Clinical Reviews in Allergy and Immunology

1080-0549/00/157–181/$16.25

Common Variable Immunodeficiency

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Introduction

Common variable immunodeficiency (CVI) and selective IgA deficiency (IgAD) are genetically and immunologically related diseases that together form the vast majority of the primary immunodeficiency states. IgAD is the more common of the two conditions occurring with a prevalence of 1:300–1:1300 in Caucasian populations *(1)*; this compares with CVI, which has a prevalence of 1:50,000–1:200,000 *(2,3)*. However, only a small fraction of those with IgAD have clinical disease and the number of individuals with CVI with symptoms probably exceeds those with IgAD. IgAD can be considered an incomplete form of CVI in which only the synthesis of IgA, rather than all immunoglobulins is defective. This in itself is a link with CVI, because IgA is the most severely affected immunoglobulin in CVI. Other links are inherent in the presence of common genetic factors as well as in the occurrence of families in which some members have CVI and others IgAD; in addition, numerous cases have been observed in which IgAD develops into CVI (see Chapter by Shroeder and review by Strober and Sneller *(1)*. Here we shall be concerned with CVI itself, but it is fair to say that many of the features of this disease are similar to those found in IgAD.

CVI was originally called acquired agammaglobulinemia, reflecting the fact that it usually became manifest after the first decade of life. Although we now know that the disease is strongly linked to genetic factors, the late onset points to the probability that an infectious agent or another environmental factor triggers and/or sustains the disease. This notion is buttressed by known instances in which hypogamma-

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globulinemia is associated with and/or caused by viral infection. For example, it is now well recognized that chronic or even acute Epstein-Barr virus (EBV) infection can lead to hypogammaglobulinemia similar if not identical to that in CVI (*4*). In addition, EBV infection in patients with a rare X-linked genetic abnormality known as X-linked lymphoproliferative disease leads in patients who survive the acute infection to either lymphoma or hypogammaglobulinemia (*5*). Although viral infection looms large as a possible precipitating factor in CVI, it is well to emphasize that there is as yet no evidence that CVI is continuously driven by an ongoing viral infection in the usual sense of the word. One possibility that should be entertained in this context and that will be discussed more fully later is that CVI is caused by an insidious viral infection that seeds cells with pathologic but noninfectious genes.

CVI has the word "variable" in its title because early on, students of the disease did not know if CVI was caused by a single defect or by many defects that each result in hypogammaglobulinemia. This question persists in the absence of the identification of one or more genetic or infectious etiologic factors. Nevertheless, it remains possible that patients present with a spectrum of immunologic abnormalities that are caused by a single defect (albeit one of variable expression and severity) and we shall consider CVI a single disease in the discussion to follow.

Diagnosis of CVI

CVI is defined very simply by the presence of IgG levels that are greater than two standard deviations (SD) below the normal mean level. In North American and European populations this translates to levels below about 250 mg/dL. Almost invariably, this is associated with similar decreases in IgM and IgA levels. Variants of CVI are characterized by IgG subclass deficiency, which usually involves decreased IgG2 and IgG4 levels alone or in association with IgAD or decreased IgG1 levels, again possibly in association with IgA (*6*). In such variants, the overall IgG level may be within normal limits and the disease is usually suspected (and immunoglobulin levels determined) in patients with bacterial infections of the lower respiratory tract; in the latter regard, radiographically documented pneumonia is a disease that only rarely occurs in a young individual with a normal immune system in the present social context. Laboratory evaluation of patients should include measurement of antibody responses to protein and polysaccharide antigens such as tetanus and diphtheria toxoids and pneumococcal polysaccharides, materials that are readily available in hospital pharmacies. Patients with CVI tested in this way fail to mount four-fold increases in antibody titer when tested 2–4 wk after antigen adminis-

tration. Such measurement of antibody responses is particularly important in the case of IgG subclass deficiency because the latter have little or no clinical significance if antibody responses prove normal. Finally, patients' peripheral blood cells should be subjected to a lymphocyte phenotype analysis to determine T-cell number and type and to determine B cell number. Such analysis allows one to distinguish CVI from several other immunodeficiency states including X-linked (Bruton's) agammaglobulinemia (XLA), which is characterized by low or absent B cell levels.

Clinical Aspects of CVI

Broadly speaking, the clinical manifestations of CVI fall into one of three categories: infections, autoimmune abnormalities, and lymphoproliferation or neoplasia. Prior to the treatment of patients with adequate immunoglobulin replacement therapy, the infections category dominated the clinical picture, although autoimmunity and lymphoproliferation were noted in patients quite early. With the advent of modern immunoglobulin replacement therapy (IVIG), it was felt that patients would be disease-free if therapy was begun early enough. It was disappointing to find, however, that CVI is still a cause of major symptomatology in spite of replacement therapy and in this way the aspects of the disease caused by concomitant T-cell defects have come to the fore.

Infections in CVI

In untreated patients with CVI, the major clinical feature of the disease is recurrent bacterial infections of the upper and lower respiratory tract. These infections are characteristically caused by "high grade" extra-cellular bacterial pathogens such as *Streptococcus pneumoniae* and Hemophilus influenza organisms that are virulent enough to cause disease even in immunologically normal individuals. CVI thus differs from other immunodeficiency states characterized mainly by T-cell abnormalities where infections with intracellular, opportunistic bacterial or viral organisms are the rule. The occurrence of infections with high grade pathogens in CVI attests to the fact that IgG immunoglobulin is the major serum opsonin, and its relative absence in CVI leads to an inability to take up and kill such pathogens.

If patients with CVI go undiagnosed and are thus left untreated, the aforementioned infections inevitably lead to severe and for the most part irreversible lung disease characterized by bronchiectasis, loss of functional lung parenchyma, and ultimately, pulmonary insufficiency. Patients who have advanced to this state are now susceptible to a much broader group of organisms, such as *Pseudomonas aeruginosa* and *Sta-*

phylococcus aureus, and are, in effect, pulmonary "cripples" who go from one serious respiratory infections to the next despite of adequate Ig replacement therapy. In recent years, this kind of situation has led to treatment of several of these patients with heart/lung or lung transplants (*7*). The progressive nature of respiratory (pulmonary) infection in CVI points to the importance of early diagnosis and prompt treatment of patients. In this regard, CVI should be considered in any patient with unexplained and recurrent lower respiratory infection.

