Perturbation of the normal immune system in patients with CLL

Francesco Forconi1,2 and Paul Moss3

1Haematology Oncology Group, Cancer Sciences Unit, Cancer Research UK Centre and National Institute for Health Research Experimental Cancer Medicine Centre, University of Southampton, Faculty of Medicine, Southampton, United Kingdom; 2Haematology Department, University Hospital Southampton National Health Service Trust, Southampton, United Kingdom; and 3School of Cancer Sciences, University of Birmingham & University Medical School, Birmingham, United Kingdom

Immune dysregulation is a cardinal feature of chronic lymphocytic leukemia (CLL) from its early stage and worsens during clinical observation, even in absence of disease progression. Although the mechanisms remain unclear, new insights are emerging into the complex relationship between the CLL clone and its immune environment. T cells are increased in early-stage disease and show progressive accumulation and exhaustion. The mechanisms that drive this expansion may include auto-antigens involved in the original clonal expansion. In addition, chronic viral infections such as cytomegalovirus generate huge virus-specific immune responses, which are further expanded in CLL. Attention is now focused largely on the direct immunosuppressive properties of the tumor. Remarkably, CLL clones often have features of the recently described regulatory B cells producing immunosuppressive IL-10. Better knowledge of the regulatory properties intrinsic to CLL cells may soon become more important with the switch from chemotherapy-based treatments, which trade control of CLL with further impairment of immune function, to the new agents targeting CLL B-cell receptor-associated signaling. Treatment with these new agents is associated with evidence of immune recovery and reduced infectious complications. As such, they offer the prospect of immunologic rehabilitation and a platform from which to ultimately replace chemotherapy. (Blood. 2015;126(5):573-581)

Introduction

Chronic lymphocytic leukemia (CLL) is a tumor of mature B cells and comprises 2 major subsets with either unmutated (U-CLL) or mutated (M-CLL) immunoglobulin variable-region genes, indicative of an origin from pregerminal center (GC) or post-GC CD5+ B cells, respectively.1,2 Clinical progression differs between the 2 subsets, with U-CLL associated with a worse prognosis.3 Heterogeneity of clinical outcome is accompanied by a variable grade of B-cell receptor (BCR) energy, measured as low sIgM levels and signaling capacity, which is evident in all CLL but is particularly prominent in M-CLL.4 Tumor cells express the phenotype of “activated” B cells,5 resembling B cells undergoing chronic antigenic stimulation.6,7 This is revealed by presumptive antigen-mediated downmodulation of sIgM, which is reversible after culture in vitro and is reflected in vivo where levels of sIgM determine the strength of signaling.5,8

Both U-CLL and M-CLL are associated with significant perturbations of the immune system, of which immune suppression is the most typical feature, from the early stages.9,10 Infections (~60%) are the leading cause of death in CLL, and the incidence of secondary malignancies is also increased.11-14 Chemotherapy will clearly contribute to immune suppression in most patients, but perturbations within immune function are present from the early stages of disease, and only ~30% of patients will require treatment.10 Hematopoietic-specific autoimmune responses are also observed in CLL and autoimmune hemolytic anemia is the most common, whereas nonhematologic autoimmune phenomena are extremely rare.15 Although clinically overt autoimmune hemolytic anemia may be observed before treatments, these disorders are often precipitated by chemotherapy, suggesting that moderate immune suppression can depress immune regulatory control of autoimmune responses. Interestingly, more intensive combination chemotherapy is protective against autoimmune phenomena and may indicate a more profound disruption of immune effector function.15

This perspective addresses the nature and potential etiology of the immunomodulation observed in association with CLL, and discusses how new targeted treatments alternative to chemotherapy hold the potential to reverse these effects.

