Twenty five years of investigations into primary immunodeficiency diseases in the Department of Immunology, The Children's Memorial Health Institute, Warsaw

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Abstract

Nine hundred and forty-six cases of primary immunodeficiency diseases were diagnosed in the Department of Immunology of the Children's Memorial Health Institute in Warsaw during 1980-2006. The highest frequency was that of antibody immunodeficiencies: 547 (57.8%). Predominantly T cell deficiencies were recognised in 80 (8.5%) children. In 29 children severe combined immunodeficiencies with different genetic defects were diagnosed, and in 18 of them hematopoietic stem cell transplantation was done. In the group of phagocytic disorders (95; 10% of patients), neutropenia was the most common - 46 cases, followed by chronic granulomatous diseased recognised in 41 patients. In the group of other well-defined PIDs - immunodeficiencies associated with chromosomal instability were the most common (152; 16% of patients). Ataxia – teleangiectasia was diagnosed in 92 cases and, next, Nijmegen Breakage syndrome in 57 children. Hyper IgE syndrome was diagnosed in 22 patients, and Wiskott-Aldrich syndrome in 19 boys.

Key words: *primary immunodeficiencies, T cells defects, B cells defects, phagocytic defects, haematopoietic stem cell transplantation*

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Since 1980 the Department of Immunology has been the reference centre for diagnosis and therapy of children with primary immunodeficiencies (PID's) from the whole of Poland. In 1988 the first registry, containing 103 cases of primary immunodeficiency diseases, was published [1]. Dynamic developments in molecular and genetic techniques led to great progress, in knowledge the pathogenesis of PIDs, and recognition of new PID types, as well as improvement in treatment protocols. The national programme of substitutional replacement therapy was initiated and conducted by the Department of Immunology, CMHI in 1993. The programme and distribution of intravenous gammaglobulins to immunology centres in Poland was financed to early the 2000s by the Ministry of Health, and now by the National Health Insurance system. Subcutaneous replacement immunoglobulin therapy was introduced in our Department of Immunology in 2001 for the first time in Poland, and has been continued successfully [2]. Increasing experience in hematopoietic stem cell transplantation (HSCT) gives a great chance for correction of the most severe forms of PIDs.

The number of diagnosed and treated PID patients is systematically growing year by year. Only a few patients with agammaglobulinaemia were diagnosed in the early 80s, but in 1988 there were 103 cases of different types of PID, followed by 608 patients in 2000 [1,3,4].

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This paper contains the clinical, immunological and genetic data of primary immunodeficiency patients treated in our department between the years 1980-2006. To date, 946 children with congenital immunodeficiencies have been diagnosed.

Antibody deficiencies

Antibody deficiencies comprise the largest group of primary immunodeficiencies diagnosed in our department (table 1). They result from inborn defects of the immune system, especially from B cell development. Since the first description of antibody deficiency made by Bruton in the early 50s, a great wealth of information has been accumulated. The last few years have unravelled the application of molecular and genetic techniques for identifying these disorders. Approximately 85% of all agammaglobulinemia cases are caused by mutations in a gene encoding Bruton's tyrosine kinase (*btk*), which consists of 19 exons and spans 38 kb. The other 15% of patients with agammaglobulinaemia (both boys in whom the *btk* mutation was excluded, and girls) appears to have a mutation in one of the several genes involved in the pathogenesis of autosomal recessive (AR) inherited agammaglobulinaemia, such as IGH-Cμ, λ(CD179b), CD79a, or B-cell linker protein (BLNK).

In our Department primary antibody deficiencies were diagnosed in 57.8% of patients (547 children). Hypogammaglobulinaemia was recognised in 71 children and cases of sporadic incidence (42 patients) were the most frequent (table 1) [5-9].

Immunoglobulin replacement therapy has been the mainstay of treatment for patients affected by a variety of immunodeficiencies. Antibody replacement was administered intramuscularly until 1980s, when intravenous immunoglobulin (IVIG) products were widely introduced. Since that time IVIG has become the most popular route of administration. The national programme of substitutional replacement therapy, financed by the Ministry of Health, was initiated and provided by the Department of Immunology, CMHI from the early 90s for almost 10 years. Anyway, due to systemic adverse reactions, in some patients, as well as to difficulties in vein access and the need for frequent hospital admission these have been replaced by subcutaneous infusion (SCIG) [2]. The method is associated with few systemic adverse reactions. The safety and easy infusion technique make SCIG a very suitable method for self-infusion at home. Subcutaneous replacement immunoglobulin therapy was introduced in the Department of Immunology in 2001 for the first time, and has been continuated successfully. The vast majority of children are treated with intravenous immunoglobulins. Fifteen patients receive subcutaneous immunoglobulins.

Table 1. Predominantly antibody disorders

Type of deficiency	Number of patients
agammaglobulinaemia	71
• sporadic	42
• XLA	29
dysgammaglobulinaemia	52
IgG subclass deficiency	81
IgA deficiency	123
CVID	68
transient hypogammaglobulinaemia of infancy	121
hyper-IgM syndrome	9
specific antibody deficiency	11
total	547

X-linked agammaglobulinaemia

This is a genetic disorder due to a mutation affecting the *btk* gene, coding for a tyrosine kinase involved in B-cell maturation. The gene is located at Xq21.3-22. Defects in that gene prevent B-cell development from pro-B cell to pre-B cell.