Although infections of the respiratory tract owing to encapsulated extra-cellular bacteria are predominant in CVI, infections with other types of organisms and involving other organ systems do occur with some frequency. In occasional instances these involve low-grade opportunistic pathogens, reflecting the fact that a substantial fraction of patients have demonstrable T-cell abnormalities. Among the types of infections seen in CVI are viral hepatitities, severe recurrent Herpes zoster infection (shingles) (*8*), mycoplasma pneumonia and arthritis (*9,10*), giardia lamblia enteritis (*11*), *Pneumocystis carinii* infection of the lung (*7*), fungal infections caused by *Candida albicans* (and giving rise to mucocutaneous candidiasis in some cases), cryptococcal infection, mycobacteria avium infection of the lung, and nocardia brain abscesses (*7*). Thus, the picture that emerges is that CVI patients, at least those not on adequate Ig replacement therapy, are at risk for all sorts of infections involving virtually every part of the body. The extent to which replacement therapy has, in fact, altered this picture is not yet clear. Although control of infection with encapsulated organisms is bound to have improved, control of infections with viruses and opportunistic organisms of all types are beyond the reach of Ig replacement and may be occurring with same frequency as prior to such therapy. It should be noted that the increased occurrence of viral hepatitis in CVI attributed to exposure to virus-contaminated gamma globulin preparations is likely to be far less common now than formerly, because of improved methods of virus detection.

One unusual infection occasionally seen in CVI is one that is opportunistic in its site of involvement rather in its actual occurrence. This is central nervous system (CNS) infection with enteroviruses, particularly Type II Echoviruses, characterized by chronic and severe meningoencephalitis, leading in most cases to severe loss of mental and neurologic function and not infrequently to death (*12*). In some instances, this is associated with non-CNS manifestations as well, such as a dermatomyositis-like skin disease and hepatitis. This type of infection is actually more common in patients with XLA than in CVI and thus may occur as a result of the fact that in the absence of an IgM

antibody response there is systemic dissemination of potential pathogens to organs (in this case the CNS) that are usually limited to the gastrointestinal (GI) tract. This syndrome can be identified in patients with CVI who have neurologic symptoms by examination of the CSF, which is usually positive for the enterovirus early on and is sterile but contains elevated cell counts late on.

Gastrointestinal (GI) Manifestations of CVI

Primarily because CVI patients frequent present with GI symptoms, the GI tract is often considered a major site of infection in CVI. However, as we shall see, although infections with known pathogens do occur in the GI tract, most of the GI symptomatology in CVI does not have an infectious origin.

Addressing the infectious causes of GI symptoms in CVI first, one comes to the not infrequent occurrence of enteritis owing to Giardia lamblia (*11*). This is a protozoan organism capable of infecting healthy individuals when the latter are exposed to high concentrations of the organism in infected food or water; however, such infection is usually self-limited or else is easily treated. In CVI, a different picture is obtained in that the infection occurs in the absence of high-level exposure, is more persistent, and is more difficult to eradicate with conventional therapy when it does occur. Diagnosis is made by identification of the giardial cysts or trophozoites in the stool, which is now best accomplished with a sensitive fluorescent technique (*13*). Treatment with quinacrine or metronidizole can be successful, but frequently combined therapy is necessary to achieve resolution; however, even when the latter is achieved, giardia infection often recurs.

Patients with CVI are also more subject to infections with "conventional" bacterial GI pathogens such as infection with Salmonella, Shigella and Campylobactor species. In addition, in recent years, severe diarrhea owing to infection with a fastidious gram-negative bacterial rod known as dysgonic fermentor-3 (DF-3) has been noted in several patients. Infection with this organism is identified by culture of stool on cefoperazone-vancomycin plates incubated at 35°C and, once identified, responds well to appropriate antibiotic therapy (*14*).

With the advent of treatment of CVI with intravenous Ig replacement therapy, the occurrence of gastrointestinal infections, especially giardia infections, has clearly decreased (*15*). Nevertheless, these infections continue to occur in CVI patients more frequently than in normal individuals, in spite of treatment. This argues that T cell abnormalities present in patients play a part in host defense against organisms causing GI infections.

Addressing now the noninfectious manifestations of GI disease in CVI, one comes to a major syndrome of the disease occurring in perhaps 20% of patients *(16–18)*. On the symptom level, this syndrome consists of chronic diarrhea, malabsorption marked by increased excretion of fat and abnormal D-xylose uptake and protein-losing enteropathy marked by increase loss of albumin and other proteins into the fecal stream. In some cases, these manifestations result in severe weight loss leading to the need for parenteral nutrition, nutrient deficiency leading to hypocalcemia (owing to vitamin D malabsorption), anemia (owing to folic-acid malabsorption), and hypoalbuminemia leading to widespread edema (owing to protein loss). On the histopathologic level, the syndrome is marked by the presence of mild to moderate villous atrophy and the presence of increased numbers of intra-epithelial lymphocytes and lamina propria lymphocytes, a picture similar to that seen in celiac sprue (gluten-sensitive enteropathy). However, in this case the clinical/pathological abnormalities do not respond to a gluten-free diet or even to the exclusion of all foods as they would if this were a food-hypersensitivity state. Moreover, the histologic findings are different from those in gluten-sensitive enteropathy in several important respects: first, the epithelial-cell layer at the tips of villi are preserved in CVI and contain goblet cells, while in gluten-sensitive enteropathy, the epithelial cell layer lacks goblet cells and contains epithelial cells lacking brush borders; second, in CVI there is no infiltration of the lamina propria with plasma cells, a hallmark of gluten-sensitive enteropathy; third and finally, while in gluten-sensitive enteropathy crypt epithelium is hyperplastic, in CVI there may be crypt loss and the presence of apoptotic bodies representing dying epithelial cells (*18*). Overall, these various findings suggest that the immunologic process in CVI is quite different than the one in gluten-sensitive enteropathy (and other food protein hypersensitivity states).

Another manifestation of the aforementioned syndrome, and one occurring in most but not all affected patients, is the presence of a histopathologic lesion known as intestinal nodular lymphoid hyperplasia. This consists of macroscopic collections of lymphocytes scattered through the small (and in many cases large) intestinal wall that have the appearance of nodular bumps on endoscopy and punched-out lesions on contrast radiography (Fig. 1). Examination of the cells within the nodules in a limited number of patients reveals that most of the cells consist of B cells bearing surface IgM surrounded by T cells, most of which are $CD8^+$ T cells (*19*). These findings suggest that they represent abortive attempts to form productive B-cell follicles, which are not properly downregulated by T cells. Intestinal nodular lymphoid hyperplasis is virtually pathonomonic for CVI (it does very occasion-