Epidemiology of infections in patients with CLL

Before the widespread application of automated blood counting, the predominant clinical presentation of patients with CLL was with recurrent and often severe infections by common pathogens.12 The most commonly documented infections in treatment-naïve patients are respiratory tract and urinary tract infections, predominantly secondary to bacteria (67%), viruses (25%), and fungi (7%).12,15 Staphylococcus aureus, Streptococcus pneumoniae, and Haemophilus influenzae underlie most cases of respiratory tract infection, with Escherichia coli implicated commonly in urinary tract infection.15 Herpesviruses are common chronic infections, but are found at a comparable prevalence with that of the general population.16 HSV seropositivity is almost ubiquitous and the risk of viral reactivation increases during the course of disease. Varicella zoster virus reactivation is a particular challenge, but is infrequent at diagnosis and becomes most apparent after initiation of treatment.15

Features of the innate immune response in patients with CLL

Quantitative and qualitative defects are seen within the innate and adaptive immune response in almost all patients from the time of...
diagnosis. Reduced levels of several complement proteins are observed, with specific reduction of at least some of the C1-C4 components in almost 40% of patients. The associated impairment in the ability to coat bacterial pathogens with C3b may contribute to the risk of infection. Importantly, this impairment in complement activity may also limit the therapeutic action of antibodies, which rely on complement-dependent cytotoxicity.

A range of qualitative deficiencies of neutrophil function has been described including impaired phagocytic killing of nonopsonized bacteria and a reduction in CSa-induced chemotaxis. Interestingly, the circulating monocyte count is increased by >60% in patients with CLL, but such cells carry a “nonclassical” CD14-CD16+ phenotype and have a gene expression profile associated with immunosuppressive properties. Deficiencies in β-glucuronidase, lysozyme, and myeloperoxidase have been reported, associated with a relative “refractory” state with impaired response to classic pathogen-induced inflammatory responses. Although refractory to stimuli from normal B cells, monocytes can be driven to an immunosuppressive or “nonclassical” M2 macrophage-like phenotype upon engagement with CLL-derived soluble stimuli. Natural killer (NK) cells are also increased in the circulation of CLL patients but appear to have several functional defects, including impaired cytotoxic activity, possibly primarily because of defective expression of the NKG2D coreceptor. Nevertheless, NK cells are considered important for mediating the antitumor activity of CD20-specific monoclonal antibodies.

Features of the adaptive immune response in patients with CLL

Marked dysfunction of the adaptive immune response is found in patients with CLL. Alterations within the T-cell repertoire are present at an early stage and become more apparent with disease progression. Interestingly, the absolute CD8+ and CD8+ T-cell counts are increased in early-stage disease, although the relatively higher increase in the CD8+ subset may lead to a reduction of the CD4+/CD8+ ratio (Figure 1). The mechanisms that underlie this increase in the peripheral T-cell count are unknown but may relate to increased mobilization from secondary lymphoid tissue, or to an absolute increase in T-cell numbers. Perhaps the most interesting explanation for the expansion of the adaptive immune response in the early phase of CLL would be that this represents a CLL-specific immune response. There has been a remarkable resurgence of interest in tumor-specific immune responses, particularly in disorders such as melanoma and lung cancer, and antibodies such as anti-PD-1 or CTLA-4, which overcome checkpoint blockade, are proving to be remarkably effective in managing these conditions. However, it has proven very difficult to demonstrate a CLL-specific immune response in vitro, and immunotherapy has not yet been proven in this condition. It is likely that the efficacy of checkpoint blockade will be assessed within CLL within the near future.

Chronic viral infections such as cytomegalovirus (CMV) trigger an increase in peripheral T cells in healthy donors, and may at least partly explain this feature. However, increased numbers of CD4+ T cells have been observed in the lymph nodes of CLL patients and can colocalize with CD38+ tumor cells, raising the possibility of local activation. Oligoclonal T-cell expansions are observed, but are also a feature in healthy elderly individuals and as such may simply represent a response to chronic infection.

In addition to quantitative change, CD8+ and CD4+ T cells also show functional impairment in patients with CLL (reviewed by Riches and Gribben). In particular, there is markedly impaired helper activity. Differential gene expression is also observed compared with age-matched healthy donor T cells, particularly in genes involved in cytoskeletal formation and cytotoxicity pathways. This translates into impaired immunologic synapse formation with antigen-presenting cells as a result of defects in actin polymerization. However, more studies are required to investigate T-cell function within the CLL proliferation centers of the lymph nodes. The proportion of FoxP3+ regulatory T cells (Tregs) is increased in CLL, most notably in late-stage disease, and is likely to contribute to immune suppression.

