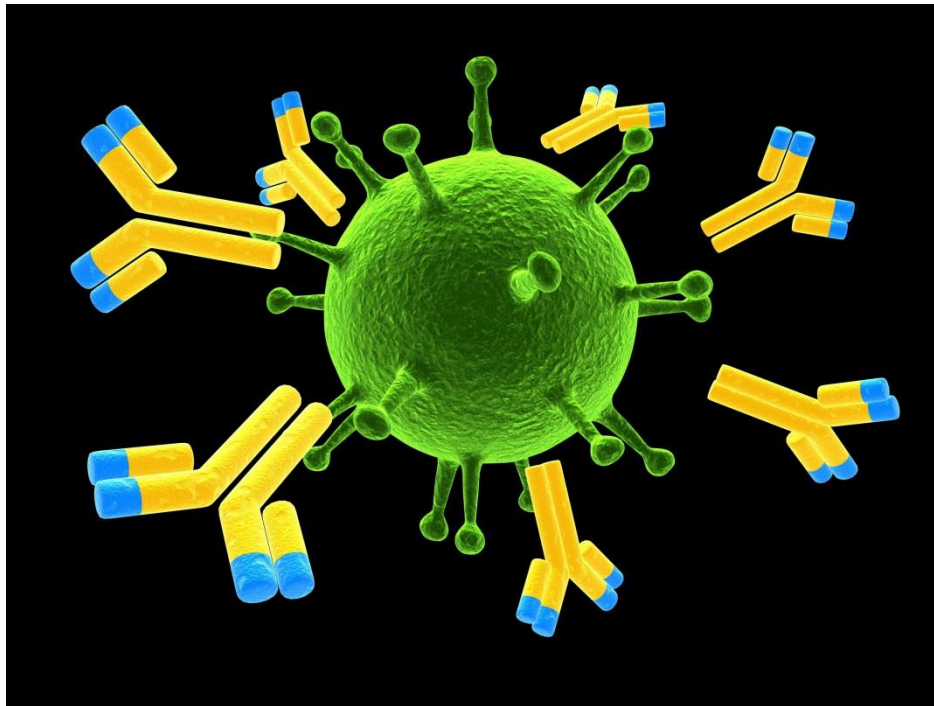


# **Non-infectious manifestations of Common Variable Immunodeficiency: The paradox of **autoimmunity** in the setting of **immunodeficiency****



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This is to acknowledge that Dr. Wysocki has disclosed that he does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Wysocki will not be discussing off-label uses in his presentation.

## **Purpose and overview:**

The purpose of this presentation is to highlight the somewhat paradoxical autoimmune, inflammatory and lymphoproliferative diseases which often occur in patients with Common Variable Immunodeficiency (CVID), their clinical presentation, and treatment. These conditions appear to be the main causes of early mortality in CVID patients, and are frequently the presenting features of the disease. The co-existence of autoimmunity with immunodeficiency is seen in other defined genetic syndromes, and some relevant examples will be discussed. This will frame a discussion on recent findings by several groups attempting to understand the genetics of CVID, a disease assumed to be polygenic in nature, and how these findings may illuminate the etiologies of autoimmune and inflammatory complications of CVID.

## **Educational objectives:**

1. Understand the diagnostic criteria and prevalence of CVID and how it is distinguished from other primary antibody deficiencies.
2. Understand the predictors of early mortality in CVID
3. Recognize the clinical and histopathologic features of the autoimmune and inflammatory complications of CVID
4. Recognize that CVID should be considered on the differential diagnosis for conditions such as autoimmune cytopenias, interstitial lung diseases, inflammatory enteropathies, and nodular regenerative hyperplasia of the liver
5. Understand the current standards in managing these complications

## **About the author:**

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## **Autoimmune and Inflammatory Complications of Common Variable Immunodeficiency (CVID)**

CVID is the most common clinically relevant primary immunodeficiency, with prevalence estimated at 1:25,000. Onset is typically in the third to fourth decades of life, and there are frequently delays in diagnosis, averaging 4-7 years<sup>1,2</sup>. Familial inheritance of CVID is the exception rather than the rule, occurring in 10-20% of cases<sup>2</sup>.

CVID is a primary humoral immunodeficiency. Other examples of primary humoral immunodeficiencies would include X-linked and autosomal recessive forms of primary agammaglobulinemia, such as X-linked agammaglobulinemia (XLA). In these rare diseases, mutations occur in genes critical to early B cell development in the bone marrow. The result is a complete absence of mature B cells in the periphery and a resultant lack of production of any antibody isotype. Patients are left susceptible to various bacterial and viral infections which require antibodies for effective opsonization or neutralization<sup>3</sup>. Other single-gene primary humoral immunodeficiencies include the Hyper-IgM syndromes, which result from X-linked or autosomal recessive mutations in genes encoding proteins essential to B cell maturation events which occur in the periphery, in germinal centers, including class-switch recombination, memory formation and somatic hypermutation<sup>4</sup>. In these diseases, B cells are often normal in number in peripheral blood, but due to the inability to class-switch and progress through later stages of B cell maturation, levels of IgG, A and E are very low, and specific antibody responses to pathogens and vaccines, are ineffective. The genetic etiologies of CVID are not well understood, although as we'll discuss, we are learning more. In most CVID patients, impairment in later B cell maturation can be seen, including low proportions of class switched B cells, and poor memory B cell development<sup>5,6</sup>. Total B cell numbers in most patients are normal or slightly reduced.