X-linked agammaglobulinaemia (XLA) – the prototypical antibody deficiency was recognised in 29 boys. The genetic confirmation of a mutation in the *Btk* gene was obtained from 18 boys. Currently under our control there are a few families affected by this disease. This is why it was possible to include them in the programme of genetic studies, sponsored by an EU grant, realized with other well-known European centres for immunological and genetic studies in primary immunodeficiencies: the Karolinska Instituet in Stockholm, and the Erasmus University in Rotterdam. Some of the XLA patients were investigated to show the presence and role of cells supressing immunoglobulin production. This showed evidence of two functionally distinct suppressor T and lymphokine deficiencies [7]. During the last 25 years numerous clinical trials and observations on intravenous immunoglobulin therapy (IVIG) have been done. This has provided standards for IVIG treatment and many publications [10-12]. In one of the studies the efficacy of two - dose IVIG and plasma treatment were compared. This demonstrated that in children with severe clinical symptoms, a high dose of IVIG (0.5 g/kg of body weight) led to significant improvement. But, in XLA patients with fewer symptoms the same high dose did not result in further amelioration in comparison with low dose therapy (0.15 g/kg of body weight). In any case, high-dose IVIG therapy hase been proved to be beneficial in treating children with agammaglobulinaemia and progressive encephalitis caused by the ECHO virus.

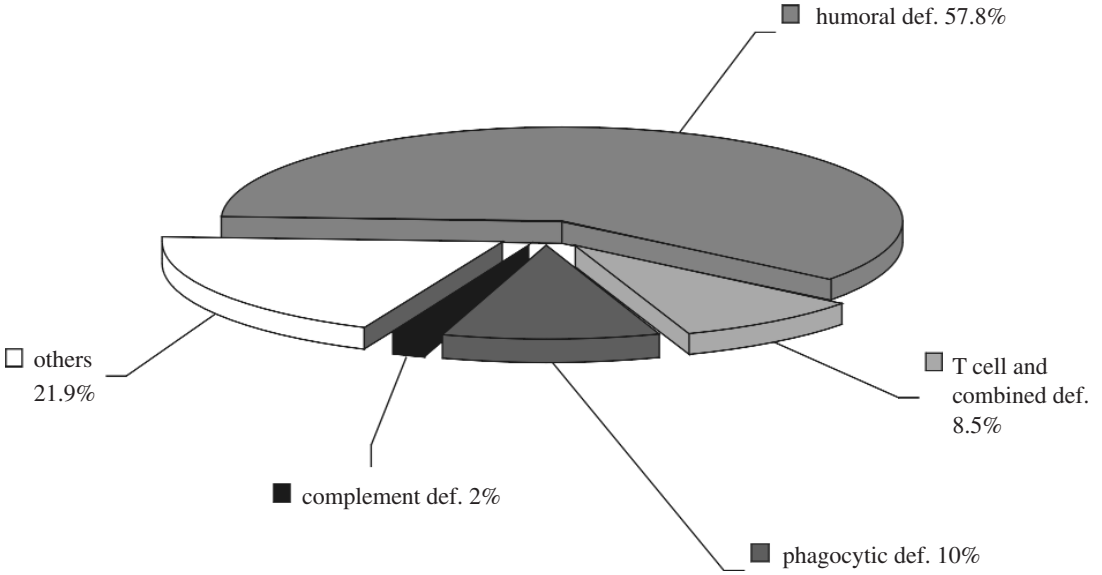

Fig. 1. Percentage of different PIDs groups in CMHI registry

BLNK deficiency. In one male patient suffering from recurrent respiratory tract infections, with no B cells and low Ig levels, a mutation was found in BLNK gene.

IgG subclass deficiencies

IgG subclass levels are related to allotypes of IgG. Different racial groups may present therefore with different normal ranges, depending on the prevalence of different allotypes. The clinical symptoms of IgG subclass deficiency can occur at any age. Recurrent infections may be a feature, particularly for IgG2 + IgG4 deficiency. IgG4 deficient hosts can present with bronchiectasis. Long-term progression to CVID is a possibility.

IgG subclass deficiencies were recognized in 81 children. The 16 patients were diagnosed to have IgA and IgG subclass deficiency, while 5 had a combination of IgG subclass deficiency and another PID, such as complement deficiency. The determination of serum IgG subclass levels in our patients was initiated in the late 80s; with Professor Martha Eibl from the Institute of Immunology at the University of Vienna [13,14]. Later, in the early 90's, the ELISA method of IgG subclass level determination was developed in the Department of Clinical Immunology. Independently, the antibody response against common polysaccharide antigens (of *Haemophilus influenzae* type b and *Streptococcus pneumoniae*) were provided together with Helen Griffith from the Radcliffe Hospital, Oxford. All investigations were carried out on patients with severe recurrent chest infections, whose serum immunoglobulin levels were in the normal range. In very early studies the most common situation was IgG3 deficiency. Further investigations showed IgG 1 to be the most frequent IgG subclass defect in children with a normal IgG level, followed by IgG2 and IgG3 deficiencies [15-17]. Low levels of the IgG4 subclass were also very often observed, but selective IgG4 deficiency is very difficult to interpret, since its levels vary widely in normal persons, and some have no demonstrable IgG4 by standard techniques.

