

Pediatric Systemic Lupus Erythematosus (SLE) As an Onset of Common Variable Immunodeficiency (CVID): The Double Link between Immunodeficiency and Autoimmunity

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Abstract

Recurrent, unusual and complicated infections are the hallmark of immunodeficiency. Primary Immunodeficiency (PID) is caused by a genetic defect impairing the immune response. Common Variable Immunodeficiency (CVID) is classified as a primary immunodeficiency with prevalent antibody defect, manifesting with respiratory and gastrointestinal infections. However, CVID is characterized by an increased prevalence of autoimmune diseases too. Here, we describe a child presenting an abnormal pattern of infections and autoimmune phenomena, fulfilling diagnostic criteria for systemic lupus erythematosus (SLE). No classified PID could be demonstrated for several years, until she developed an antibody deficiency much later than the onset of the aforementioned clinical issues. Actually, a deficit of B memory cells, being useful to classify CVID after the diagnosis is made, was evident since the first immunological investigations performed before the onset of the deficit of serum immunoglobulin. This observation supports the vision of CVID as a disorder whose primary defect cannot be limited to B cell only. That can explain the variable clinical picture of CVID, including both infections and autoimmune disorders, which can arise even before serum immunoglobulin level is compromised.

Keywords: Autoimmunity; Immunodeficiency; Infections; Memory B cells

Introduction

Immunodeficiency (ID) is a medical condition deriving from the impairment of immune defenses against infectious agents and, then, increased susceptibility to infections is its hallmark. As concerns the etiology, IDs are grouped in two broad categories: i) secondary IDs, being the consequence of acquired factors, such as other diseases, malnutrition or pharmacological treatment, usually impairing more components of immune defenses; ii) primary IDs, deriving from a constitutional defect of the functioning of the innate and/or adaptive immune system. Numerically, the former group largely outgrows the latter, showing a global prevalence of at least 1:10,000 and manifesting mainly in the pediatric age. Depending on the specific genetic/constitutional immune defect, in primary IDs, the susceptibility to the several types of infectious agents can be differently broad and severe [1,2].

Normal children can be interested by recurrent respiratory infections and the suspicion of immunodeficiency can arise from the site, the severity and the infectious agents, which can also address toward the type of primary ID [3,4].

Primary IDs resulted to be associated to a greater incidence of allergic and/or autoimmune diseases too. The immune system plays its function of defense against non-self-agents through a complex network of cells and molecules interactions. Whenever there is a defect in one or more nodes of this network, the homeostasis of the whole

immune system can be altered, leading to autoimmunity, in addition to the unusual susceptibility to infections [5,6].

Autoimmunity is the emergence of auto-reactive lymphocytes and, whenever these auto-reactive clones escape the control of mechanisms of peripheral tolerance, variable tissue injuries and/or interferences in physiologic functions can be produced, leading to autoimmune diseases [7]. Autoimmunity can be associated also to the production of immunoglobulin's reacting with self-antigens, namely autoantibodies. In systemic lupus erythematosus (SLE), some autoantibodies are considered to play a pathogenetic role as well, through the deposition of immuno-complexes in several tissues. Indeed, SLE is a multi-systemic disease being able to damage many organs: in pediatric SLE, skin, blood and kidneys are the sites being involved most commonly [8-10].

The greater incidence of autoimmune diseases in primary IDs can be elicited by the susceptibility to infections itself, through their potential role in triggering autoimmunity, or it may be directly linked to the primitive immune defect itself and the consequent immune dysregulation. Indeed, some primary IDs present autoimmune manifestations as a prominent clinical issue (e.g. APECED, IPEX, ALPS, etc.); moreover, complement deficiencies and humoral immune defects (including common variable immune deficiency, CVID) resulted to be particularly prone to develop a series of autoimmune diseases [11]. Among those, SLE is one of the most frequent and is consistently associated to complement system immunodeficiency: a complete defect of one of precocious components of classic pathway of complement system (C1q, C1r, C1, S, C4, C2) is the strongest genetic risk factor for SLE [12,13].