Diagnostic criteria (ESID/PAGID)<sup>7</sup>:

Age >4

- Low **total IgG** (2 SD below mean for age)
- AND Low **total IgA** and/or **IgM**
- AND **abnormal responses to vaccines**
- Exclusion of "defined causes" of hypogammaglobulinemia

CVID is frequently complicated by a wide range of autoimmune and inflammatory conditions, including antibody-mediated autoimmunity (cytopenias and organ-specific disease), inflammatory enteropathy, granulomatous lymphocytic interstitial lung disease (GLILD), and liver disease, as well as lymphomas, which significantly shorten survival<sup>8-10</sup>. We will discuss the clinical features of these complications, and current treatment strategies. The mechanisms through which the autoimmune and inflammatory complications of CVID develop are not clear,

but there has been a great deal of work on this in recent years, which we will attempt to summarize. A number of recent gene discovery studies have identified mutations in CVID patients with autoimmune, inflammatory and lymphoproliferative phenotypes, with autosomal dominant inheritance patterns, sometimes found as sporadic mutations. The penetrance of disease is often incomplete, arguing that these may be disease modifying genes, predisposing to autoimmune/inflammatory/lymphoproliferative disease but requiring additional genetic or environmental insults for full manifestation of the above phenotypes. Potential treatment strategies are already emerging as a result of these studies, and more findings similar to these are undoubtedly coming.

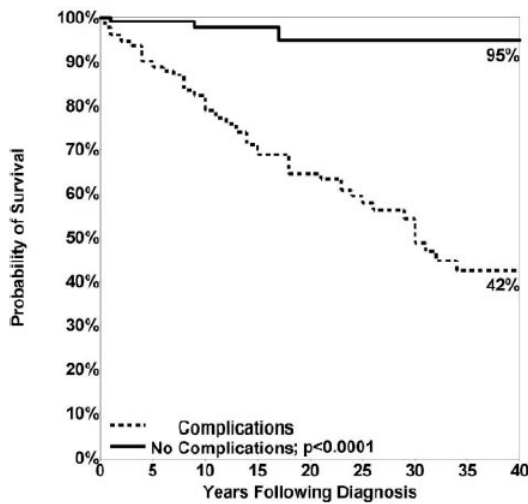


Figure 1. Mortality in CVID patients with "infections-only" (no complications) versus those with autoimmune and inflammatory complications<sup>8</sup>.

	HR	95% CI	P
Any complication	10.96	(3.46, 34.69)	< .0001*
Gastrointestinal disease	2.78	(1.44, 3.59)	.0004*
Liver disease/hepatitis	2.48	(1.51, 4.09)	.0003*
Lymphoma	2.44	(1.43, 4.16)	.0010*
Chronic lung disease (structural and functional)	2.06	(1.34, 3.16)	.0010*
Malabsorption	2.06	(1.11, 3.81)	.0218*
Splenectomy	1.69	(0.91, 3.12)	.0957
Cancer	1.51	(0.795, 2.87)	.2084
Autoimmunity	1.36	(0.87, 2.12)	.1735
Granuloma	1.27	(0.65, 2.48)	.4939
Bronchiectasis	0.76	(0.39, 1.48)	.4235

Figure 2. Non-infectious complications of CVID and risk of mortality<sup>8</sup>.

## Specific Autoimmune and Inflammatory Complications

### Autoimmune Cytopenias

Autoimmune disease in CVID occurs at a higher incidence than that in the general population <sup>2</sup>. Various systems are affected but autoimmune cytopenias are the most common. In a retrospective chart review of 326 patients with CVID, 35 patients (11%) had a history of autoimmune hematologic disease. Fifteen had immune thrombocytopenic purpura, 9 had autoimmune hemolytic anemia, and 11 had Evans Syndrome. Interestingly, fifty-four percent of patients had the first episode prior to the diagnosis of CVID, and 32% were diagnosed concurrently at the time of CVID diagnosis, arguing that **autoimmune cytopenias are frequently among the presenting features of CVID**. Only 14% developed autoimmune cytopenias after diagnosis <sup>11</sup>, prompting speculation that immunoglobulin replacement therapy may decrease the occurrence of AIHA/ITP.

Immune cytopenias associated with CVID are treated similarly to those in patients without CVID, although immunosuppressive therapies in these already immunocompromised patients increase infectious risks. Therapies include systemic corticosteroids, high-dose intravenous immunoglobulin (1 mg/kg), anti-rhesus D Ig, and sometimes additional immunomodulators in refractory patients. In one study of patients with hematologic autoimmunity, institution of IVIG was correlated with fewer reoccurrences of hematologic disease <sup>12</sup>. In a review of 21 patients with CVID and ITP, 13 patients received corticosteroids alone and 54% achieved at least a partial response. 8 patients received IVIG at 1-2 g/kg alone or in combination with steroids with a short-term response rate of 50%. Therefore, corticosteroids, either alone or in conjunction with IVIG are helpful in a proportion of CVID patients, but these patients are frequently refractory to first-line therapies.

Classically, patients refractory to first line therapies required splenectomy, which in immunocompetent patients generally provides long lasting remission of ITP<sup>13</sup>. Splenectomy can be considered in CVID patients who fail first line therapies. There has been debate about its safety in CVID patients. In the largest reported retrospective analysis of 45 European CVID patients who'd had splenectomy, 80% had the procedure for refractory autoimmune cytopenias, and 75% of these responded. A 2.47% annual rate of overwhelming post-splenectomy infections (OPSI) was observed, which was higher than that of splenectomized patients without CVID. This did not, however, appear to translate into an increased mortality rate as compared with non-splenectomized CVID patients with autoimmune complications<sup>12</sup>. In another series 10% of splenectomized patients had pneumococcal sepsis and 16% had postoperative complications<sup>2,14</sup>.

Since the early 2000s, B cell depletion with Rituximab has become a prevalent and efficacious therapy for ITP in immunocompetent patients who are either refractory to splenectomy, are poor candidates for splenectomy, or prefer to avoid this surgery<sup>15</sup>. As B cells in CVID patients are dysfunctional, and per standard of care, CVID patients are generally on immunoglobulin replacement therapy, B cell depletion with Rituximab was felt a reasonable option, particularly given concerns about the safety of splenectomy and broader cellular immunosuppressants in these patients. In the largest retrospective analysis of 33 patients at multiple institutions, treated with Rituximab for CVID-associated cytopenias, the overall initial

response rate was 85% including 74% complete responses. 59% of initial responders had durable responses. Of 10 relapsing patients, 9 were retreated and 7/9 responded to re-treatment. Severe infections after rituximab occurred in 8 adults (24%). Notably, four of these patients were not on IVIG, and one of the four had a prior splenectomy. The rate of serious infections in this study was overall higher than seen in safety studies in Rheumatologic disease and lymphoma, which range from 7-20%<sup>16,17</sup>, but with typical prophylaxis infection may have been prevented in half of these patients.