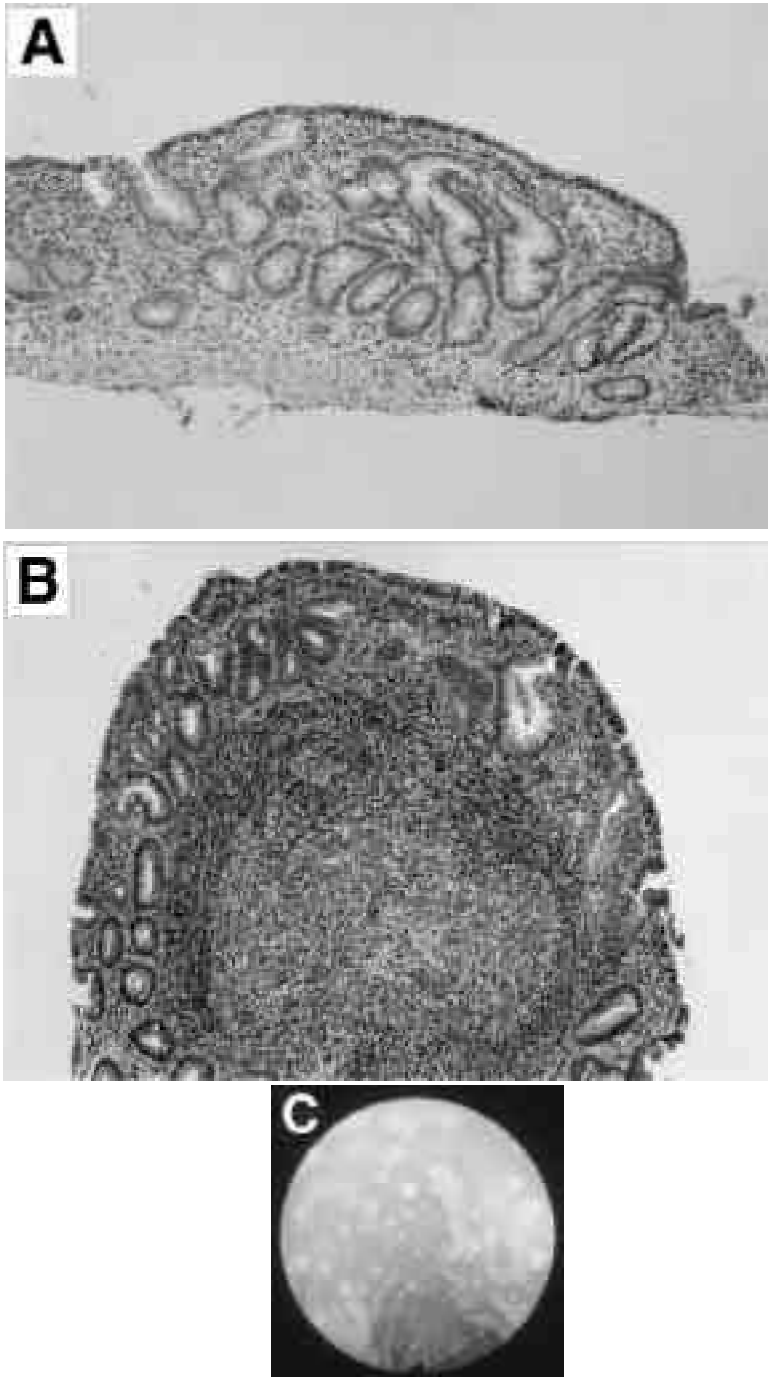

Fig. 1. Histologic and endoscopic findings in CVI patients with GI syndromes. (A) Cross-section of jejunal epithelium showing partial villous atrophy. (B) Photomicrograph showing nodule of intestinal nodular lymphoid hyperplasia. (C) Endoscopic findings in CVI showing multiple nodules of intestinal nodular lymphoid hyperplasia.

ally occur in otherwise normal individuals); in addition, it does not in itself cause GI symptoms and its main clinical significance is that it may be associated with an increase risk of intestinal lymphoma (*20*,*20a*).

For many years it was felt that the origin of the aforementioned syndrome was the presence of one or more infectious agents or, in a related vein, the presence of bacterial overgrowth. That this is not the case, however, is shown by the fact that patients with the syndrome do not manifest consistent improvement with antibiotic therapy, and although increased numbers of bacteria are seen in the upper intestine of CVI (and IgA deficient) patients, including bacteria that are normally found only in the lower intestine, these levels do not give rise to symptomatic overgrowth (*21*). A more likely possibility is that the syndrome arises from T-cell dysregulation and a type of autoimmune attack on the intestinal wall. This possibility is supported by the fact that the syndrome does not occur in XLA, an immunodeficiency characterized by an impairment of B-cell function as great or greater than that seen in CVI, but is not characterized by a T-cell abnormality (*22*). In addition, the syndrome can be correlated with the presence of other, non-GI evidences of autoimmunity (*see* below) and CVI patients with the syndrome are more likely to have T-cell dysfunction than CVI patients without the syndrome (*7*). Finally, several patients with very severe diarrhea and malabsorption have responded at least partially to corticosteroid therapy. The latter, however, cannot be recommended as more than a short-term or emergency treatment because there is ample documentation of the fact that such treatment subjects the patient to increased risk of life-threatening infection (*7*). If indeed diarrhea, malabsorption, and intestinal nodular hyperplasia are manifestations of an autoimmune attack on the intestinal mucosa, one should be able to find immune effectors with epithelial specificity in CVI. To date, this possibility has received little investigation except for one or two reports of patients with antibodies that react with intestinal epithelial cells (*23*). It is not likely, however, that antibodies are the basis of these lesions because CVI patients have impairment in their ability to produce antibodies and certain features of the histopathology of the small intestine in CVI resemble that of graft-v-host disease (GHVD), a condition that is mainly T cell-mediated (*18*,*24*). This in fact correlates with the observation that most of the cellular infiltration in the lamina propria consists of T cells.

To round out the spectrum of small intestinal disease in CVI, mention should be made of a recent report of a single patient who had villus atrophy similar to that described earlier, but in this case the atrophy was associated with an heavy infiltration of foamy macrophages similar to those found in Whipple's disease and in patients with chronic granulomatous disease (CGD) (*18*). Whipple's disease was not present,

however, since the macrophages did not contain PAS-positive bacilli or bacillary debris indicative of an infection with *Trophorema whippeli*, the organism now know to cause Whipple's disease. This patient again points out that the histopathologic findings in CVI may superficially resemble those in other diseases yet have a unique character of its own. It seems likely that the lesion here is a variant of the syndrome described earlier and again is owing to a immune dysregulation, although infection with an usual organism cannot be ruled out.

The discussion so far has been concerned with small intestinal abnormalities associated with villous atrophy. It is important to note, however, that inflammatory lesions involving the gastric mucosa as well as ileal and colonic mucosae occur as well. Gastric lesions consist of gastritis leading in most cases to gastric atrophy and achlorhydria (*25*). However, in contrast to immunologically normal individuals with this pathologic constellation, the patients are usually free of *Helibacter pylori* infection and histopathologic examination of the gastric lesions reveals a pan-gastritis with no antral sparing (as in gastritis of immunologically normal individuals) (*18,26*). In addition, the gastritis is marked by a nonspecific inflammation of the oxyntic glands as well as focal apoptosis of glandular epithelial cells concentrated in the neck areas, a picture reminiscent of mild GVHD. Importantly, the gastritis associated with CVI is associated with the development of pernicious anemia which, as discussed later, also differs from that found in immunologically normal individuals (*26,27*).