Here, we describe a clinical case endowing infectious susceptibility and autoimmune phenomena, which existed in a diagnosis of primary ID only after a complex clinical history.

Case Report

A 6 years female child went to our attention in order to receive an immunological evaluation before undergoing a surgical procedure (Functional Endoscopic Sinus Surgery, FESS) to solve a severe form of chronic rhino-sinusitis, complicated with diffuse nasal polyps. Indeed, patient's personal history was characterized by recurrent and unusual infections. Although the pregnancy and the neonatal period were both regular, the patient developed a serious pneumonia at the 4th month of life, which was characterized by a diffuse and interstitial pattern, leading to a severe acute distress respiratory syndrome, despite the prompt administration of antibiotics (macrolide + 3rd generation cephalosporin). The infectious work-up was consistent with a primary cytomegalovirus (CMV) infection. Therefore, the infant received therapy with gancyclovir, achieving a progressive recovery. Moreover, oral candidiasis had been reported since the first year of life, recurring in the following years along with upper airways infections and aphthous stomatitis. These episodes became more and more frequent and the patient showed also intermittent diarrhea, which evolved to a chronic pattern sometimes. Therefore, even before coming to our attention, the child underwent to immunological investigations, but those did not unveil any substantial alteration, as regard blood cell count (including B, T and NK cells), serum immunoglobulin's and lymphocyte proliferation test. Despite that, at the age of 4-5 years, upper airways infections acquired the features of recurrent acute sinusitis, leading to severe and persistent nasal obstruction and chronic sinusitis, as CT confirmed. Moreover, medical therapy was not sufficient to control appropriately the sinus disease and the otolaryngologist indicated a surgical procedure, namely FESS, after an appropriate immunologic re-evaluation.

Our immunological work-up included: i) blood cell count, serum immunoglobulin and complement, lymphocyte immune-phenotype and lymphocyte proliferation test (Table 1 and 2); ii) allergic evaluation, through RAST (negative); iii) screening for cystic fibrosis, through sweat test and research of most common genetic defects (negative); iv) analysis of morphology and activity of respiratory cilia from nasal brushing (normal). At this time point, the child was in a good clinical condition and, at the physical exam, the most remarkable findings were the oral candidiasis, the ogival cleft, the diffuse adenopathy (cervical, axillary, inguinal) and the chronic cough associated to non-fixed respiratory sounds. Therefore, we added to the work-up a chest CT, showing some bronchiectasies in the left lung and some sub-pleuric nodules, as a consequence of previous inflammatory processes.

The surgical procedure was well tolerated. However, after around 1 week, the patient started complaining fatigue and general malaise accompanied by an increase of adenopathy and enlargement of spleen and liver. Blood cell count evidenced moderate anemia and further investigations suggested an ongoing immune-mediated hemolysis. During the following days, patient's clinical condition worsened, because of the appearance of fever, the progression of anemic status and the finding of pericardial fluid and interstitial pneumonia. Again, immunological investigations were repeated, without finding any significant alteration in serum immunoglobulin and blood lymphocytes levels. However, the autoimmunity work-up showed a clear positivity for anti-nucleus antibodies (ANA) and anti-native

DNA antibodies (ds-DNA). Moreover, urine examination showed mild proteinuria, which was constituted by albumin, but also by antibody light chains (both κ and λ), being oligo-clonal, as immune-fixation demonstrated.

Blood count	
RBCs	6,980.000/ μ l
Hb	13.4 g/dl
PLTs	301.000/ μ l
WBCs	12.170/ μ l
Lymphocytes	47.70%
Neutrophils	43.60%
Monocytes	6.70%
Eosinophil's	1.30%
Basophils	0.70%
Lymphocyte proliferation <i>in vitro</i>	
PHA	normal
PMA	normal
ConA	normal
Pockweed	normal
CD3	normal
Lymphocyte Immunephenotype	
CD3+	57% (3.889/ μ l)
-CD4+	17% (987/ μ l)
-CD8+	40% (2.322/ μ l)
-DR+	10% (581/ μ l)
-CD56+	8% (464/ μ l)
CD3-CD8+	2% (116/ μ l)
CD3-CD56+	13% (755/ μ l)
CD20+	13% (755/ μ l)
NBT test	normal
Serum Immunoglobulin	
IgM	152 mg/dl
IgG	1317 mg/dl
IgA	57 mg/dl
IgE	3 ng/ml