Other treatments given as second and third line included azathioprine, danazol, cyclosporine, cyclophosphamide, dapsone, and vincristine although they were associated with higher rates of adverse effects. Cyclosporine has not been effective in resolving the cytopenias<sup>14,18,19</sup>.

### Inflammatory Enteropathy

Patients with CVID suffer non-infectious gastrointestinal disease in 9-15% of cases, and inflammatory GI disease and malabsorption have been shown to increase mortality in CVID<sup>1,8</sup>. A number of studies have characterized the clinical and histopathologic aspects of the non-infectious enteropathy associated with CVID<sup>20-27</sup>. The clinical findings most commonly reported in CVID- enteropathy are chronic diarrhea, malabsorption, dyspepsia, abdominal pain, wasting/low BMI and anemia<sup>22,24</sup>. It is important to note that a number of studies have highlighted the association between the occurrence of enteropathy in CVID and other autoimmune and inflammatory complications such as autoimmune hemolytic anemia and or thrombocytopenia, neutropenia, arthritis, thyroiditis, pernicious anemia, and numerous others<sup>1,21,24,28</sup>. In fact the rate of occurrence of autoimmunity in the subset of CVID patients with enteropathy in Freiburg, Germany was noted to be higher than in their overall CVID cohort, arguing that this is a sub-population of CVID patients at high risk<sup>28</sup>.

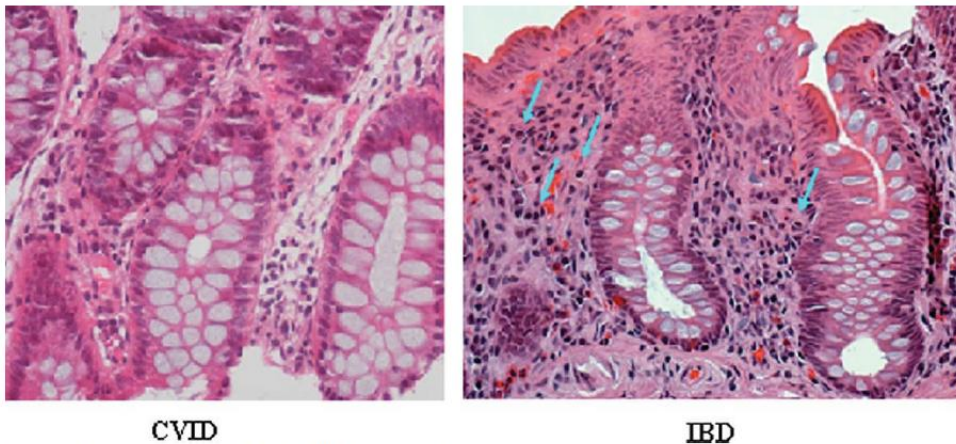


FIGURE 5. Intestinal histology (H&E 40×). CVID intestinal biopsies lack intestinal plasma cells. This is in contrast to specimens collected from IBD patients as shown by the arrows.

**Figure 3. Villous blunting, CD8+ T cell predominant lymphocytic infiltrate and almost universally, a paucity of plasma cells, are typical of CVID-enteropathy<sup>27</sup>.**

Histologically, a paucity of plasma cells in the GI mucosa has been a unifying finding among many of these studies<sup>20,23-25,27</sup>. Findings in the small intestine include villous blunting

ranging from mild to subtotal in 30-87% of patients studied<sup>20-22,24,26</sup>, increased intraepithelial lymphocytes<sup>20,24,26</sup>, mild duodenitis<sup>20,22,24</sup>, and nodular lymphoid hyperplasia<sup>22,24</sup>. Findings in stomach include chronic gastritis, as frequently as 80% in one study, ranging from mild gastritis to severe mucosal atrophy in the body and antrum<sup>20,24</sup>, with vitamin B12 deficiency and serologic evidence of pernicious anemia reported. In the colon, GVHD-like lesions with epithelial apoptosis and variably dense mononuclear cell infiltrates with obliteration of occasional glands and crypts is noted<sup>21,24,26</sup>.

The pathophysiology of CVID-enteropathy remains unclear. Parallels have been drawn with celiac disease, given the “sprue-like” histologic pattern. However, the paucity of mucosal plasma cells, associated GVHD-like lesions, and in most cases lack of responsiveness to gluten withdrawal argue against this being celiac disease (CD) superimposed onto CVID. A number of studies have examined the similarities between CVID-enteropathy and celiac disease (CD). The group in Freiburg, Germany<sup>28</sup> retrospectively examined 20 patients with enteropathy, from their cohort of 250 CVID patients, to determine HLA DQ2 and DQ8 frequency, and whether it predicts response to gluten withdrawal. Only 4/20 had either HLA genotype. Two of the 4 responded to gluten withdrawal. In other cohorts, a low frequency of patients were shown to respond to gluten-withdrawal, and HLA genotypes were not predictive<sup>24,25</sup>.

T cells seem to drive the inflammation in this process. Mannon et al<sup>26</sup>, noting a previously identified TH1-bias in peripheral mononuclear cells from CVID patients<sup>29</sup>, isolated lamina propria mononuclear cells (LPMCs) from biopsy samples from 7 CVID patients with chronic diarrhea and malabsorption (88% had villous blunting and increased IELs), and 6 CVID patients without GI symptoms. These cells were stimulated in culture with either TLR ligands as innate stimuli, or anti-CD3/CD28 antibodies to stimulate the TCR, and cytokine production in supernatants was measured. Significantly higher IL12 (with innate stimuli) and IFN-gamma (with TCR stimulation) was noted from LPMCs from CVID patients with enteropathy. When compared to LPMCs from Crohn’s patients, similar levels of IL12 and IFN-gamma were produced, but significantly less IL23, 17 and TNF. Thus, this study suggests CVID-enteropathy to involve a TH1/Tc1 mediated inflammatory process driven by IL12 and IFN-gamma, which is distinct from Crohn’s disease.