To investigate the efficacy of IVIG, a group of children with IgG subclass deficiency, including some with IgG3 deficiency, were treated with an immunoglobulin product containing only a trace amount of IgG3 [15-17]. The results showed a significant improvement, documented by a reduction in infections and days on antibiotics and on steroids. Data obtained on a subgroup of IgG3 deficient patients were analysed separately, but also showed a significant amelioration. The mode of action cannot be attributed to replacement of the respective isotypes, but is probably due to the effect of other biological properties of IVIG, such as anti-inflammatory activity, in preventing repeated viral infections. Low concentration or no detectable IgG subclasses can coexist with IgA deficiency. In our material, only 16 childern were diagnosed to have IgA and IgG deficiency together.

IgA deficiency

Selective IgA deficiency is the most common primary immunodeficiency, but mostly passes unnoticed. The frequency of that form of PID depends on the racial group, and appears as often as 1 in 400 – 18 500 individuals. In

Caucasians, 1 in 700 persons have no demonstrable serum IgA. Most of these individuals (about 2/3) have no apparent disease. There is an increased incidence of allergic diseases, connective tissue diseases, coeliac disese, and pernicious anaemia. Infections occur in some patients. Patients with IgA deficiency should be screened for evidence of other humoral defects, particularly IgG subclasses and specific antibodies.

We have recognized an isolated IgA deficiency in 123 patients (13%), and combined IgA and IgG subclass deficiency in 16 patients, which places this defect in the first position by frequency. Some of the patients were studied to evaluate the transport mechanism of IgA across enterocytes [18].

Transient hypogammaglobulinaemia of infancy

Transient hypogammaglobulinaemia of infancy is thought to be due to a delay in immune development, leading to a prolongation of the physiological trough of antibodies after the age of 6 months, when the maternal antibodies have largely disappeared. In such patients the nadir of serum Ig concentration is 2SD below normal range. The initiation of antibody production may be delayed for as long as 36 months, and even sometimes longer, and is ultimately manifested by increasing levels of serum IgG. The usual presentation is with bacterial infections occurring after 6 months of age. By definition all patients recover. If there is no recovery, then the patient should be regarded as having common variable immunodeficiency or another type of humoral deficiency. Unless there is some other underlying defect, the condition corrects itself and requires no treatment. Children who have recurrent lower respiratory tract infections hardly ever need intravenous immunoglobulin therapy temporarily.

In our registry 121 children (12.7%) were diagnosed to have transient hypogammaglobulinaemia of infancy.

Dysgammaglobulinaemia and common variable immunodeficiency

Less commonly, different dysgammaglobulinaemias (IgG/IgA, IgG/IgM, IgG or IgM) as well as common variable immunodeficiency, were recognised, respectively, in 52 and 68 of all immunodeficient patients. The cause of CVID is not clear. There are some hypotheses suggesting the role of an environmental factor (virus?) triggering the disease in a genetically susceptibile individual. There is also some evidence for a genetic background (linked to MHC), and there may be a family history of other PID (especially IgA or IgG subclass deficiency) in up to 59% of patients. Usually patients present with recurrent bacterial infections such as XLA. However, autoimmune problems, particularly thrombocytopaenia, haemolytic anaemia, and organ-specific autoimmunity (e.g. thyroid, diabetes, vitiligo, alopecia) are common and may precede the recurrent infections. Granulomatous disease with lymphadenopathy and spleno- and/or hepatomegaly, often involving the lungs, is common in severe cases of CVID (approximately 25%). It should be distinguished from sarcoidosis and, in boys, from XLP (X-linked lyphoproliferative disease). In some patients a mutation in the ICOS gene was found. The earlier diagnosis is made, the better the prognosis. The treatment is the same as for XLA: intravenous or subcutaneous immunoglobulins, antibiotics, physiotherapy and steroids in granulomatous disease. In some patients with splenomegaly and hypersplenism a splenectomy may be the treatment of choice. Regular screening for malignancies should be maintained.

Hyper IgM syndrome

There are nine patients with hyper-IgM syndrome (HIGM) diagnosed according to the definition proposed by ESID. Among them there are six male patients from four families, who suffer from genetically-confirmed HIGM1. Molecular analysis was carried out, thanks to co-operation with Professor Jack van Dongen from Erasmus University, Rotterdam. Four different mutations of CD40L were identified. Among the other three patients, in two male patients HIGM1 and HIGM3 were excluded. Both of them and the female patient are awaiting genetic analysis of other autosomal recessive types of hyper-IgM syndromes. One of the patients with significant microcephaly, growth retardation, hypogammaglobulinaemia with elevated IgM level and impaired lymphocyte subset was found to suffer from a newly-discovered primary immunodeficiency dependent on a mutation in the Cernunnos gene. Genetic analysis of this patient was performed in the Necker Hospital, Paris, thanks to co-operation with Doctor Ann Durandy. There are still doubts about the qualification of this type of PID, but it will probably fall be qualified as a severe combined immunodeficiency.