Table 1: First-level immunological work-up

Thus, the child was diagnosed as having systemic lupus erythematosus (SLE), based on the criteria of the American College of Rheumatology: i) history of persistence/recurrence of oral ulcers; ii) serositis (pericarditis); iii) immune-mediated cytopenia (hemolytic

anemia); iv) ANA positivity; v) high-titer anti-dsDNA positivity; v) malar rash. Thus, the patient received an appropriate course of steroid therapy, which solved completely the acute clinical picture, and a maintenance treatment with hidroxy-cloroquine and low-dose prednisone (5 mg/die) was started. After several months of relative wellbeing, the patient developed an intense febrile illness, which was not associated to any positive microbiologic or serologic infectious test and did not respond to anti-microbial therapy.

In vitro production of immunoglobulin(mcg/ml/10e6 CD19+)	
-IgM basal [stimulated]	36 (274)
-IgG basal [stimulated]	26 (47)
-IgA basal [stimulated]	1.4 (8.2)
Memory B cells (% CD19+)	
CD20+CD27-IgD+	94
CD20+CD27+IgD+	4.2
CD20+CD27+IgD-	1.7
Memory T CD4 cells (% CD4+)	
CD45RA+	80
CD45RA+/RO+	30
CD45RO+	50
Memory T CD8 cells (% CD8+)	
CD45RA+	93
CD45RA+/RO+	10
CD45RO+	17
Complement system function [test WIELISA]	
Classical pathway	normal
Alternative pathway	normal
Lectinic pathway	normal

Table 2: Second-level immunological work-up

Actually, the clinical progression and an appropriate laboratory monitoring allowed us to evidence the characteristics of macrophage activation syndrome (MAS), which was successfully treated according to the current protocols. After this episode, the patient re-started the maintenance therapy and received a careful follow-up, including periodical immunological investigations. Indeed, she received also a complete study of complement function, which resulted normal. During the follow-up, the child (aging 8 years at that time) developed persistent hypo-gammaglobulinemia, fulfilling the diagnostic criteria of Common Variable Immunodeficiency (CVID): i) reduced IgG and IgA levels, in presence of normal or slightly decreased values of IgM; ii) reduced ability to develop specific antibodies after active immunization (tetanus, diphtheria and pertussis). Intra-venous replacement immunoglobulin therapy was instituted, in addition to maintenance therapy for SLE.

Discussion

Here, we described a patient where infections and autoimmune phenomena resulted to be embedded in a very complex clinical picture. Since her first years of life, the child showed a striking predisposition to develop infectious illnesses, which were mostly located in the respiratory system. They never reached aspects of great severity, such as meningitis/encephalitis or sepsis, but the frequent involvement of lungs (including CMV pneumonia), the occurrence of oral candidiasis after the first year of life, the recurrent/chronic diarrhea and, finally, the development of chronic sinusitis with extensive nasal polyps resulted to be quite concerning, of course. However, despite all the above clinical issues, no classified primary ID could have been demonstrated, through several and repeated immunologic investigations. According to clinical criteria (e.g. warning signs or red flags, edited by Jeffrey Modell Foundation), a condition of immunodeficiency was very likely and other diseases, resembling ID clinically (such as mucoviscidosis and primary ciliary dyskinesia), have been ruled out as well.

A secondary ID was considered in the differential diagnosis, because of the onset of autoimmune manifestations consistent with a diagnosis of SLE. Patients affected with SLE can develop symptoms of immunodeficiency, which is often due to drug therapy. However, several functional defects have been described apart from the ongoing treatment, namely reduced phagocytic activity, lymphopenia/neutropenia, altered T-cell activation and reduction of NK cells, by instance [14,15]. Therefore, SLE is associated to a greater occurrence of infections, which represent a major cause of morbidity and mortality and can promote the occurrence of a complication known as macrophage activation syndrome (MAS), which was manifested by our patient too [16,17]. Actually, the diagnosis of SLE was made at 7-8 years of age, which is much later than the onset of the infectious burden, being recorded since the first year of life. Thus, SLE and related therapy (including steroids) could not justify the whole clinical history, in light of current knowledge.