Currently the approach to treatment is largely based on anecdotal evidence and expert opinion. It has long been noted that this process is steroid responsive<sup>30</sup>. In the French cohort above, steroids were given to 8 patients (prednisone n=1, budesonide n=7). Steroids induced responses in all 8 with significant reductions in diarrhea and malabsorption, and reduction or discontinuation of parenteral nutrition in 3. Notably, anti-TNF agents were attempted as steroid-sparing therapies in 2 patients but were “insufficient”. Steroid related complications requiring discontinuation included osteoporosis (n=1) and infections (n=2). All other patients remained on budesonide for 6 months (n=2), 3 years (n=1), and 6 years (n=2). IVIG therapy had no effect on GI symptoms<sup>24</sup>. There are other case reports and small case series suggesting safety and possible efficacy with TNF inhibitors<sup>31,32</sup>, although in one case series, while all 3 patients treated with infliximab had reductions in stool frequency and weight gain, none showed histologic response<sup>31</sup>. Other expert recommendations for steroid-sparing therapy in CVID-enteropathy include azathioprine and 6-mercaptopurine<sup>7</sup>.

## Granulomatous Lymphocytic Interstitial Lung Disease (GLILD)

Non-infectious lung disease in CVID includes bronchiectasis, which occurs frequently (11-25% in large CVID cohorts)<sup>1,33</sup>, and interstitial or infiltrative lung diseases including isolated granulomata, and a more diffuse process which involves loose granulomata and a mixed infiltrate of T cells, B cells, histiocytes and stromal cells which has recently been named granulomatous interstitial lung disease, or GLILD<sup>10</sup>. Our discussion will focus primarily on GLILD, which has been shown to significantly impact mortality in CVID patients<sup>10</sup>.

The association between hypogammaglobulinemia and non-infectious infiltrative lung disease has been noted since the 1970s when cases of associated lymphoid interstitial pneumonia in hypogammaglobulinemic patients were identified<sup>34-36</sup>. As was the case with enteropathy in CVID, frequent or chronic infection was felt to underlie these inflammatory diseases. However, comparisons of the frequency of interstitial lung disease (ILD) in CVID and XLA patients revealed that while similar proportions of patients are affected by infectious pneumonia, CVID patients suffered interstitial lung disease at rates of 5-25%, but no ILD was noted in XLA patients<sup>37,38</sup>. The authors speculated that T cell abnormalities or autoimmune related inflammation may play roles in chronic lung disease in CVID patients.

The frequency of GLILD in CVID is not well understood. In the one study which examined this process in depth, 25% of the total CVID cohort had diffuse findings on chest CT suggestive of ILD. All of these patients underwent lung biopsy. Of these, nearly  $\frac{3}{4}$  (18% of the overall CVID cohort) had GLILD on histopathology. The remaining 7% had BOOP, hypersensitivity pneumonitis or metastatic gastric cancer<sup>10</sup>. This retrospective study was done at a large pulmonary referral center, and therefore there may be referral bias. Other retrospective analyses have examined the frequency of granulomatous disease in the lung in CVID patients. In these studies, the frequency of GLILD ranges between 4.7% and 6.9%<sup>39,40</sup>. Notably, a large proportion of patients with GLILD had granulomata at extrapulmonary sites as well<sup>10,39,40</sup>, and there is a higher frequency of autoimmune diseases in these patients than seen in the CVID cohorts as a whole<sup>39</sup>.

From a clinical perspective, GLILD presents most frequently with dyspnea<sup>10,39,40</sup>. Clubbing and splenomegaly were additional exam findings noted in association with GLILD<sup>10</sup>. Pulmonary function tests show restrictive physiology with diffusion defects<sup>10,37,39,40</sup>. Several retrospective studies have characterized CT imaging findings in GLILD. In the largest study, noted above, consolidation, ground glass and reticular abnormalities were found in 80-100% of patients<sup>10</sup>. In a subsequent study, findings included multifocal consolidations, micronodules with lower lung zone predominance, ground glass attenuation, mediastinal lymphadenopathy and splenomegaly<sup>41</sup>. High resolution CT scan has been shown to be a more sensitive assessment for GLILD than pulmonary function testing, and one group noted **that CT findings in GLILD were indistinguishable from extranodal B cell lymphoma in the lung, arguing in favor of biopsy to establish the diagnosis in patients with these CT findings**<sup>10,42</sup>.



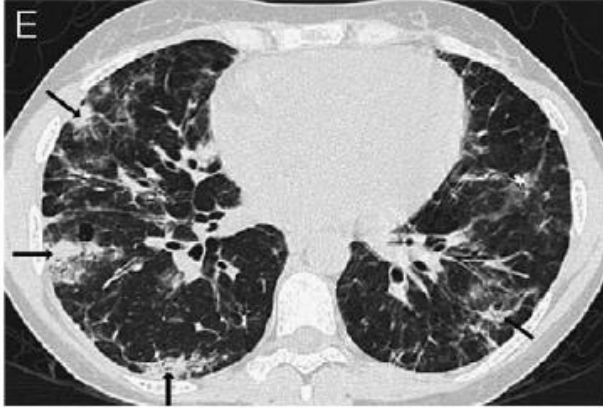


Figure 4. Diffuse reticulation, nodules with lower lung zone predominance, ground glass opacities, mediastinal>hilar adenopathy, splenomegaly, characteristic imaging findings in CVID-associated GLILD<sup>10,41</sup>.