Specific antibody deficiency

It has been known for decades that some individuals suffering from recurrent respiratory infections have normal Ig levels, but they fail to respond to certain antigens, particularly to polysaccharide antigens. This syndrome is probably much more common than realized.

In our registry we have 11 patients with a specific antibody deficiency, mostly against *Haemophilus influenzae* type b and/or *Streptococcus pneumoniae*. In some of these children a diminished serum IgG2 subclass level was found.

Antimicrobial prophylaxis in antibody-deficient children

There is no need to treat every patient with primary antibody deficiency with IVIG or SCIG. The majority of those diagnosed to have IgA deficiency, IgG subclass deficiency, or transient hypogammaglobulinaemia will never need it. The first choice for treatment is vaccinations against widespread pathogens, such as *H. influenzae* type b and *Streptococcus pneumoniae*. It is also very helpful to test for

Table 2. Predominantly T-cell deficiencies

Type of deficiency	Number of patients
severe combined immunodeficiency – SCID	29
primary CD4 lymphopaenia	4
CD3 deficiency	1
DiGeorge syndrome	37
chronic mucocutaneous candidiasis	9
total	80

specific antibody deficiency. Many of the vaccinated children had a significantly reduced number of infections or even stopped them. In the remainder, protracted antibiotic prophylaxis should be considered. Usually, an antibiotic in a half dose once a day is recommended for several months. In children showing an improvement, 2-3 months summer intervals in antibiotics are introduced. Despite patients with the above-mentioned immunodeficiencies, we recommend protracted antibiotic therapy in children with agammaglobulinaemia and other severe immunodeficiencies as an additional, helpful treatment to IVIG. Antibiotic prophylaxis is also prescribed in individual children with asplenia, who have not produced specific antibodies against polysaccharide antigens, and who suffer from recurrent infections. This is also obligatory for asplenic infants.

T cell defects and combined immunodeficiencies

T cell defects and combined immunodeficiencies were diagnosed in 80 (8.5%) children (table 2).

Severe Combined Immunodeficiency (SCID)

Severe Combined Immunodeficiency (SCID) is a group of rare PIDs, but the most severe forms of PIDs of different genetic cause. In the natural course of the disease most patients die before the end of the second year due to infectious complications. The opportunistic infections, among them generalised BCG infection following vaccination against tuberculosis, are the major cause of fatal outcomes. In our centre 29 cases of different forms of SCID were recognised. Thank to co-operation with Prof. JJ van Dongen from the Erasmus University in Rotterdam, in 19 cases molecular investigation was performed which led to establishing a final genetical diagnosis in 13 children. In 5 patients the most often X-linked form of disease, caused by mutations in the common gamma chain gene, was confirmed: in 3 cases RAG2 gene, in 2 patients RAG1 gene, in 2 patients IL-7 receptor gene and in one boy Artemis gene mutations were found [19]. In some cases final confirmation of SCID on a genetic level was essential for a decision about transplant procedure, as well as for genetic counselling. From 1998 the first successful bone marrow transplantation in SCID boy was performed in the Bone Marrow Transplant Unit in Wroclaw, headed by Prof. A.Lange. From this time seventeen other SCID patients have been transplanted with a good outcome [20,21].

Di George syndrome (DGS)

Di George originally described one phenotype of what is now realized to be a wide array of developmental defects, probably due to more than one genetic lesion. Presentation involves combinations of such features as: hypocalcaemia and tetany, cardiac abnormalities, dysmorphic faces, highly variable immunodeficiency, and absence or reduction of thymic size. The diagnosis is done by clinical suspicion, but all suspected patients should be screened for 22q11 deletion. The immunological defects in DGS patients are variable and tend to improve with age.

In the Department of Immunology, CMHI, we have 37 patients with clinical features of DGS. Among them there are 12 girls (32%) and 25 boys (68%) at ages between 8 months and 20 yrs. In 26 out of 37 microdeletion of chromosome 22q11 was found by FISH (fluorescence in situ hybridization) (70%). In the next 3 patients with clinical features of DGS FISH was negative, while in 4 this was not done. Five children died in infancy, mostly due to severe cardiac disorders and co-existing overwhelming infections. There is no contact with 3 other patients, who have probably died.

Cardiac defects were diagnosed in 86% of patients with clinical features of Di George syndrome which corresponds to the 74% described in the literature. Hypocalcaemia and hypoparathyroidism were found in 46% of our patients vs 49%. The third common feature of DGS, dysmorphic faces, were recognized in 85% [22].