Another interesting aspect was the precocious CMV infection: it rarely causes symptomatic and severe disease, such as pneumonia, in the immune-competent host.18 Congenital CMV infection was ruled out and, thus, the patient was supposed to have developed a post-natal infection. Precocious CMV infection can associate to cellular immunodeficiency [18], but the patient did not show a severe combined immunodeficiency (SCID) and no significant abnormalities have been found through the analysis of lymphocyte phenotype and in vitro proliferation. Conversely, it is known that herpes viruses, including CMV, interact with immune system in a complex manner: several immune cells (including monocytes, macrophages and dendritic cells) represent an important viral target and site of latency; moreover, CMV genome has been showed to encode also several products, including analogues of cytokines, which can interfere with the immune responses. Although CMV is known to induce unbalances of T cells (especially in term of CD4/CD8 ratio) and hypo-responsiveness to mitogens and antigens in lymphocyte during the early phases of the infection, actually no long-term immune-dysregulation could be demonstrated. However, some evidences are emerging, that CMV might alter the homeostasis of the immune system somehow, but that does not seem to be enough in order to provide a sufficient explanation of the complexity of the clinical history we reported, based on the current knowledge [19,20]. Thus, the hypothesis of a secondary ID (because of infections or rheumatic diseases or drugs) is not sufficiently supported in this clinical case.

Several primary IDs have been consistently associated to the development of SLE, such as some complete defects of one of precocious components of the classic pathway of complement system, complete IgA deficiency and chronic granulomatous disease. By instance, C1q, C4, C1r-s and C2 deficits have an occurrence of SLE equal to 93%, 75%, 57% and 25%, respectively. An altered clearance of immune complexes has been supposed as a main pathologic mechanism, although a direct role of complement system in the lymphocyte immune tolerance (through the elimination of auto-reactive lymphocytes) emerged from experimental animal models [21,22]. Moreover, many other primary IDs are less frequently reported to develop SLE, including several antibody defects (IgG subclass deficiency, hyper-IgE syndrome, hyper-IgM syndrome and CVID) [12].

A complement deficiency was excluded, through a complete analysis of all components of complement system. Moreover, antibody deficiency was excluded many times during infancy and childhood, until she developed a significant (IgA + IgG) hypogammaglobulinemia, when she was around 9 years. Which was later than onset of both the infectious issue and autoimmune phenomena? Only at this time, the immunological features fulfilled the criteria for a diagnosis of CVID.

In addition to the respiratory and gastro-intestinal recurrent infections (and also an increased risk of malignancy), CVID is characterized by a significant prevalence of autoimmune manifestations: 25-30% cases usually develop autoimmunity during the course of the disease and many are immune-mediated cytopenias (anemia and thrombocytopenia) [23].

Several functional and phenotypic abnormalities of lymphocytes have been described in the setting of CVID, which are not limited to B cells, but involve T cells, monocyte-macrophages and antigen-presenting cells too. All these observations supported a vision that CVID is a heterogeneous group of diseases, deriving from different pathogenetic defects leading an impaired production of antibodies, in addition to other and variable clinical manifestations. This statement is supported by the genetic of CVID, which resulted to be very variable and linked to a complex (and largely unknown) multi-factorial causal pattern. Indeed, several molecular mutations (at least five) have been found in the small part (around 10% cases) of CVID where a genetic aberration could have been demonstrated [24-26].