Granulomatous disease in CVID patients is frequently misdiagnosed as sarcoidosis. In one CVID cohort, granulomatous disease was diagnosed prior to the diagnosis of CVID in 38% of patients, the delay ranging 1-18 years<sup>39</sup>. A recent case control study examined clinical characteristics which distinguish pulmonary disease in CVID from pulmonary sarcoidosis. Distinguishing features included significantly increased frequencies of recurrent infections, autoimmune disease, and extrathoracic disease in CVID patients. Velcro rales, splenomegaly and hepatomegaly on exam were significantly increased in CVID. Imaging differed significantly on a number of levels. Hilar lymphadenopathy was significantly less frequent in CVID, the distribution of micronodules was significantly different, being perilymphatic in 100% of sarcoidosis patients, but randomly distributed in the majority of CVID patients. Bronchiectasis was a significantly more frequent finding in CVID. Death during follow up was significantly more frequent in CVID (30% vs 0%  $p < 0.001$ )<sup>43</sup>.

The histopathology of GLILD was studied in depth by Maglione et al<sup>44</sup>. It has been described as an expansion of bronchus associated lymphoid tissue (BALT), or tertiary lymphoid neogenesis in the lung, with development of ectopic germinal centers, which may present in a number of histologic patterns, including those of lymphocytic interstitial pneumonia (LIP), follicular bronchiolitis, nodular lymphoid hyperplasia and reactive lymphoid hyperplasia. All of these include mixed infiltrates of T cells, B cells, APCs, stromal cells and loosely organized granulomata, and all of which fall under the umbrella term GLILD. In characterizing the histopathology in GLILD, Maglione et al noted peribronchial lymphocytic infiltrates organized into CD20+-rich B cell follicles surrounded by more diffuse T cell zones. Follicles were Ki67 and Bcl6+, and had CD23+ follicular DCs, identifying these as actively proliferating ectopic germinal centers. The ratio of T and B cells was variable, but in general there was an overall predominance of CD4+ T cells. Tertiary lymphoid neogenesis occurs in other autoimmune and inflammatory diseases, including Sjogren's disease and rheumatoid arthritis. Notably, these diseases have been shown to be responsive to B cell depletion therapies, arguing that these ectopic B cell rich germinal centers may drive the expansion or persistence of the infiltrative process. Also notably, GLILD or pulmonary lymphoid hyperplasia has not been demonstrated in XLA, where B cells are absent, despite frequent and often chronic pulmonary infections in that disease, arguing that B cells may be central drivers of the process.

The pathophysiology of GLILD is not well understood. In murine models, the development of bronchus associated lymphoid tissue is dependent on development of follicular dendritic cells (FDCs), through lymphotoxin alpha/beta (LT $\alpha\beta$ ) and CXCL13. In mice, BALT is maintained by dendritic cells (DCs) and LT $\alpha\beta$ , and inducible DC depletion or lymphotoxin inhibition can induce its regression. Treg have been shown to inhibit the formation of BALT in mice (reviewed in <sup>45</sup>).

An uncommon human TNF-alpha polymorphism was shown to correlate significantly in one study with the occurrence of granulomatous disease and splenomegaly in CVID. The majority of patients with granulomatous disease had granulomata in the lung<sup>46</sup>. A role for chronic viral infection in GLILD pathogenesis was proposed, and one group has shown an association between PCR positivity for HHV8 and GLILD, while others have not seen the same association<sup>47,48</sup>. Lastly a role for stromal cell function in the pathogenesis of GLILD is suggested by the finding of a SNP in the FGF14 gene found to be highly associated with LIP in CVID patients in the GWAS study by Orange et al<sup>49</sup>.

Regarding the treatment of GLILD, there are no controlled or comparative treatment studies. The treatment is therefore largely based on anecdotal data and expert opinion. Steroids are the typical first line agents. In one retrospective cohort study, corticosteroids were the most frequently used medications in treating pulmonary granulomatous disease (the majority of patients having CT findings consistent with GLILD). Almost 50% failed to respond to steroid. There were 25 treatment responses in total (in 30 patients with lung disease), 5 complete remissions (3 treated with steroid, 1 with methotrexate, 1 cyclophosphamide). Partial responses occurred with steroid, rituximab, hydroxychloroquine, and cyclophosphamide. There were treatment failures with steroid, hydroxychloroquine, cyclophosphamide, infliximab and Imuran. Adverse responses to treatment were seen almost exclusively with steroids, all were infections<sup>40</sup>. Chase et al<sup>50</sup> took a multi-agent approach to GLILD therapy. Given the above noted histopathology of GLILD, they hypothesized that a combination of B cell depletion therapy and a T cell targeted cellular immunosuppressant would be efficacious. They published a retrospective review of their experience with 7 patients treated with this regimen. Six of the 7 patients had GLILD based on both radiographic and biopsy criteria, one on radiographic criteria alone. Over approximately 2 years of treatment, they found significant improvements in CT scores, FEV1 and FVC with their regimen. There were no serious infections or other treatment related adverse events. There were no comparisons to either rituximab or azathioprine monotherapy. Given the association of TNF-alpha polymorphisms with granulomatous disease in CVID, a number of case reports have examined TNF-alpha inhibition in CVID. In a small case series, Franxman et al<sup>51</sup> reported treatment of 5 CVID patients with granulomatous disease at diverse sites with infliximab. Three of 5 patients in the series had involvement of the lung, two with CT findings consistent with GLILD. All 3 had both subjective improvement, and objective improvement in FEV1 and FVC. One patient had improvement in DLCO. There were no infectious complications. One of the three patients discontinued therapy due to a serum sickness-like reaction.

## **Liver disease**

The frequency of non-infectious liver disease in CVID patients is unclear. Given historical cases of hepatitis C transmission through IVIG products, monitoring of liver function tests in these patients has become standard of care. Liver disease complicating CVID has been shown to be associated with increased mortality in two large cohort studies. In Resnick et al, liver disease significantly increased mortality with HR 2.48<sup>8</sup>.

