



Primary immunodeficiencies (PIDs) presenting with cytopenias

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Autoimmune manifestations are increasingly being recognized as a component of several forms of primary immunodeficiencies (PID). Defects in purging of self-reactive T and B cells, impaired Fas-mediated apoptosis, abnormalities in development and/or function of regulatory T cells, and persistence of immune activation as a result of inability to clear infections have been shown to account for this association. Among autoimmune manifestations in patients with PID, cytopenias are particularly common. Up to 80% of patients with autoimmune lymphoproliferative syndrome (ALPS) have autoantibodies, and autoimmune hemolytic anemia and immune thrombocytopenia have been reported in 23% and 51% of ALPS patients, and may even mark the onset of the disease. ALPS-associated cytopenias are often refractory to conventional treatment and represent a therapeutic challenge. Autoimmune manifestations occur in 22% to 48% of patients with common variable immunodeficiencies (CVIDs), and are more frequent among CVID patients with splenomegaly and granulomatous disease. Finally, autoimmune cytopenias have been reported also in patients with combined immunodeficiency. In particular, autoimmune hemolytic anemia is very common among infants with nucleoside phosphorylase deficiency. While immune suppression may be beneficial in these cases, full resolution of the autoimmune manifestations ultimately depends on immune reconstitution, which is typically provided by hematopoietic cell transplantation.

Although primary immunodeficiencies (PIDs) are typically marked by increased susceptibility to infections, autoimmune manifestations are increasingly being recognized as an important component of several forms of PID.^{1,2} Various mechanisms have been implied in the pathophysiology of autoimmune manifestations in patients with PID, including defective negative selection of autoreactive T lymphocytes in the thymus, defects in the number and/or function of regulatory T cells, impaired apoptosis of autoreactive lymphocytes, breakage of tolerance due to increased load or decreased clearance of apoptotic cells and pathogens, and increased homeostatic lymphoid proliferation and cytokine secretion associated with lymphopenia (reviewed in Liston et al,³ Oliveira and Gupta,⁴ Westerberg et al,⁵ and Goyal et al⁶).

Here we will briefly discuss three forms of PIDs in which autoimmune cytopenias are particularly common: autoimmune lymphoproliferative syndrome (ALPS), common variable immune deficiency (CVID) and selected forms of combined immunodeficiencies characterized by “leaky” defects in T cell development. While autoimmune manifestations are not typically part of the clinical features observed in patients with severe combined immunodeficiency (SCID), cytopenias have been described in some forms. Autoimmune cytopenias are very common also in patients with the Wiskott-Aldrich syndrome and with hyper-IgM syndromes, as discussed in a series of excellent reviews.⁷⁻¹¹

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Autoimmune Lymphoproliferative Syndrome

ALPS includes a genetically heterogeneous group of disorders characterized by lymphadenopathy, splenomegaly, autoimmune cytopenias, and increased occurrence of lymphoid malignancies associated with defective apoptosis.¹² Most often ALPS is due to genetic defects that affect the apoptosis extrinsic pathway, such as mutations in CD95 (*TNFRSF6*),¹³ or more rarely in CD95L (*TNFSF6*),¹⁴ caspase 8,¹⁵ or caspase 10.¹⁶ These defects can be inherited as autosomal-dominant or autosomal-recessive traits; however, somatic mutations in CD95 have been also reported,¹⁷ providing evidence that impairment in Fas-mediated apoptosis confers a selective advantage to mutated cells that tend to accumulate with time. In addition to these ALPS-causing genetic defects, a significant number of patients with clinical and immunological features of ALPS carry no known gene defects. Some of them show impaired Fas-mediated apoptosis,¹⁸ but others do not, suggesting that

defects in other apoptosis-triggering pathways may also be involved. Indeed, one patient with an activating mutation in the *NRAS* gene, leading to a defect in IL-2 starvation-induced apoptosis, has been described.¹⁹