As for colonic and ileal disease in CVI, a substantial number of patients have been said to have ulcerative proctitis or colitis or Crohn's disease (*15,18,28*). In recent descriptions of the histologic changes found in colonic lesions, nonspecific and superficial infiltration of lymphocytes as well as neutrophils and eosinophils, increased numbers of intra-epithelial cells and apoptosis of cells in glandular crypts and loss of crypts was found; however, crypt architecture were intact and crypt abscesses were not seen (*15,18*). This histologic picture resembles that of so-called microscopic colitis (*15,29*). Ileal disease, on the other hand, was marked again by nonspecific, nongranulomatous transmural inflammation, fibrosis, and strictures (*15,18*). Whether these colonic and ileal changes are indicative of true ulcerative colitis and Crohn's disease or a T cell-mediated autoimmune reaction unique to CVI and related in type, if not in degree, to the immunologic process taking place in the upper small intestine remains to be seen. The same can be said for the aforementioned gastric lesions.

Autoimmune Disease in CVI

In describing GI manifestations of CVI, we have already launched our discussion of autoimmunity in the disease. Autoimmunity occur-

ring in CVI is a much broader phenomenon, however, and it is not unreasonable to say that CVI is almost as much a disease of immune dysregulation as it is a disease of immunodeficiency. The basis of such autoimmunity is not at all clear. One factor is that CVI is associated with particular MHC haplotypes that are also associated with autoimmune states (*see* article by Shroeder). Thus, it is likely that there is a genetic basis for the autoimmunity, which may be related to the genetic basis of the immunodeficiency. Another factor is that patients with CVI (as well as those with IgAD) lack a normal mucosal IgA response and may fail to exclude entry of environment proteins at mucosal surfaces. This can lead to immunization by environmental antigens that crossreact with self-antigens and thus the induction of immune elements that mediate autoimmunity. Finally, as noted later, CVI is also marked by the presence of T-cell abnormalities and thus CVI patients may have less ability to counter-regulate (suppress) autoimmune responses.

Excluding autoimmune GI manifestations, autoimmune diseases occurs in about 20% of patients (*7,30,31*). The most common of these are autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura, which occur either separately or in some cases together (Evan's syndrome). These disease manifestations are associated in both patients with CVI and immunologically normal individuals with antibodies to the respective blood elements. This is also the case in the several cases of neutropenia occurring in CVI, which were shown to be associated with the occurrence of antigranulocyte antibodies (*30,32*). It is paradoxical that CVI, a disease in which the hallmark abnormality is B-cell dysfunction and the inability to produce antibodies, is associated with disease manifestations that are caused by the presence of autoantibodies. It should be recalled, however, that CVI patients can usually produce substantial amounts of immunoglobulin and, as we shall see, patient B cells can be induced to produce immunoglobulins in vitro to levels higher than those seen in vivo under appropriate conditions. Thus, it becomes more understandable that the patients can mount autoimmune responses. Conventional treatment of these forms of autoimmunity involves the use of corticosteroids and cytotoxic agents. As alluded to earlier, however, such agents render the CVI patient highly susceptible to life-threatening infection and must be used with extreme caution. High dose intravenous Ig has been used to good effect in one patient with autoimmune hemolytic anemia and thus is an attractive first line of therapy for this manifestation of CVI (*33*).

Another autoimmune disease occurring quite frequently in CVI is pernicious anemia (*26,27*). This disease occurs much earlier in CVI patients than in immunologically normal individuals reflecting the early onset of chronic gastritis described earlier (*27,26*). Unlike pernicious

anemia in immunologically normal individuals and in contradistinction to the situation in autoimmunity involving blood elements, pernicious anemia in CVI is often not associated with the presence of autoantibodies (antibodies to intrinsic factor or gastric parietal cells) and thus is more likely caused by a T cell-mediated destruction of gastric epithelial cells producing intrinsic factor (*27*). Many other autoimmune diseases have also been reported in patients with CVI, including Grave's disease and hypothyroidism, juvenile rheumatoid arthritis (JRA), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), primary biliary cirrhosis, sicca syndrome, glomerulonephritis, and vasculitis (*7*). Thus, while a limited number of particular autoimmune manifestations dominate in CVI, the tendency toward autoimmmunity is, in reality, quite general.

Lymphoproliferation, Lymphoma, and Carcinoma in CVI

As in other immunodeficiency states, patients with CVI are at an increased risk for the development of neoplasias, either because of faulty immune surveillance or because the disease is associated with chronic viral infections and proliferative states that increases the chance of multiple gene mutations leading to neoplasia. The latter is illustrated by the fact that about 30% of patients in CVI manifests benign lymphoproliferative disorders characterized by splenomegaly, diffuse lymphadenopathy, or both (*7*). On a histologic level this takes several forms. The most benign consists of reactive follicular hyperplasia in which the architecture of the lymphoid tissue is preserved and the only abnormality is one of increased tissue mass (*34*). The next level consists of atypical reactive lymphoid hyperplasia, in which the architecture of the lymphoid tissue is effaced, in some cases with a polymorphonuclear infiltrate. This type of hyperplasia can be distinguished from the lymphoma by the fact that the cellular infiltrate consists of both T cells and B cells and is not monoclonal. The origin of benign lymphadenopathy in CVI is unknown, but probably relates to the presence of subtle viral infection. This is well-illustrated in one patient who had widespread lymphoproliferation associated with a documented cytomegalovirus (CMV) infection; this patient was treated with intravenous (iv) gancyclovir but did not resolve the lymphoproliferation (*35*). Despite the experience with this patient, lymphoproliferation can spontaneously recede in CVI and it does not necessary herald a malignant condition (at least in the short run). In general, it is important to explore thoroughly lymphoproliferation in CVI to rule our frank lymphoma and to spare the patient unnecessary treatment for presumed lymphoma when it does not exist.

As for lymphoma itself in CVI, these clearly occur at greater frequency in patients than in normal individuals, although the limited

data presently available does not allow a clear risk assessment as yet. The latter is bounded by one study showing a 30-fold increased risk and another showing a 400-fold increased risk (*36*,*37*). In most cases the lymphomas were non-Hodgkin's lymphomas of a B cell type, but other types of lymphoma were also seen including one case of Waldenstrom's macroglobulinemia and one case of T-cell lymphoma (*7*,*38*). Most cases were extra-nodal in location, with several in the intestinal tract. In addition to lymphoma, CVI patients are subject to increased risk of carcinoma of varying type and location. These included several cases of stomach adenocarcinoma, a disease usually encountered in a much older patient population (*7*).