Several studies have focused on the frequency, and pathology of non-infectious hepatic complications of CVID. Malamut et al<sup>52</sup> performed a retrospective analysis of patients with primary hypogammaglobulinemia and liver disease from a French patient cohort including 51 patients with liver abnormalities (40 CVID, 8 Hyper-IgM, 3 XLA) and 43 control patients without liver abnormalities (31 CVID, 4 Hyper-IgM, 8 XLA). In total, twenty three patients were biopsied. Twenty (87%) had a pathologic entity called **nodular regenerative hyperplasia (NRH)**, characterized by diffuse transformation of the hepatic parenchyma into small, regenerative nodules, without significant fibrosis<sup>53</sup>. The majority (87%) had moderate or marked intrasinusoidal lymphocytic infiltrates of CD8+ T cells. Other features included non-caseating granulomata in 43% portal vein endotheliitis in 43%. Importantly, **portal hypertension**, a known complication of NRH, was present in 50%, with patients manifesting ascites, esophageal varices, elevated hepatic venous pressures and portal gastropathy. Also, a substantial proportion of patients (75%) had associated autoimmune disease. Overt hepatic failure was rare. There were no differences between CVID patients with or without liver disease with regard to time on IVIG, thus there did not seem to be either a causative or treatment effect of IVIG on NRH. This study argued that NRH may be the predominant non-infectious hepatic complication in CVID, leading to portal hypertension in half of affected patients.

Two subsequent studies examined NRH in CVID cohorts in the UK and United States, and included data on the frequency of this issue in the total CVID cohort. In the UK cohort<sup>54</sup>, 43% had abnormal LFTs. Approximately half were biopsied. Patients with viral hepatitis were excluded. Thirteen out of 16 patients with non-infectious liver disease who were biopsied had evidence of NRH on histopathology, a frequency of 12% in the total CVID cohort, felt to be an underestimate as half of the patients with abnormal LFTs were not biopsied. It was noted that the lab abnormality most closely associated with biopsy-proven NRH was a **progressive elevation of alkaline phosphatase**. Half of these patients had hepatomegaly, 18% in this study had signs of portal HTN (ascites or variceal bleeding). There was one death from liver failure.

Subsequently, Fuss et al<sup>55</sup> published a detailed analysis of hepatic complications in CVID patients. Again, elevated alkaline phosphatase was the most prominent lab abnormality. Notably, this was first noted in the NRH patients a mean of 7.8 years after diagnosis of CVID arguing that this complication may evolve after CVID presents. In all, 14 out of 261 CVID patients were found to have NRH, when biopsied to examine the etiology of abnormal liver function tests, for a frequency of 5% in this cohort. Notably, imaging has been shown to have poor sensitivity and specificity in diagnosis of NRH. In biopsies, NRH is characterized by nodular areas of enlarged hepatocytes organized into 2 cell thick plates alternating with compressed liver cell plates. In three patients there were spotty lobular lymphocytic inflammatory foci. In six there was mild to moderate interface hepatitis. In four patients, NRH occurred in conjunction with interface hepatitis with bridging necrosis, and prominent bridging periportal and perisinusoidal fibrosis, which was termed "autoimmune hepatitis (AIH)-like" disease. Of note there was a paucity of plasma cells in biopsies. Interestingly, in one patient, followed with

serial biopsies over six years, the disease was shown to progress from mild NRH to this more severe AIH-like illness. Corroborating the Malamut study, the interface hepatitis in these patients was shown to involve CD3+CD8+ T cells and interferon gamma expression. Furthermore, patients with this AIH like disease, unlike typical NRH, developed hepatic synthetic dysfunction in addition to portal HTN. The course in these 14 patients was shown to follow three possible patterns. Three patients developed lab and biopsy evidence of NRH which remained static. These patients did not require treatment. Six patients developed progressive disease, evolving slowly to involve signs and symptoms of portal HTN with hypersplenism, and resultant neutropenia and thrombocytopenia. These patients ultimately required treatment for portal HTN and/or cytopenias. Two of these patients required shunts to relieve portal HTN, which was also noted to improve neutrophil and platelet counts. Lastly, five patients had a more rapidly progressive course with evolution of frank hepatic dysfunction with portal hypertension and synthetic dysfunction, within 1-2 years after onset of abnormal labs. These patients required treatment with immunosuppressant medications. Two patients in this group were treated with prednisone and Imuran, with resultant improvement in portal pressures, although cytopenias were not improved and both died from progressive liver dysfunction and infection. One patient died of systemic infection prior to start of immunosuppressive therapy. One patient was reported to achieve stable control with steroid and 6-MP. This patient was noted to be young (age 8) at the time of diagnosis of liver disease, and the authors speculate that early diagnosis may be important in successful treatment of this disease. Regarding the association of NRH with other complications, 4/14 patients were noted to have associated malabsorptive enteropathy, 8/14 had other associated autoimmune diseases including ITP, AIHA, arthropathies, vasculitis, psoriasis and scleroderma.

NRH occurs in other disease settings as well, including autoimmune and hematologic illnesses, infections and in the setting of treatment with certain medications, including thiopurines (azathioprine, 6-mercaptopurine, 6-thioguanine), HAART medications (didanosine) and chemotherapeutics (reviewed in<sup>53</sup>). It accounts for 27% of cases non-cirrhotic portal hypertension<sup>53</sup>. The prevalence of NRH in autopsy studies is approximately 2%, and in those studies appears to be enriched in persons over 80 years old, and in patients with systemic arteritis, polymyalgia rheumatica, tumor infiltration and mineral oil deposition, as well as cardiac disease, pulmonary diseases associated with pulmonary hypertension, and systemic amyloidosis<sup>56,57</sup>. Interestingly, the frequency with which portal hypertension is found in association with NRH found at autopsy was low, at 4.7%<sup>56</sup>, arguing that both the frequency and severity of this entity may be increased in CVID patients.