The defective immune system affected 69% of children, but it must be said that the majority of them had slight to moderate defects. The mean number and percentage of CD3 lymphocytes as well as the lymphoproliferative response to PHA in DGS patients were slightly diminished. Only one child presented with CD3+T cells less than 500, which was one of the definitive criteria to recognize DGS by ESID. The next three patients presented with as low as 501 – 540 of CD3+ T cells. In 11 children a low response to PHA was observed. The mean lymphoproliferative response to PHA was 8 094 (range 1 783-31 147) compared to a control group of healthy children: mean 24 657 (range 16 735-48 396).

Low IgG levels were found in 4 children, who were temporarily in need of IVIG therapy. None of these needed permanent substitution therapy.

One patient, a 5-week-old girl, developed severe Aspergillus infection, a total CD3 count of 62% and 355 cells as well as low Ig levels were observed; she was successfully treated with voriconazole and intravenous immunoglobulins. During almost 2 years of observation her CD3+ T cells and Ig levels have risen significantly enough to stop IVIG treatment.

The majority of DGS patients have the same microdeletion of 22q11.2 in locus D22S75 Great clinical variability was observed and the genotype/phenotype correlation was difficult to establish.

Chronic mucocutaneous candidiasis (CMC)

The cause of CMC is not well known. There are autosomal dominant and recessive forms as well as sporadic cases. An abnormal gene on chromosome 21q22.3, coding for the protein known as AIRE (autoimmune regulator), is found in autoimmune polyendocrinopathy candidiasis ectodermal dysplasia (APECED) - one of the forms of CMC.

Chronic mucocutaneous candidiasis (CMC) is a complex disorder in which patients suffer from persistent or recurrent infections of the mucous membranes, skin and nails, in the majority due to the *Candida* species. It can be associated with an endocrinopathy. We recognised CMC in 9 patients. They had slight to moderate T-cell dysfunction: decreased T-cell subset number, and a reduced lymphoproliferative response to mitogens, antigens, or allogenic cells. In two of them hypoparathyroidism was found, and in one diabetes type I. Another had familial CMC (mother, and grandmother on the maternal side were affected).

In one boy with candidiasis, ectodermal dyspalsia and poliendocrinopathy genetic confirmation was carried out.

Phagocytic immunodeficiencies

The third largest group among the primary immunodeficiencies consists of disorders in the phagocytic system – 95 (10%) patients (table 3).

Primary neutropaenia

Chronic neutropaenia is a rare disorder in children. It is defined by an absolute neutrophil count (ANC) below 1500 cells per cubic milimetre of blood (under 2 years of age – below 1000), lasting at least 6 months. Congenital disorders are more important in the differential diagnosis of neutropaenia in neonates and young children than in teenagers, although acquired causes are more common during childhood.

The four main forms of hereditary neutropaenia are: chronic benign familial neutropaenia, Shwachman-Diamond syndrome, cyclic neutropaenia and severe congenital neutropaenia, also referred to as Kostmann syndrome. Chronic inherited neutropaenia may be associated either with a primary immunodeficiency (such as: HIGM type I and III, XLA, myelokathexis and WHIM, Chediak-Higashi syndrome, ALPS, FHL, Griscelli syndrome type II, reticular dysgenesis) or with a metabolic disorder (glycogen storage disease type 1b, Barth syndrome, organic acidosis like MMA, IVA, PA).

Among children diagnosed in our department, 50 were given a diagnosis of primary neutropaenia [23,24]. The investigation revealed 4 cases of Kostmann syndrome, 1 child with Shwachman-Diamond syndrome, 20 cases of severe chronic neutropaenia and 25 patients with chronic benign neutropaenia. Autoimmune primary neutropaenia was confirmed (due to GIFT, GAT or MAIGA tests) in 6 children.

Therapeutic management depends on the clinical entity, patient's age and severity of clinical course. It includes antibiotic prophylaxis, human recombinant granulocyte colony stimulating factor (rHuG-CSF) and bone marrow transplant from an HLA-identical related donor, especially in children suffering from the disease connected with a high risk of malignant myeloid transformation, such as Shwachman-Diamond and Kostmann syndrome. One of our patients, diagnosed with Kostmann syndrome, died of chronic myeloblastic leukaemia; another underwent HSCT,

Table 3. Phagocytic disorders

Type of deficiency	Number of patients
primary neutropaenia	46
chronic granulomatous disease	41
Chediak-Higashi syndrome	1
Griscelli syndrome	2
Shwachman syndrome	1
myeloperoxidase deficiency	1
IFN γ receptor deficiency	2
Il 12 receptor deficiency	1
total	95

Table 4. Other PIDs

Type of deficiency	Number of patients
complement deficiencies other well-defined PIDs:	**16**
hyper-IgE syndrome	22
DNA – breakage disorders:	**152**
ataxia-teleangiectasia	92
NBS	57
bloom syndrome	3
WAS	19
XLP	6
prepuberal periodontitis	3
Papillon-Lefevre syndrome	2
autoimmune and immunedysregulation syndromes:	2
ALPS	1
APECED	1
total	224

but unfortunately developed a fatal fungal infection soon after the procedure. Only 10 children (all with Kostmann syndrome and 6 with ANC<500/ml and severe bacterial infection) recquired rHuG-CSF therapy, which did not cause any important adverse events [25,26]. About 20 children, mostly the youngest, had to receive an antibiotic prophylaxis. Either rHuG-CSF treatment or antibiotic prophylaxis caused a lower frequency and severity of infectious complications and stopped chronic gingivitis - a common problem in patients afflicted with severe chronic neutropaenia.