Additional Clinical Features of CVI

Certain additional clinical features of CVI are worthy of mention. One is the not infrequent occurrence of patients with noncaseating granulomatous infiltration of many organs including the liver, the lungs, the lymph nodes, and the skin (*39*,*40*). Although such lesions require careful work-up to rule out mycobacterial or fungal infection as a cause, in most cases they turn out to be benign lesions that do not progress and/or spontaneously disappear. The origin of these lesions is not known, but, as already mentioned in the earlier discussion, an autoimmune etiology seems likely. This is supported by the fact that the occurrence of granulomas correlate with both the presence of T-cell abnormalities and the presence of other autoimmune manifestations (*40*). Finally, it should be noted that in some rare cases, these lesions lead to local tissue destruction and require corticosteroid therapy.

A second additional clinical feature of CVI, and one already alluded to, is arthritis. This can be the result of an infection (bacterial or mycoplasmal) or, more often in this era of IVIG treatment, to autoimmune factors (*41*,*42*). In the latter case, the disease is nondestructive and generally involves the large joints in an asymmetric fashion.

Prognosis in CVI

CVI is a life-long disease that, with only rare and poorly understood exceptions, does not spontaneously remit. When untreated, it is a life-threatening condition that can lead to severe and irreversible pulmonary disease within several years of an initial lower respiratory infection. Although intramuscular immunoglobulin slowed this progression, it did not eliminate it, and it was only when IVG replacement was introduced that one could be truly confident that one could prevent the development of irreversible lung disease in most patients. Thus, the clinical rule of thumb is to start patients on IVIG as soon as is

feasible, but certainly after the first lower respiratory infection. Once patients are on such therapy, pneumonia can still occur, but this is the exception rather than the rule. Likewise, patients on therapy develop bacterial infections involving other organs only rarely. As for the dose of IVIG to be administered to CVI patients, experience has shown that maximal clinical effects are achieved when enough IVIG is administered to obtain a "trough" IgG level of 500 mg/dL; this is usually obtained with a dose of 200–400 mg/kg/mo (*43*).

Unfortunately for patients with CVI, the benefits of IVIG do not extend to other features of the disease, such as the various autoimmune manifestations and the increased risk for the development of neoplasms, particularly lymphomas. In this context, in the fairly sizable subgroup of patients with the CVI who manifest the aforementioned GI syndrome, the latter occurs despite of IVIG therapy and is not ameliorated by increased IVIG therapy. This, in fact, provides further support for the idea that this syndrome does not have an infectious etiology. The various noninfectious complications of CVI have, when taken together, a significant impact on survival of patients. Thus, as noted by Cunningham-Rundles, mortality of patients on IVIG therapy is significantly increased when compared to age-matched normals or even patients with XLA (*7*). This latter difference is highly suggestive that abnormalities in T-cell function in CVI (which is not present in XLA), through their effects on autoimmunity and tumor surveillance is a significant factor in patient survival.

A final point concerning patient prognosis is the occasional occurrence of opportunistic infection in CVI. As noted already, this is also presumed to be a manifestation of impaired T-cell function and hence can occur spontaneously in patients in otherwise good health. However, it will occur more frequently in those patients treated with immunosuppressive agents or corticosteroids, usually in dealing with GI manifestations of CVI or autoimmune phenomena (*7*). Although this cannot be avoided in some case, such therapy should used only when other alternative therapy is not available and then only in a time-limited fashion.

Recently, an attempt to improve T-cell function in CVI through the administration of IL-2 linked to polyethylene glycol (PEG-IL-2) has been carried out by Cunningham-Rundles and her colleagues (*44*). It was felt that such therapy would also improve B-cell function on the assumption that at least some of the B-cell dysfunction seen in CVI are secondary to poor helper T-cell activity. So far, short-term therapy with PEG-IL-2 has led to measurable improvement in both B- and T-cell function, but whether these therapeutic approaches can have significant effect on the disease over the long term requires further study.

Immunologic Function in CVI

Although the molecular defect or defects present in CVI have yet to be defined, a great deal of knowledge concerning B- and T-cell function in CVI has been accumulated and it is now possible to make more educated guesses about the etiology of the disease than previously. In the discussion to follow, we will take the position enunciated earlier that CVI (along with IgAD) is essentially a single or unitary disease entity, albeit one that varies somewhat from patient to patient because of quantitative differences rather than qualitative differences in the central immunologic abnormality. It should be noted, however, that this position does not preclude the possibility that there exists occasional patients within the CVI group as currently defined that have a disease caused by a markedly different abnormality that necessitates their separation from the main body of patients.

The modern study of CVI began in earnest in the late 1960s and early 1970s with the advent of techniques for the culture of peripheral lymphoid cells. These studies were constantly refined over the ensuing quarter century as newer methods of culturing and co-culturing defined cell populations became available, along with more specific ways of stimulating the cultured cells. It thus became possible to determine if CVI was primarily a B-cell defect, or, alternatively, a defect of T-cell helper function or T-cell suppressor function. In the discussion to follow, we shall organize these studies by focusing first on B cell function and then on T-cell function in CVI. Following this informational groundwork, we shall attempt to put together a reasonable hypothesis concerning the underlying defect in CVI.

B Cells and B-Cell Function in CVI

A defining feature of CVI is the presence of some level of B cell abnormality as defined by in vitro studies in which the B cells are stimulated by "B cell" stimulants such as Staphylococcus aureus Cowan 1 (SAC), anti-μ antibody or anti-CD40 antibody, each in combination with various cytokines. At one end of the spectrum are patients who present with the most profound B-cell abnormalities marked by reduced B-cell numbers or normal B cell numbers associated with reduced B cell proliferative capacity *(45)*. This sort of abnormality is seen in a relatively small minority of patients and is suggestive of a B-cell defect affecting B-cell development; however, there is no compelling evidence that this is a distinct form of CVI. At the other end of the spectrum are those patients with normal B-cell numbers and proliferative capacity but with B cells that fail to produce normal amounts of Ig in vitro. This group comprises the majority of patients and can be further subdivided into those patients whose cells fail to produce any Ig in vitro and those that

produce normal amounts of IgM along with reduced amount of IgG and IgA.

The above picture of in vitro Ig production in CVI was obtained mainly with studies in which patient B cells were stimulated with SAC or with anti-μ antibody (as well as certain cytokines) (*45,46*). In 1991, Banchereau et al. introduced a new stimulation system in which the B cells were stimulated with anti-CD40 antibody in conjunction with IL-4 or IL-10 (*47,48*). Such in vitro B cell stimulation mimics in vivo B-cell stimulation because B cells interacting with T cells induce the expression of CD40L on the latter, which then back-stimulates B cells via CD40. Studies of CVI B cells conducted by Eisenstein et al. as well as other investigators have shown that single or repeated stimulation of CVI B cells by this method results in secretion of IgM at a level more or less comparable to control B cells in most patients with CVI (*49–52*). In addition, this form of stimulation leads to about half normal levels of IgG production and some IgA production, the latter almost never found with other forms of B-cell stimulation. These results suggest that defective B-cell function in vivo can to some extent be overcome by certain types of stimulation of B cells in vitro. This, in turn, implies that the B-cell defect in CVI can either be "bypassed" by accessory differentiation mechanisms or that the defect is quantitative in that synthesis of certain key differentiation components is decreased (but not absent) and can be artificially increased in vitro.

