional anomalies of the airways in cases 2 and 3. Inasmuch, bacterial infections were the most important reasons for

exacerbations of their pulmonary disease. In a study by Umetsu *et al.* [9], authors reported 20 children with IgG subclass deficiency and recurrent sinopulmonary infections, among whom as many as 25% had an IgG3 production defect. The leading clinical manifestation of IgG3 deficiency were upper airways infections – recurrent otitis media and sinusitis were present in 100% of children; pneumonia was reported in 55% of children. In a state-of-the-art review produced by Sotomayor *et al.* in 1989 [10], the essential role of pediatricians and pediatric pneumonologists in recognizing pulmonary manifestation of IgG3 deficiency was stressed. This opinion was then supported by DeBaets *et al.* [11], who demonstrated the correlation between IgG3 subclass deficiency and airways infections; this immune defect was present in 17% of 3-4 year old children suffering from recurrent bronchitis episodes. Similarly, in the study by Bossuyt *et al.* [12] IgG3 deficiency was found to be one of immunological susceptibility factors to recurrent respiratory infections as assessed in 20% of children. Ozkan *et al.* [13] evaluated a group of 225 children aged 6 months to 6 years with recurrent upper respiratory tract and pulmonary infections in relation to IgA and IgG subclass deficiency. These authors demonstrated chronic pulmonary damage on chest radiograms and computed tomography (CT) scans occurring five times more frequently in IgG subclass deficiency than in IgA deficiency. Among the overall number of 22 patients with recurrent respiratory tract infections and coexisting antibody production defects, two children with IgG3 deficiency presented bronchiectasis and one had lung fibrosis, whereas bronchiolitis obliterans was present in two patients with combined IgG3 and IgG2 deficiency. The results of this study point to the correlation between IgG3 deficiency and airway infections with long-term pulmonary sequelae. In contrast to these data, in the study by Aghamohammadi *et al.* [14] evaluation of humoral immunity in a large group of 103 patients with recurrent upper respiratory tract infections revealed IgG3 deficiency in only one case. In the report of 30 children with recurrent sinusitis by Shackelford *et al.* [15], IgG2 deficiency was detected in seven patients and none had IgG3 deficiency.

It is worth noting that IgG3 subclass deficiency is frequently reported in young children and may reflect a transient humoral immune deficiency, as it was shown in two studies by Turkish investigators, Karaca *et al.* [16] and Kutukculer *et al.* [17]. Serum IgG subclass levels reached the normal range for age in 30% of patients until about 6 years of age. In the patients studied, regularly repeated measurements of IgG subclass concentrations remained at a decreased level. However, in case study 2, because of the young age of the patient (currently 24 months), the normalization of IgG3 subclass level is still possible, but difficult to predict.

Moreover, in several of these studies, the relationship between the humoral immune defect and the clinical course of asthma in children were investigated. In the above publications by Umetsu *et al.* [9] and by Kutukculer *et al.* [17] asthma was a common clinical presentation in children with IgG3 deficiency, diagnosed in 50% and 24% of cases, respectively. The aim of the study by de Moraes *et al.* [18] was to determine possible dysfunction of humoral immunity in asthmatic children, making them susceptible for recurrent respiratory tract infection and worse clinical evolution. Immunoglobulin G3 subclass deficiency was the most frequently found immune defect, assessed in 10 of total 41 children. Interestingly, this abnormality was detected in children with and without recurrent airways infection and therefore, according to the results of this study, appeared not to be a suitable predictor of development of infections in children. These results are consistent with judgement of other investigators that determination of IgG subclass levels is not helpful in the general assessment of immune function and provides no information about patient's capacity to produce specific antibodies to protein and polysaccharide microbial antigens and in fact does not identify patients at risk of infection convincingly [19, 20]. Therefore, despite the widely performed measurement of IgG3 subclass level, its isolated deficiency is still debated. Armenaka *et al.* [21] observed the normalization of IgG3 levels during the treatment of sinusitis, hence he suggested that IgG3 deficiency may result from its excessive consumption during infection. Therefore it is necessary to repeat the measurements of IgG subclass level after the infection as it was carried out in our patients.

The problem of laboratory data interpretation is even more complex, due to lack of reliable age-dependent reference values and unsatisfactory standardization of IgG subclass assays [22].

Furthermore, the indications for the treatment and the choice of an appropriate therapy remain a matter of controversy. According to the recommendations concerning immunoglobulin replacement therapy in immunodeficient children provided in 2008 by Garcia-Lloret *et al.* [23] and issued by the National Institutes of Health, in the absence of impaired antibody responses, the significance of a depressed level of any of the IgG subclasses is unclear and immunoglobulin replacement is not indicated. However, Barlan *et al.* [24] described the treatment of 22 IgG3 deficient pediatric patients suffering from recurrent upper respiratory tract infections, referred to prophylactic therapy with oral trimethoprim/sulfamethoxazole. Twelve of these children did not respond to the antimicrobial prophylaxis and were treated with intravenous immunoglobulins (IVIG), which led to a significant amelioration of airway infections. Likewise, Meyts *et al.* [25] described 6 pediatric cases with selective IgG3 subclass deficiency and recurrent respiratory tract infections, in whom the introduction of immunoglobulin treatment was considered after a failure of antibiotic therapy had been evaluated and this therapeutic option with IVIG proved to be highly effective. In the three cases presented, the decision about intravenous im-

munoglobulin replacement therapy was made on clinical, microbiological, immunological and radiological findings. In case study 1 a significant argument for IVIG treatment was a coexisting chronic progressive interstitial lung disease with poor prognosis. In case studies 2 and 3, congenital anatomical and functional respiratory tract defects posed a striking predilection to bacterial infections and were considered as an indication for immunoglobulin therapy. The alleviation of respiratory symptoms, the reduction of the antibiotic therapy frequency, as well as decreased hospitalization rates, admitted as the endpoints serving for the evaluation of IVIG treatment effectiveness, were observed in all patients.

Conclusions

Selective IgG3 subclass deficiency (albeit frequently detected in children suffering from recurrent respiratory infections) and its clinical relevance are usually neglected in pediatric practice. However, this immune defect may have an important impact on chronic lung disease in children, predisposing them to airway infections and exacerbations of primordial illness. An increased awareness of pediatricians and pediatric pneumonologists is necessary in order to monitor IgG3 deficient patients periodically under a clinical immunologist's supervision. If infections are frequent and severe, in these patients individualized treatment with prophylactic antibiotic and/or immunoglobulin replacement therapy should be considered.

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