The underlying etiology of NRH, in a broad sense, seems to be related to disturbances in blood flow and perfusion in the hepatic microvasculature, leading to a local hyperplastic response in hepatocytes<sup>53</sup>. This can occur secondary to congenital anomalies in portal vasculature, hematologic/prothrombotic disorders, toxic, or immunologic injury to endothelial cells<sup>53,58,59</sup>. T cells may play a role in pathogenesis in some patients. Similar to findings in CVID patients in the studies cited above, Ziolkowski et al<sup>59</sup> found CD8+T cell infiltrates adjacent to apoptotic endothelial sinusoidal cells in 32% of NRH patient biopsies, and the majority of these patients had associated diseases including CVID, autoimmune disease, malignancy or infection. The specific role these CD8+T cells play in this disease is unclear. Cytokines including interferon gamma<sup>55</sup> and interleukin 6<sup>60</sup> have been postulated to play roles in this disease. Autoantibody,

particularly antiphospholipid antibodies contributing to prothrombotic states, have been shown in association with NRH and may play a role in some patients<sup>61</sup>. In their GWAS study Orange et al found highly significant associations of two SNPs on chromosome 1 with NRH in CVID patients, the specific identity and function of which are unknown<sup>49</sup>.

As alluded to above, the treatment of NRH focuses on management of portal hypertension. Immunoglobulin replacement therapy for the underlying CVID does not seem to impact NRH, as discussed above. Immunosuppressant medications for the more severe AIH-like variant may be effective in the setting of early detection and initiation of therapy, and therefore vigilance is recommended in patients with abnormal liver function tests, particularly a persistently escalating alkaline phosphatase. The outcome and prognosis of this disease appears to be highly variable as illustrated in the studies above and in prior studies in non-CVID patients with NRH<sup>62</sup>.

### **Mechanisms of Autoimmunity, Inflammation and Lymphoproliferation in Immunodeficiencies**

Autoimmunity and primary immunodeficiency are frequently and seemingly paradoxically linked. This relationship highlights the inherent mechanistic links between proper development of the various elements of the immune system, and the selection and regulatory mechanisms which maintain self-tolerance.

In early stages of adaptive immune cell (T and B cell) development in thymus and bone marrow, as antigen receptors are rearranged, a series of checks and balances occur to ensure that cells with antigen receptors which bind self-antigens too avidly are eliminated. This is best explained through an affinity model, in which binding of the newly rearranged antigen receptor to self-antigen/MHC with overly high affinity results in signaling events leading to either receptor editing, or apoptosis. A third possibility in developing thymocytes may include “clonal diversion” in cells binding self-antigen with intermediate affinity, leading to their differentiation into Treg. The process of negative selection thereby requires a) exposure to a broad array of self-antigens during immune cell development, b) proper signaling through the antigen receptor and through various costimulatory and cytokine receptors, and c) intact apoptotic signaling mechanisms. Therefore, defects, for example, in antigen receptor or cytokine receptor signaling, may lead to both ineffective development of a diverse array of T or B cells, and simultaneously to escape of self-reactive clones into the periphery.

Additional checks and balances also exist in the periphery to inhibit the function of self-reactive clones that do manage to escape negative selection. These include various types of regulatory T cells and myeloid cells, which inhibit the effector function of T and B cells, as well as mechanisms within lymphoid tissues to exclude these aberrant self-reactive clones from follicles, by virtue of altered growth factor receptor expression. Examples of critical mechanisms in self-tolerance, and syndromes of immunodeficiency and/or immunodysregulation which result from genetic lesions in these pathways, are denoted in figure 1, and are reviewed in<sup>63</sup>.

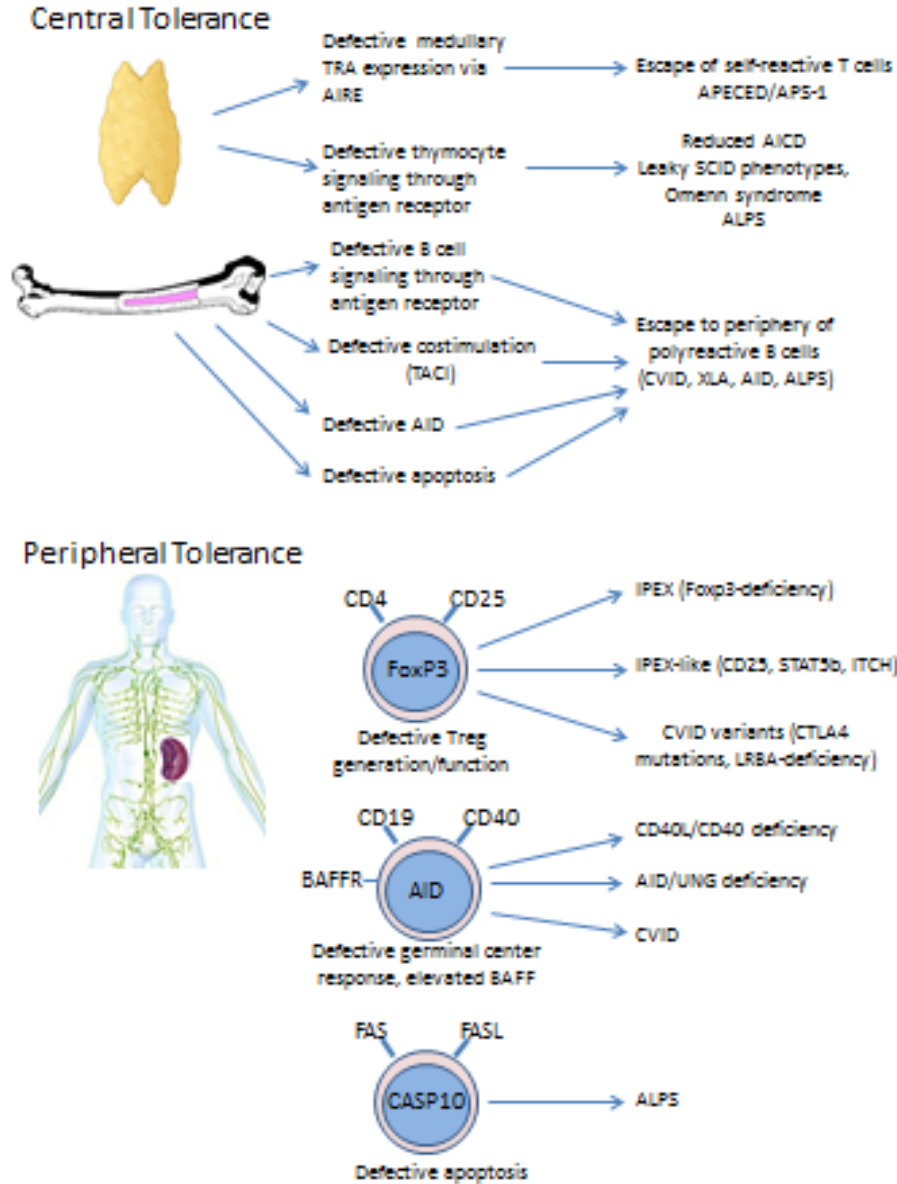


Figure 5. Genetic defects in immune development and immunoregulation and resulting diseases.