A characteristic feature of ALPS patients is a high percentage of circulating TCR $\alpha\beta^+$ CD4 $^-$ CD8 $^-$ double-negative (DN) T cells^{20,21} that express a restricted T-cell receptor (TCR) repertoire that may possibly recognize self-antigens.²² It has been hypothesized that these DN T cells represent T lymphocytes targeted to Fas-mediated apoptosis.^{20,21} Overall, an increased proportion of DN T cells and elevated plasma levels of IL-10 and FAS-L represent useful biological markers of ALPS.²³

Up to 80% of patients with ALPS have detectable autoantibodies, most commonly anticardiolipin or direct Coombs antibodies.²⁴ Autoimmune hemolytic anemia and thrombocytopenia have been observed in 23% to 51% of the patients in different series, and autoimmune neutropenia is also common (19%-27%).²⁴⁻²⁶ Importantly, autoimmune cytopenias may mark the clinical onset of the disease, even in the absence of signs of lymphoproliferation. Accordingly, patients with Evans syndrome should always be evaluated for ALPS.^{27,28}

The autoimmune cytopenias of ALPS are often severe and refractory to treatment. For these reasons, splenectomy has frequently been used in the past in the management of ALPS; however, this approach carries significant risks of sepsis. Successful results have been reported with mycophenolate mofetil (MMF) and sirolimus.^{29,30} Use of rituximab, with the intent to ablate autoreactive B cells, has been associated with neutropenia and persistent hypogammaglobulinemia.²⁹ A management plan has been recently proposed²⁹ that includes use of oral prednisone, followed by intravenous methylprednisone if no response is observed. MMF has been proposed as the first-line drug for breakthrough cytopenias, with the possible use of alternative immunosuppressive drugs, and eventually rituximab, in patients who do not respond to MMF. Splenectomy should be reserved for the most severe cases.

Autoimmune Cytopenias in CVID

CVID includes a heterogeneous group of conditions characterized by reduced levels of serum immunoglobulins and primary antibody failure.³¹ Genetic defects in TAC1 (*TNFRST13B*), ICOS, BAFF-R and CD19 account only for a minority (15%-20%) of cases of CVID; the molecular pathophysiology of the remaining cases remains undefined and could be polygenic. Increased susceptibility to bacterial infections is a hallmark of CVID and was a prominent cause of death until immunoglobulin substitu-

tion therapy, antibiotic prophylaxis and prompt treatment of infections came into clinical practice, permitting prolonged survival and a better definition of the incidence of non-infectious complications in CVID.³²

Autoimmune manifestations occur in a substantial proportion of patients with CVID, ranging from about 22% to 48% in different series from different countries.³²⁻³⁶ They are more frequent among patients with granuloma, with up to 50% of these patients suffering from autoimmune manifestations. Although a higher prevalence of autoimmunity among females with CVID has been reported by some studies,³⁷ this notion has been challenged by more recent observations.³⁸

Among autoimmune manifestations, cytopenias are particularly common and (especially in the case of immune thrombocytopenic purpura [ITP] or autoimmune hemolytic anemia) may mark the onset of the disease.³³ In a series of 326 patients, the prevalence of hematological autoimmune manifestations was 11%, ITP being the most frequent cytopenia.⁵ A multicenter retrospective study in France involving 105 patients with CVID described an even higher prevalence of ITP (20%),³⁹ whereas a lower frequency of autoimmune hemolytic anemia and thrombocytopenia (4% and 7%, respectively) has recently been reported in a large European collaborative study.³² Overall, the prevalence of autoimmune cytopenias among patients with CVID is 100- to 1000-fold higher than in the general population. The occurrence of autoimmune cytopenias in CVID does not correlate with organ-specific autoimmunity, but tends to correlate with splenomegaly.³²