One conclusion concerning the observation that CVI B cells exhibit increased function in response to anti-CD40 stimulation that is not justified is that CVI is caused by lack of CD40L expression in T cells. Thus, although levels of CD40L expression on CVI T cells may be decreased at least in some patients (*see* further discussion later), anti-CD40 does not fully correct B-cell function in CVI. Furthermore, in a key experiment conducted by Eisenstein et al., it was shown that activated normal T cells did not induce CVI B cell to differentiate into Ig-secreting cells when co-cultured with the latter (*16,53*). Thus, on a functional level, the defect in CVI clearly resides mainly in the B cells rather than in the T cells.

The failure of CVI patient B cells to produce Ig is in reality a dual defect, in that patient B cells display both an inability to undergo terminal differentiation and to undergo switch differentiation. The former is indicated by the fact that in vivo IgM B cells, which do not require switch differentiation, do not produce normal IgM levels. The latter is indicated by the fact that isolated B cells from patients consist largely of $sIgM^+$ B cells and few if any $sIgG^+$ or $sIgA^+$ B cells (*49*). In addition, in vitro stimulation of IgM production is always more nearly normal than stimulation of IgG and IgA production, as already alluded to earlier. This defect in Ig switch differentiation is highlighted by recent studies

in which it was shown that in cultures of CVI B cells stimulated by anti-CD40 and IL-10, in which some level of IgG and IgA was produced, the synthesis of the IgG_4 subclass followed by that of the IgG_2 subclass was the most severely deficient and the synthesis of the IgA_2 subclass was more severely deficient than that of the IgA_1 subclass (*50*). In this study it was also noted that this apparent hierarchy of Ig isotype secretion in CVI corresponds to the sequence of Ig heavy-chain constant region (C_H) gene segments of the 300 kilobase region on chromosome 14 where C_H gene segments are located. In this regard, it is known that C_H genes are located in one of two larger domains, a first domain containing in order μ, δ, γ3, γ1, ψε, and α1 C_H genes and a second and downstream domain containing in order ψγ, γ2, γ4, ψε, and α2 C_H genes. It is thus apparent that the C_H genes in the second domain are more affected than the C_H genes in the first domain.

The reason CVI patients display the aforementioned pattern of Ig isotype production (as well as the related fact that IgAD patients frequently display IgG_2 and IgG_4 subclass deficiencies) is unknown. One possibility is that CVI involves differential defects in the transcriptional activation of switch regions located 5′ to each of the C_H genes that govern switching to the latter genes. While such switch regions contain certain common sequences, they also contain unique sequences and it is thus conceivable that the most severely affected C_H region share particular switch region sequences that are specifically targeted in CVI. This possibility seems unlikely, however, because no patterning of switch-region sequences to explain the secretion of Ig in CVI has yet been found. Another possibility is based on the fact that switch differentiation is a sequential process that involves looping out and deletion of C_H genes, starting with genes at the 5′ end and proceeding toward the 3′ end of the immunoglobulin gene complex. Since each deletion event is associated with a round of proliferation, a defect in the survival of B cells following stimulation may explain the increasing difficulty of differentiation as one proceeds to the 3′ end of the C_H gene segment. Such a defect would also explain the recent finding that some CVI patients manifest decreased IgV gene somatic hypermutation and affinity maturation, because this could also be the result of a defect in B-cell survival (*54*).

Given the fact that in most CVI patients various stimuli induce normal proliferation, it is not too surprising that in several studies that focused on the signaling function in CVI B cells, such function was found to be normal. Thus, in one study in which the production of inositol (Ins) (1,4,5) P_3 was measured in EBV-transformed CVI B-cell lines following anti-Ig stimulation, it was found that levels of Ins (1,4,5) P_3 induced in CVI were comparable to that in controls (*55*). In another

study, in which patient B cells were stimulated with phorbol dibutyrate and ionomycin, stimuli that bypass the BCR and directly activate protein kinase C (PKC), again CVI B cell proliferation was similar to that seen in normal B cells (*56*). Of interest, in this latter study, stimulation by phorbol ester plus ionomycin, although eliciting normal proliferation, did not elicit normal Ig secretion, pointing to a downstream B-cell defect independent of the biochemical pathways involved in cell proliferation.

If indeed the majority of CVI patients display normal B-cell proliferation responses and initial signaling events, one must look further "downstream" for a B-cell differentiation defect resulting in deficient Ig production. Unfortunately, these are difficult to identify given the fact that CVI B cells do undergo some level of differentiation in vitro and thus that any defect present is partial at best. In one study by Kamiko et al., the Ig enhancer region of CVI B cells was examined and it was shown through polymerase chain reaction (PCR) amplification of various parts of this region that the Ig enhancer was normal in size and in sequence (*57*). In addition, in unpublished data from this laboratory, major Ig gene-transcription factors such as Oct-2 or Bob-1 were found to be normally activated in stimulated CVI B cells as defined by electrophoretic mobility shift analysis (EMSA). Finally, although patient cells are deficient in Cμ, Cγ, and Cα mRNA after pokeweed mitogen stimulation, a relatively weak B-cell stimulant, they contained normal or near normal amounts of such mRNA when stimulated with anti-CD40 and IL-10, i.e., under conditions in which they secreted more normal amounts of Ig protein (*49,57*). Thus, the molecular pathways of Ig synthesis in CVI appear grossly intact, qualitatively if not quantitatively.

One interesting possibility that might explain the defects in CVI B-cell function is that anti-apoptotic mechanisms normally brought into play by stimulation of cells are defective and that although CVI B cells exhibit normal levels of proliferation when the latter is measured at 48–72 h, they nevertheless do not survive long enough to undergo switch or terminal differentiation. This explanation fits with the fact that a subset of patients actually display decreased B-cell proliferation, because the latter may actually reflect decreased cell survival in such patients. It also fits with the hierarchy of Ig production described earlier because as mentioned, expression of more downstream C_H genes would require greater B-cell survival than expression of more upstream C_H genes. More direct evidence for this possibility comes from studies of Saxon et al. who have shown that a subset of CVI patients with reduced levels of circulating B cells express increased amounts of Fas antigen and decreased amounts of CD38, a molecule highly expressed on germinal-center B cells that acts as an inhibitior of apoptosis (*58*).