Central tolerance in CVID may be modified by mutations in the TNFRSF13B gene encoding TACI (Transmembrane activator and CAML-interactor), a costimulatory receptor shown to be involved in class-switch recombination. Using a candidate gene approach, heterozygous mutations in TACI were found in 8-10% of CVID patients and appear to increase the predisposition to autoimmunity<sup>64</sup>. TACI mutations are shown to compromise central tolerance<sup>65</sup>, and may thereby increase the risk of autoimmunity in these patients by increasing the frequency of self-reactive B cells escaping the bone marrow.

Peripheral tolerance has been shown to be compromised in CVID patients regardless of

TACI mutations<sup>65</sup>, and a number of potential lesions may contribute to this. The frequency and function of Treg cells is depressed in CVID patients with autoimmune cytopenias, thyroiditis, polyendocrinopathy, arthritis and inflammatory manifestations including granulomatous disease and enteropathy<sup>66-68</sup>. Pro-survival signaling and entry of self-reactive cells into otherwise restricted B cell follicles are allowed via elevated circulating BAFF levels in CVID patients<sup>69,70</sup>. Calcium signaling through the BCR is dysfunctional in a subgroup of CVID patients prone to autoimmunity and infiltrative disease<sup>71</sup>. This correlates with poor class-switching and memory formation, and expansion of anergic, CD21lo B cells, with altered trafficking properties<sup>6,72,73</sup>. Interestingly, similar CD21lo populations have been shown in SLE and RA, and in fact infiltrate synovial tissues<sup>72-74</sup>. The specific role of these cells is unclear.

More recently, whole exome sequencing approaches have shed light on two additional genes which may be important break-points in tolerance in a proportion of CVID patients with autoimmune, inflammatory and lymphoproliferative complications. By sequencing families with multiple affected family members, with a phenotype of hypogammaglobulinemia, autoimmunity, inflammatory disease, and lymphoproliferation, gain-of-function mutations in PI3K-delta were found exclusively in affected family members, inherited in an autosomal dominant pattern<sup>75,76</sup>. Activating PI3K-delta mutations were subsequently found at a rate of 0.7% in a large European CVID cohort<sup>77</sup>. Notably, the patients screened were NOT selected for the above phenotype. This argues that the frequency of these mutations in CVID patients with severe autoimmune/inflammatory/lymphoproliferative phenotypes is likely significant. PI3K-delta is a membrane associated lipid kinase which phosphorylates phosphatidylinositol residues at the cell membrane to activate downstream AKT signaling. This in turn is a pro-survival signaling molecule, inhibiting apoptosis, and activating mTOR signaling<sup>78</sup>.

Using the same approach, heterozygous mutations in CTLA4, a membrane protein upregulated on activated T cells, and constitutively expressed on Treg, were identified in CVID patients with autoimmunity, enteropathy, GLILD and lymphoproliferation<sup>79,80</sup>. These mutations had not been listed in dbSNP database. Interestingly, screening a cohort of 71 unrelated CVID patients with a similar phenotype, revealed 5 with CTLA4 mutations, for a frequency of 7% in this cohort. The disease phenotype is incompletely penetrant – there are thus far 20 cases in 10 different families (3 cases are sporadic), and 9 healthy “carriers” in these families. The mutations affect CTLA4 surface expression and cause haploinsufficiency. Interestingly, while CTLA4 haploinsufficiency does not cause a phenotype or any detectable immunologic dysfunction in mice, in humans (both affected patients and healthy carriers) Treg suppressive function is impaired<sup>80</sup>. The incomplete penetrance of disease, is as yet not understood.

## Conclusions

CVID is the most common clinically relevant primary immunodeficiency, affecting 1:25,000.

- Despite routine use of IV and subcutaneous immunoglobulin therapy for infectious prophylaxis, **survival in patients with CVID is significantly shorter than the general population. This has been shown to be affected primarily by non-infectious complications of the disease.**

- These non-infectious complications **frequently present well before the diagnosis of CVID is made**. CVID should be on the differential diagnosis for patients presenting with autoimmune cytopenias, granulomatous disease, interstitial lung disease, inflammatory enteropathy, non-infectious/autoimmune hepatitis and nodular regenerative hyperplasia. These autoimmune and inflammatory conditions often trend together in CVID patients, and the presence of one of these should prompt close surveillance for others.
- The treatment of these conditions usually requires the use of immunosuppressant medications in already immunocompromised patients, putting patients at increased infectious risk and necessitating aggressive prophylaxis and again, close surveillance. Furthermore, there are shown to be high failure rates with typical first line agents such as glucocorticoids, often necessitating either additional, or alternative, immunomodulatory drugs.
- Unfortunately, robust comparative treatment studies do not currently exist to guide decisions when treating these illnesses – currently treatment decisions are based on retrospective analyses, anecdotal data, and expert opinion. This field sorely needs multi-institutional collaboration for the study of treatment protocols, to best establish standards of care and make real advances.
- Through the study of familial variants of CVID, and well defined sub-phenotypes with immunodysregulation, advances are being made in understanding the disease modifying mutations in these patients which predispose to autoimmunity, inflammation and lymphoproliferation. Furthermore, these studies are illuminating potential targeted therapies for these conditions in CVID patients.

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