A reduced number of CD4 $^+$ CD25 $^{\text{hi}}$ Foxp3 $^+$ cells, associated with lower levels of Foxp3 expression, has been recently reported in patients with CVID with autoimmune manifestations.^{40,41} Autoimmune cytopenias are particularly common among patients with CVID with reduced numbers of switched memory B cells and an increased proportion of CD19 $^{\text{hi}}$ CD21 $^{\text{lo}}$ lymphocytes.⁴² The presence of TAC1 mutations represents another risk factor for autoimmune cytopenias.^{31,43} Increased serum levels of BAFF and APRIL have been observed in patients with CVID⁴⁴ and could support expansion and antibody secretion by autoreactive B-cell clones. Lower proportions of circulating CD8 $^+$ lymphocytes were also found to be associated with autoimmunity.³²

Treatment of autoimmune cytopenias in CVID is usually based on steroids.³¹ Data on the use of rituximab remain anecdotal.³⁵ In the absence of data on the efficacy and safety of splenectomy in patients with CVID, this approach should be reserved to patients with autoimmune cytopenias that are refractory to medical management.

Autoimmune Cytopenias in Combined Immunodeficiencies

Human severe combined immune deficiency (SCID) comprises a heterogeneous group of genetically determined disorders that are characterized by the virtual absence of T cells and severe numerical and/or functional B-cell deficiency. Therefore, as a rule autoimmune manifestations are not part of the clinical features observed in patients with SCID. However, genetic defects that severely compromise, but do not abrogate, T- and B-cell function may also result in an increased occurrence of autoimmunity, including cytopenias.^{45,46} The molecular and cellular mechanisms that account for autoimmunity in “leaky” forms of SCID are the focus of a series of recent studies. B-cell hyper-reactivity, with aberrant T cell–mediated immune regulation, may play a role. Additional mechanisms may include impaired lymphostromal cross-talk in the thymus, with defective expression of autoimmune regulator (AIRE) protein and inadequate induction of Treg cells.⁴⁷ Furthermore, some forms of SCID reflect genetic defects that affect V(D)J recombination. Hypomorphic mutations in these genes may allow for residual T- and B-cell development, associated with impaired receptor editing, thus leading to accumulation of autoreactive B cells.⁴⁸ Although autoimmunity may complicate virtually any form of “leaky” SCID, it is particularly common in some of these disorders.

Deficiency of purine nucleoside phosphorylase (PNP) in humans causes accumulation of deoxyguanosine triphosphate (dGTP) that is toxic to lymphocytes, resulting in a progressive and severe decline in the number and function of T lymphocytes, whereas in most patients B cells are marginally affected.⁴⁹ Apart from increased susceptibility to infections, typical clinical features of PNP deficiency include progressive neurological deterioration and autoimmunity. Approximately one third of the patients develop autoimmune manifestations, which can also be the presenting feature. Autoimmune hemolytic anemia is particularly common, but ITP and neutropenia have been also reported.⁵⁰⁻⁵² These complications have been attributed to B-cell hyper-reactivity resulting from a loss of T-cell regulation.

Autoimmune cytopenias have also been reported in several patients with delayed- and adult-onset adenosine deaminase (ADA) deficiency in whom residual ADA activity is present.^{53,54} We have observed several cases of autoimmune hemolytic anemia in patients with complete ADA deficiency, upon initiation of enzyme replacement therapy (Notarangelo, unpublished observation).

Transient autoimmune thrombocytopenia has been reported in patients with partial DiGeorge anomaly,^{55,56} and autoimmune cytopenias represent one of the most common

complications early after thymus transplantation for DiGeorge syndrome.⁵⁷

Autoimmune cytopenia has been also described in patients with hypomorphic mutations in genes involved in V(D)J recombination, including the *RAG1* and *RAG2* genes,⁵⁸⁻⁶⁰ as well as *DCLRE1C* (Artemis),⁶¹ *NHEJ1* (Cernunnos),⁶² and *LIG4/3* genes, that encode for essential components of the non-homologous DNA end-joining pathway. Although steroids are often effective in the treatment of autoimmune manifestations in patients with combined immune deficiency, complete and definitive resolution of these complications depends on successful immune reconstitution by means of hematopoietic cell transplantation.

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