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is a rare, inherited, genetically heterogenous disorder with impaired intracellular killing of microorganisms due to ineffective respiratory burst. The disease is characterised by severe recurrent or persistant infections caused by catalaso-positive bacteria and fungi and diffused granulomata. The disease is inherited in X-linked or autosomal recessive patterns. X-linked inheritance occurs more often and is characterised by a more severe clinical course than the autosomal recessive form.

Our Polish Immunodeficiency Registry includes 41 CGD patients: 31 patients with a definitive diagnosis confirmed by an abnormal NBT slide test (less then 5%) and genetic analysis and 10 with probable diagnosis with characteristic medical history and abnormal NBT slide test without genetical confirmation, according to European Society of Immunology (ESID) criteria [27-31]. There are 22 cases of the X-linked form of CGD with the mutation in he *CYBB* gene, and 9 of autosomal recessive trait. In 3 cases a mutation in the *CYBA* gene was found and in 6 in *NCF 1*. Our observations confirm the fact that X-linked inheritance occurs more often than the autosomal recessive. We have only 4 CGD girls and the others are boys. In the prevention of bacterial and fungal infections in CGD patients antibiotic prophylaxis is used. All our patients are on trimethoprim/sulfamethoxazole and ketokonazol or itrakonazol prophylaxis and the reduction of the occurence of severe infections is noted.

HLA-identical BMT as the treatment was conducted only in one case, unfortunately with graft rejection. He also required interferon gamma as a prophylactic agent. Twenty-eight of our CGD patients are in a good condition, 11 of them are dead and 2 patients are lost to follow-up. Temporary therapy with interferon gamma had been provided in a few patients, only in one with a significant improvement [32]. Sucessful treatment of refractory autoimmune thrombocytopaenia with rituximab and cyclosporine A was observed in one patient [33]. Following ESID recommendations, we have modified and implemented the diagnostic and therapeutic criteria for CGD patients. Twenty-five years of experience in the therapy of fungal infections in these patients, especially of invasive Aspergillosis, has provided a good outcome due to multi-drug antifungal therapy with 40% of cases surviving [34].

Congenital asplenia

Congenital asplenia was rare in the group of phagocytic disorders – we diagnosed only 4 patients; it was usually connected with an inherited anomaly of the cardiovascular system.

Chediak-Higashi syndrome

Chediak-Higashi syndrome was recognised in only one patient, with the characteristic picture of giant lysosomal granules found in peripheral blood and in bone marrow smears. The boy died at the age of 6 years because of severe infections, without the chance for BMT at that time.

Griscelli syndrome type II

Griscelli syndrome type II, with typical features including partial albinism, immunodeficiency (absent cytotoxic activity of NK cells) and central nervous system involvement, was diagnosed in 2 girls from one family. The older died in the course of the second haemophagocytic exacerbation phase, before planned HSCT. The younger sister is still asymptomatic expecting HSCT procedure. The diagnosis was based on microscopic investigation of their hair and subsequently confirmed by genetic investigation in the Necker Hospital in France by Genevieve de Saint Basile.

Shwachman-Diamond syndrome

Shwachman-Diamond syndrome was diagnosed in a child suffering from chronic severe neutropaenia, recurrent respiratory tract infections, and a failure to thrive due to pancreatic insufficiency causing steatorrhoea and staturoponderal retardation. The ill boy is waiting for the results of a genetic investigation aimed at the SBDS gene mutation, provides by Professor Lashlo Marodi, Hungary, before considering HSCT as the only curative treatment, as SDS is connected with a high risk of malignant myeloid transformation (approximately 30%).

Other well-defined PID's

Immunodeficiencies associated with chromosomal instability

Combined immunodeficiencies associated with chromosomal instability were diagnosed in 152 children. Among them, the most frequent ataxia-telangiectasia, was recognised in 92 cases, and second was Nijmegen Breakage Syndrome in 57 children. Bloom's syndrome was diagnosed in only three children. It constitutes the largest group of patients with DNA repair disorders in the European registry.

Ataxia-telangiectasia syndrome

Ataxia-telangiectasia (AT) is a rare, autosomal recessive neurodegenerative disorder characterised by the progressive cerebellar ataxia, telangiectasies, and immunodeficiency.

Other signs of AT are chronic sinopulmonary infections, cancer predisposition, and hypersensitivity to ionising radiation. Lymphocytes show frequent chromosomal breaks, inversion and translocations involving sites of the T cell receptor genes, and immunoglobulin gene complexes in chromosomes 7 and 14 [35].