T-Cell Responses in CVI

In the past decade and a half, it has become well established that the more or less obvious B cell defects of CVI are accompanied by relatively subtle but nevertheless definite T cell defects.

Early studies along these lines provided evidence that CVI T cells manifest reduced proliferative capacity when stimulated with standard mitogens. This was followed by a number of studies in which it was shown that CVI T cells produced reduced amounts of IL-2 in mRNA and protein when stimulated with a variety of T cells stimulants such as phytohemagglutinin (PHA), superantigen (staphylococcal enterotoxin B; SEB), or anti-CD2 (*59–62*). This defect could not be attributed to abnormalities in T-cell phenotype because they were usually found in patients with normal numbers of circulating T cells and normal CD4/CD8 ratios; in addition, because CVI patients have a tendency to manifest increased numbers of CD45RO T cells, the cytokine production abnormality could not be attributed to a defect in T-cell maturation.

Interestingly, the aforementioned proliferation and cytokine secretion defects can be "overcome" by more powerful T-cell stimulation. Thus, IL-2 secretion was normalized when cells were stimulated with certain potent anti-CD3 antibodies, phorbol ester (PMA) plus ionomycin or anti-CD3 plus PMA (*59–62*). Such correction of the defect raises the question of whether CVI T cells have an early activation defect. Evidence for such a defect has, in fact, come from Fisher et al., who have shown that $CD4^+$ T cells from a large subset of CVI patients manifest reduced intracellular free calcium when stimulated with recall antigens (tetanus toxoid) or superantigen, but normal free calcium when stimulated with PMA and ionomycin (*63*). In addition, they found that following superantigen stimulation, reduced IL-2 production by peripheral T cells was associated with reduced levels of inositol phosphates (*55*). Taken together, these studies suggest that early activation defects in CVI T cells explain the lower T-cell proliferation and reduced cytokine production, at least in some patients. However, additional study is necessary to establish this point because cytokine production has been observed in patients whose T cells proliferate normally and manifest normal expression of IL-2 receptor mRNA (*59,61*). In addition, proliferation defects in CVI are known to be more or less correctable by the addition of exogenous IL-2 (*64*). It is thus possible that the more important defect with respect to cytokine production lies "downstream," in consonance with the type of defect likely to be present in patient B cells. One possibility here is that CVI T cells, when stimulated by antigen (via antigen-presenting cells), are subject to reduced co-stimulation because they express reduced amounts of CD40L, as discussed later. Finally, a T-cell cytokine production abnormality in CVI cannot be attributed to defective antigen-presenting cell (APC) function because

patient CD4 T cells exhibit comparable levels of IL-2 production when stimulated with PHA in the presence of CVI or normal accessory cells (*59*). In addition, it has been shown that antigen presentation by CVI monocytes and B cells is not impaired (*55,65*).

The cytokine production defect in CVI is not limited to IL-2. Not only the production of IFN-γ, but also the production of IL-4 and IL-5 have been shown to be decreased when CVI T cells are exposed to the same stimulants as was utilized in the demonstration of the IL-2 defect. In the case of IFN-γ, however, the defect may be secondary to an IL-2 production defect, since addition of exogenous IL-2 to cultures of patients cells corrected the IFN-γ production deficiency (*59,61*). The production of yet other cytokines by CVI T cells is still uncertain owing to variability in results and the production of these cytokines by non-T cells. Such is the case for IL-10, for which both increased and decreased production have been reported (*66,67*). Abnormalities in cytokine production other than IL-2 have led to the hypothesis that failure to secrete one or more cytokines critical to B-cell differentiation is the primary defect in CVI. However, this seems unlikely because while CVI T cells may produce less than optimal amounts of cytokines under some in vitro conditions, in the cell mixing studies already mentioned they fully support normal B-cell Ig synthesis; thus, even if production of some cytokines are reduced, such reduction is not sufficient to cause decreased B-cell help.

Although, as mentioned, most patients have normal $CD4^+$ T cell number and normal $CD4^+/CD8^+$ T cell ratios, two important exceptions to this generalization exist. First, a subgroup of patients with low $CD4^+$ T-cell levels have been observed in patients who also manifest low B-cell numbers, splenomegaly, and increased serum tumor necrosis factor (TNF)/neopterin and β_2 microglobulin (*68*). Whether these patients represent a distinct form of CVI with a different pathogenesis from the main body of patients remains to be seen, because these presumably unique manifestations could still be secondary to the presence of infection. Second, a larger subgroup of patients, perhaps 20–25% of the whole, have low CD4/CD8 ratios associated with elevated levels of $CD8^+$ cells, the latter expressing markers suggestive of previous activation (CD45RO and HLA-DR) (*69*). As shown by Jaffe et al., $CD8^+$ T cells from these "$CD8^{hi}$" CVI patients manifest poor proliferative responses when stimulated with anti-CD3 plus PMA compared to $CD8^+$ T cells from CVI patients with normal CD4/CD8 ratios as well as controls. In addition, although $CD8^+$ T cells from $CD8^{hi}$ CVI patients express reduced amounts of IL-2 at both the mRNA and the protein levels, they express increased levels of IFN-γ, IL-4, and IL-5 mRNA (*70*).

In further, more functional studies, $CD8^+$ T cells from $CD8^{hi}$ CVI patients were found to exhibit increased cytotoxic function, as mea-

sured in an anti-CD3 redirected toxicity assay using a P815 cell line target. In addition, in assays of the capacity of these cells to suppress B-cell Ig production, it was found that when co-incubated with normal B cells, the T cells suppress the ability of the B cells to produce IgG but had no effect on the capacity to produce IgM (*70*). This suggests that cytokines produced by these cells, most likely IFN-γ, block certain types of isotype differentiation.

The existence of CVI with increased numbers of $CD8^+$ T cells raised the possibility that these patients have a form of CVI characterized by normal B-cell function that is being suppressed by T cells, as first suggested by Waldmann et al. many years ago (*71,72*). To test this possibility, B cells from the $CD8^{hi}$ CVI cell high subgroup of CVI patients were isolated and stimulated with B-cell stimulants to determine if in the absence of their T cells (putative suppressor cells), their B cells function normally. It was found that in 3 out of the 4 patients studied, although the purified B cells produced IgM in amounts comparable to normal, the production of IgG remained severely depressed. In contrast, one case exhibited normal IgG production *(70)*. One can conclude, therefore, that even in patients with high levels of $CD8^+$ T cells with increased cytotoxic and suppressive potential, the existence of CVI owing to suppressive T cells is the exception rather than the rule. Finally, it is important to note that patients with CVI may manifest T cells (CD8+ T cells) with an increased capacity to suppress Ig synthesis as a consequence of gammaglobulin administration (*73*). Such therapy-induced suppressor T-cell activity is not associated with increased numbers of circulatory CD8+ T cells but is associated with a reduced CD4+/CD8+ T-cell ratio. The basis of this suppressor cell induction is likely to be the capacity of administered Ig to bind to T cell Fc receptors and thereby to induce the latter to manifest increased suppressor function.