Among multiple immune abnormalities recorded in CVID, special attention should be paid to the development of memory B cells. A study published in 2002 by Warnatz et al., who had initially observed a memory deficit in patients affected with X-linked hyper-IgM syndrome [27]. This and other works led to so-called "Freiburg classification" of CVID patients, based on the phenotype of CD27+ memory B cells, which are divided in two main types: i) IgD+CD27+ "unswitched" memory B cells, producing IgM almost exclusively; ii) IgD-CD27+ "switched" memory B cells, being able to produce IgG and IgA, in addition to IgM [28]. Recently, thanks to a multi-centric study (EURO-Trial), considering more than 300 CVID patients, this classification was refined. First, CVID patients were divided according to the number of peripheral B cells: <1% (B- patients) or >1% (B+ patients). The latter group of patients showed a deficit in the memory compartment, being more or less severe. SmB+ patients conserved >2% of switched memory B cells out of the total CD19+ B cells, whereas SmB- patients have a number of switched memory B cells <2% [29].

The child showed a reduced B cell count (4% of peripheral lymphocytes) and, of these, a percentage <2% was constituted by switched memory B cells. Therefore, according to EURO-trial classification, the patient was in B+SmB-. Interestingly, this group was characterized by a greater prevalence of non-infectious complications, such as diffuse adenopathy, splenomegaly, granulomatous lesions and autoimmunity [29-31].

Therefore, CVID patients have been classified into subgroups according to their B cell count and their memory B cell profile and, finally, based on further B cell sub-population, such as activated B cells (IgD+IgM+CD27-CD21^{low}CD38^{low}) or transitional B cells (IgD+IgM+CD27-CD21^{+/low}CD38^{high}) [32].

In reference to the phenotype analysis of lymphocytes performed after CVID diagnosis, the patient showed a percentage of CD21^{low} B cells greater than 30%. An expansion of CD21^{low} B cell population (>10% of circulating B cells) has been associated to the presence of spleen enlargement and autoimmune phenomena [29,33].

However, in our patient, it is outstanding that the abnormalities of B memory and, particularly, the deficit of switched memory B cells, had been noticed before the development of hypogammaglobulinemia. This finding prompted two observations.

First, it might be speculated that antibody deficiency could have been the final step of a "deteriorating" process of the immune system and that some immune abnormalities, namely B memory deficit (used to classify CVID), could come up earlier. Thus, it might be useful to look for these immune aberrations in those clinical settings, characterized by increased susceptibility to infections and autoimmune phenomena, which cannot be diagnosed as a specific rheumatic disease or a well-defined primary ID.

Second, patients suffering with CVID might have a personal history of recurrent infections even before the development of a quantitative antibody deficiency, which is the hallmark of CVID, according to current diagnostic criteria. Here, it seems that a deficit of memory B-cell function, which is a fundamental component of the humoral defense, might arise before the hypogammaglobulinemia. Such an observation might provide a valuable explanation why the patient developed a clinical phenotype of recurrent/chronic respiratory infections and enteropathy, even though no quantitative humoral deficiency had been evidenced yet. It might be that the production of antibody was not qualitatively effective (because of the memory impairment); or that the B cell unbalance could reflect an impairment of other immune components, maybe a deficit in T cells helping the full development of B cell response.

In fact, the pathogenetic defects in CVID, which is considered an antibody-related primary ID, could actually be inside the T lymphocyte rather than inside B cell. By instance, among few known CVID-related genetic defects, one involved ICOS molecule, which is expressed on T lymphocyte [34]. Thus, CVID could theoretically derive from a primary defect in any component of the immune system (T lymphocytes and APCs) supporting B cell proliferation, isotype switching and memory development. Such a vision might explain those cases where recurrent infections, severe complications such as MAS and the development of autoimmunity and lymphoproliferative phenomena have occurred before or independently from the presence of quantitative antibody deficiency.

In conclusion, unusual and recurrent infections may precede the occurrence of a quantitative deficit of antibody in CVID as well as

some autoimmune phenomena do, which are known to be associated to some primary immune-deficiencies [35]. Moreover, we speculate that the B memory defect, considered useful in the classification of CVID patients (once such a diagnosis has been made) may be evidenced before the development of serum immunoglobulin deficiency. In this clinical setting, a B memory defect might alert for a potential evolution to CVID in those patients where infections and autoimmunity cannot be clearly diagnosed as specific rheumatic disease or primary immunodeficiency, in light of current diagnostic criteria.

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