The responsible gene, *ATM* (**a**taxia **t**elangiectasia **m**utated) is located on the human chromosomal region 11q22-23 and was identified in 1995 by positional cloning. The ATM protein is a 370 kDa protein kinase that plays a central role in the recognition of double-strand breaks in DNA and activation of cell cycle checkpoints.

The genetic analysis was performed with the co-operation of Prof. Richard Gatti from the University of California in Los Angeles, USA. In 24 Polish AT families twenty-six distinct Short Tandem Repeat (STR) haplotypes were identified. The most frequent mutations observed were nonsense mutations, the next aberrant splicing, and missense mutations. Almost all our patients were compound heterozygotes, only two patients being found to be homozygous for mutations. It was possible, owing to our long-term co-operation with Prof. Gatti, to perform prenatal diagnosis in two cases [36-41].

The disease frequency has been estimated at 1/40 000-1/100 000 live births. But it is believed that many children with AT, particularly those who die at a young age, are never properly diagnosed. Therefore, this disease may actually be much more common. AT is presently incurable and unrelenting, and most AT children are dependent on wheelchairs by the age of ten. Later, AT patients usually die from respiratory failure or cancer in their teens or early twenties [42].

The immunodeficiency affects both humoral and cellular immune systems. The numbers and function of circulating T lymphocytes are generally diminished. We found that patients with AT had a severe deficiency of CD4+/CD45RA+ lymphocytes, a decreased number of CD4+ cells, and CD19+ lymphocytes reduced. The response of peripheral lymphocytes to phytohemaglutinin (PHA) is abnormal. Evaluation of humoral immunity revealed the following disorders: reduced or absent IgA, deficiency of subclass IgG (mainly IgG2 and IgG4), but in some patients, only a decreased IgG level. An elevated serum alpha-fetoprotein was found in 95 % of patients [43].

Nijmegen Breakage Syndrome

Nijmegen Breakage Syndrome (NBS) is a rare, autosomal recessive disorder characterised by primary microcephaly, typical face, growth retardation, mental retardation, radiation sensitivity, radioresistant DNA synthesis, cytogenetics aberrations (involving chromosomes 7 and 14), immunodeficiency, and increased incidence of cancer. The disorder was first described by Weemaes in 1981 in Nijmegen, in Netherlands [44]. Two years later Bernatowska and colleagues found two, and then four more patients [45,46]. Up to now most *NBS* patients are from Poland. The NBS gene is located at human chromosome 8q21, and encodes a protein called nibrin, which belongs to the family of cell cycle checkpoint proteins [47]. The gene for this disorder was isolated and a single mutation in exon 6(657del5) was found in 95% of European NBS patients, all of them of Slavic ancestry.

Most patients with NBS have recurrent respiratory infection. Recurrent bronchitis and pneumonia result in bronchiectasis, and premature death from respiratory failure. There is profound susceptibility to malignancy, predominantly of lymphoid origin. In 38% of our patients lymphomas were diagnosed [48]. Two of them have developed the same type of cancer twice. The propensity to malignancy is greater than that of AT. Immunoglobulin levels are low, and antibody responses are decreased. IgG subclass deficiency has been described. Some of the patients have an elevated IgM. The numbers of circulating T lymphocytes are generally diminished, and leucopaenia is often found with lympho-paenia. There is low blastic transformation of the lymphocytes in response to mitogens [49].

Guidelines for diagnosis and therapy of patients with ataxia-telangiectasia and Nijmegen breakage syndrome

Early diagnosis of AT is important for genetic counselling, appropriate care, and avoidance of unnecessary tests. The Department of Immunology at the Children's Memorial Health Institute has prepared a folder with brief short information about ataxia-telangiectasia and Nijmegen breakage syndrome, necessary for diagnosis of the diseases, and disseminated it among general practitioners, paediatricians, and medical doctors in Poland. We have also created criteria for the definitive, probable and possible diagnoses of AT and NBS based on clinical, laboratory features, cytogenetic testing and DNA analysis. Thses criteria are very helpful in differential diagnosis of AT and NBS.

Polish nation-wide meetings of parents with children suffering from ataxia-telangiectasia syndrome

Parents of children suffering from a serious disease feel very helpless, and if the disease is a rare one, like the syndrome of ataxia-telangiectasia, additionally often feel left alone with their problems. The Clinical Department of Immunology at the Children's Memorial Health Institute and the Association of Friends to Children with Immunological System Deficiencies have organised twice, in 2004 and 2005, meetings for parents and their children affected by AT syndrome. The parents were really grateful for the opportunity to participate in the meeting. They realized that they were not alone with their problems and that they had the possibility of learning more about their children's disease. At present the parents involved are establishing a Fund as well as their own website.

Bloom's syndrome

Bloom's syndrome is a rare autosomal recessive disorder, estimated at 1 in 160 000 births, characterised by low birth weight, growth retardation, a sun-sensitive erythematous skin lesion, immunodeficiency, and increased susceptibility to malignancies. The gene mutated in Bloom's syndrome is located at chromosome band 15q26.1, and encodes a nuclear protein similar to those in a helicase domain. We registered 3 patients with genetically confirmed Bloom's syndrome, of which one boy is on IVIG substitution therapy.