On the basis of the very different cytokine production patterns seen in $CD8^{hi}$ CVI patients, as well as initial studies showing that $CD4^+$ T cells in such patients produced normal amounts of IL-2, it was suggested that $CD8^{hi}$ CVI patients formed a distinct subgroup that was clearly distinguishable from the patients with normal CD4/CD8 ratios whose T cells usually manifest reduced lymphokine production. In later studies, however, it was clearly shown that $CD8^{hi}$ CVI patients with high $CD8^+$ T-cell levels also had $CD4^+$ T cells that manifest defective lymphokine production (*63*). Thus, it now seems more likely that this group is not a distinct subset of patients.

In the earlier discussion, it was mentioned that CVI T cells express decreased amounts of CD40 ligand. Thus, in a study performed by Farrington et al., 13 of 31 CVI patients exhibited decreased CD40 ligand (gp39) mRNA expression when stimulated by PHA plus PMA and the

surface expression of CD40 ligand was correspondingly decreased (*74*). In follow-up studies, this finding was corroborated but it was also found that, as in the case of reduced IL-2 production, stimulation of CVI T cells with a PMA plus ionomycin (as opposed to anti-CD3/anti-CD28) led to correction of the defect (*75,76*). It should be noted that while CD40 ligand expression in CVI is reduced at least under some conditions, no mutation in the CD40 ligand gene has been found as in patients with X-linked hyper IgM (XHIM) syndrome. Nevertheless, this abnormality may well contribute to or even explain the abnormality in cytokine production seen in CVI because CD40 ligand interaction with CD40 on APCs is necessary for expression of co-stimulatory molecules, such as B7-1 and B7-2 (CD80/CD86, respectively), and thus may lead to reduced stimulation of T cells in CVI (*77,78*). Indeed, in the study by Farrington et al., there was a correlation between reduced CD40 ligand mRNA expression and IL-2 production of CVI T cells (*74*). Whether the defect in CD40 ligand expression in CVI represents a primary T cell activation defect or a more "downstream" transcriptional defect remains to be seen; given the earlier discussion, it would seem the latter possibility is more likely than the former.

Hypotheses Concerning the Molecular Defect in CVI

The earlier description of the B- and T-cell defects in CVI are incomplete with respect to the fact that they do not provide a clear-cut overview as to the nature of the underlying defect present in most, if not all, patients. In speculating about what this defect might be, it is well to recall that CVI is in fact usually a disease that occurs after an initial period of normal B-cell function, suggesting that an environmental factor, most likely a viral infection, initiates and/or sustains the disease. As noted previously, precedent for this possibility comes from the fact that chronic or acute EBV infection can lead to typical CVI and there exists a genetic disease, X-linked lymphoproliferative syndrome, in which initial EBV infection is followed by severe disease culminating in the occurrence of hypogammaglobulinemia or lymphoma. This occurrence of CVI following EBV infection, in one case in association with the presence of a genetic defect, suggests by analogy that CVI is also owing to encounter with a virus in a genetically susceptible host. In the latter regard, it is now well-recognized that CVI has several genetic antecedents (*see* review by Schroeder).

Because CVI is not an infectious disease in the usual sense, and indeed no evidence of a conventional viral infection has been found in patients with the disease, what sort of viral infection could be present to cause the disease? Here one might postulate that the disease is caused by integration of viral genes in B-cell precursor cells, which perma-

nently alters B-cell development and/or B-cell function in the absence of constant reinfection of mature B cells by viruses. Such integration leads in a minority of instances to B-cell proliferation defects and/or defects in B cell development, hence, the occurrence of B cell-deficient CVI patients. In most instances, however, it does not interfere with B-cell development or proliferation, but instead has its main effects on B-cell differentiation after the cell has reached maturity. It might be postulated that the spectrum of disease arises from the differences in the site of integration. If the latter is near a strong endogenous promoter, more interfering viral product is produced and more severe B-cell disease occurs. Conversely, if it is near a weak endogenous promoter, less-severe B-cell disease occurs (or only IgAD occurs).

One might also postulate that different amounts of viral genome are integrated in various patients and in a subset of patients, one or more viral products are produced that can give rise to surface expression of a viral protein that evokes a $CD8^+$ T cell cytotoxic response. The reason why this response does not lead to secondary B-cell depletion is unclear, but may arise from the fact that the viral product evoking the $CD8^+$ response does not provide a sufficient target for actual cytotoxic B-cell death. On this basis, the existence of a subpopulation of patients with high $CD8^+$ T-cell levels in the absence of an ongoing viral infection of a conventional nature is strong evidence for the viral hypothesis.

Assuming the validity of the aforementioned hypothesis, one might next ask how the integrated viral gene or genes actually affect B-cell differentiation. The answer to this question is also unclear but it is not unreasonable to go back to a point made earlier and to postulate that it causes a defect in B-cell survival leading to premature death of the cells as they undergo either switch differentiation or terminal differentiation. This explanation explains two important facts concerning CVI B-cell phenomenology: 1) that B cell differentiation defect is hierarchical in that IgA and certain IgG subclasses are more abnormal in vitro than IgM and certain other IgG subclasses, a fact best explained by assuming that cells must survive longer to differentiate into IgA and IgG_2, IgG_4 B cells than IgM and IgG_3, IgG_1 B cells; and 2) CVI B-cell dysfunction is at least partially reversed by stimulation with CD40L and IL-10, stimulation that is inherently anti-apoptotic in nature.

If indeed one or more integrated viral genes are the cause of CVI, is the virus involved in fact EBV? Although this possibility is attractive, it is associated with several difficulties. The first is that the EBV encodes anti-apoptotic genes rather than pre-apoptotic genes. Thus, if EBV were involved, one would have to postulate that a mutated EBV gene (or a mutated virus) is responsible for disease. The second is that EBV gains entry into the cell via CD21, a cell surface marker apparently

late in ontogeny. Thus, to explain the entry of the virus into an early progenitor cell, one would have to postulate a new means of viral entry. Finally, as noted earlier, CVI also involves T-cell abnormalities and it is logical to assume that the latter are also owing to integrated viral gene(s). However, no known means of EBV entry into T cells is presently known.

Finally, to prove the earlier "viral hypothesis of CVI," one must at least establish the presence of abnormal viral genome in CVI B cells. This may be difficult, however, if only one or two genes of the virus are necessary to sustain the disease. The best approach, therefore, is to study CVI by genetic subtraction techniques so as to discover genes appearing in CVI B cells not present in normal B cells.

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