Wiskott-Aldrich Syndrome (WAS)

In the group of other well-defined PIDs, 19 boys with Wiskott-Aldrich syndrome were registered. In 5 of them mutation analysis confirming WASP gene mutation was performed by Professor JJ van Dongen from Erasmus University in Rotterdam. In three patients without typical triad of symptoms (thrombocytopaenia, infections and atopic-like dermatitis), genetic confirmation was essential for establishing the diagnosis. Three boys from this group are now after succesful haematopoietic stem cell transplantation. Despite the high risk of premature death due to infection, bleeding or malignancy, in four cases parents did not agree to the transplantation procedure, considering the high risk of this treatment.

Complement deficiencies

The genetic deficiencies of all complement components together with their inhibitors have been described in literature. Almost all of them are autosomal recessives, except C1 inhibitor deficiency, which is autosomal dominant, and properdin deficiency, which is X-linked. There is an increased susceptibility to SLE and SLE-like syndromes, and to recurrent pyogenic and neisserial infections in patients with deficiencies of particular complement deficiencies.

Complement deficiencies are relatively rare in our registry. Some of them with C1 inhibitor deficiencies are seen in the Department of Allergology, while those with SLE or SLE-like syndromes are in the Department of Rheumatology. Sixteen children, mostly with C1 inhibitor deficiency, associated with hereditary angioedema (HAE) or Quincke's disease have been described in a separate paper [50].

Six children were diagnosed as having classic pathway component defects, particularly C4 and C3. In one boy C3 and C4 deficiency were established together with a slight diminution of IgG3 and IgG4 subclasses. He had complained of recurrent severe chest infection and asthma. All patients with complement deficiency are more or less susceptible to infection and to the development of immune complex disease. In C3 defects *Haemophilus influenzae* and *Streptococcus pneumoniae* are often pathogens, while in C5–C9 defects *Neisseria* plays an important role as a cause of infection.

In patients with complement deficiencies, immunizations against *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* are recommended. Sometimes anti-microbial prophylaxis with antibiotic is required. The patient mentioned above with C4, C3, IgG3 and IgG4 deficiency is on IVIG therapy, with good results.

X-linked lypmhoproliferative disease (XLP)

XLP is a very rare genetic disease, leading to failure to handle EBV infection correctly. The defect is localised on chromosome Xq26. Patients are usually asymptomatic until EBV is encountered. Three outcomes are possible when infected with EBV: fulminant EBV, EBV infection and lymphoma, or immunodeficiency with usually profound hypogammaglobulinaemia. A diagnosis of XLP was made in 6 of our patients. Three of them came from one family. Two older brothers developed lymphomas, and one of them had a successful bone marrow transplant. The third underwent BMT, but due to GVHD localised in the liver and intestines, he died waiting for a liver transplant (LTX). A 17-year-old boy died due to lymphoma.

The next patients underwent LTX twice due to liver insufficiency.

Hyper IgE syndrome

Recurrent, mostly staphylococcal infections, inflammatory changes of the skin looking like atopic dermatitis, and an extremely elevated level of IgE in serum in children with a typical facial phenotype are representative symptoms of Hyper IgE syndrome. This rare PID of unknown genetic cause was diagnosed in 22 patients [51].

In the group of other well-defined PIDs we rarely observed such immunodeficiencies as **prepuberal periodonitis** and **Papillon-Lefevre syndrome**, respectively in 3 patients. Chronic peridontitis was a big problem in both diseases.

Autoimmune and immunodysregulation syndromes

This group of immunodeficiencies was separated recently in ESID qualification, containing three types of PID: ALPS, APECED and IPEX. In our centre we recognised one patient with ALPS and one patient with APECED (described above).

ALPS

Autoimmune lymphoproliferative syndrome (ALPS) is characterized by autoimmune features and lymphoproliferation, and is generally caused by defective Fas-mediated apoptosis.

The leading features of ALPS are: lymphadenopathy, splenomegaly, accumulation of nonmalignant CD4-CD8-TCRαβ+T cells, autoantibody production and autoimmune haemolytic anaemia. According to criteria used by the NIH Group, patients with ALPS must have defective Fas-mediated apoptosis compared with healthy controls. Fas is encoded by the gene TNFRSF6 located on chromosome 10q24.1.

In one of our patients with lymphadenopathy, splenomegaly and autoimmune haemolytic anaemia, the Fas mutation was found, thank to co-operation with Francoise Le Deist from the Necker Hopital in France.

Summary

During the past twenty-five years, the biggest number of Polish PID patients were diagnosed and treated in the Department of Immunology, CMHI. A national programme of substitutional replacement therapy for patients with primary immunodeficiencies from the whole of Poland has been introduced by the Department. Our experience in the field of PID has a good track record and hopefully will, with close cooperation on a national and international level, the basis for implementation of adequate diagnostic and treatment strategies throughout Poland and Europe.

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