The major clinical parameter that correlates with infection risk in CLL is hypogammaglobulinemia. Levels of at least one serum immunoglobulin are diminished at an early stage of both CLL and small lymphocytic leukemia (SLL), despite initial low tumor load (Figures 2 and 3). The severity increases with the duration and progression of disease, and progresses to involve all Ig classes (IgG, IgA, and IgM), with a particularly marked fall in IgG3 and IgG4 subclasses. A direct correlation has been observed between low levels of IgG and IgA and increased morbidity and mortality from common bacterial infections, whereas the reduction in serum IgM levels seen early in disease does not seem to influence infection risk. However, trials of prophylactic intravenous immunoglobulins for CLL patients with hypogammaglobulinemia have not proven of major benefit, and one factor may relate to the low levels of IgA within the current therapeutic products.

Although the degree of hypogammaglobulinemia does not appear to differ between the U-CLL and M-CLL subsets, retrospective studies have documented an association between infection risk and immunoglobulin heavy chain variable (IGHV) gene status. In a study of 231 patients, those with U-CLL had increased infectious morbidity and mortality and shorter time to first infection compared with those with M-CLL. Another study of untreated patients showed that U-CLL tumors were correlated with shorter time to onset of recurrent infection. However, U-CLL progress more rapidly, and it remains difficult to exclude that the differences depend on different tumor load in the subsets.

Antibody responses to primary and secondary challenge with antigen are often inefficient from the early stages in CLL. Vaccination is most successful if immunoglobulin levels are preserved, and if conjugated vaccines are used. Notably, administration of live vaccines is potentially dangerous in patients with CLL. Unfortunately, modern conjugated vaccine preparations, which recruit T-cell “help” are still not generally capable of fully overcoming suboptimal immune responses. In a recent study, an adequate response to an initial dose of 13-valent pneumococcal conjugate vaccine was found in only 58% of CLL patients compared with all healthy age-matched controls. This study also observed a very poor increment of plasmablasts within peripheral blood at 1 week after vaccination. Overall the clinical observations suggest that both a direct suppression of B-cell function and an impairment of cognate T-cell help contribute to the poor response to vaccines. Conjugate preparations help to overcome some of these limitations but are not capable of restoring normal function.

Mechanisms of immune perturbations: tumor cells, infection, or both?

The degree of tumor burden is a dominant factor in relation to preservation of immune function. The most notable example is in relation to
progression of hypogammaglobulinemia (Figure 2) or efficiency of vaccine response. An obvious explanation might be anatomic replacement of immune cells in primary and secondary lymphoid tissues by tumor cells. However, impairment of immune function is observed even during early-stage disease (Figure 2), when the degree of tumor infiltration within the marrow is relatively minimal.9 Reduced antibody levels are also seen in SLL despite minimal involvement of the hemopoietic compartments (Figure 3). As such other factors must play a significant role.

There is strong evidence for the importance of infection as an etiological factor in the development of mature B-cell tumors. Examples include the role of HTLV-1 (human T-cell leukemia virus) in adult T-cell leukemia/lymphoma, Epstein-Barr virus in endemic Burkitt lymphoma, and Borrelia burgdorferi in nasal-type B-cell lymphoma.45 No such direct causative link has been established between a specific infective agent and CLL, although disease risk is increased in those with hepatitis C infection.46 Nonetheless, it is possible that viral or bacterial infections may lead to the emergence of pathogen-reactive B cells, which subsequently undergo transformation.6,47 Reactivity of CLL-derived Ig from a small subset of M-CLL using IGHV3-7 with fungal β-(1,6)-glucan has also been reported.48 Interestingly, viral infection and autoimmune conditions can also expand populations of regulatory CD5+ B cells (Bregs),49 with which CLL cells share many features and may be related (discussed in the next section).

Viral infection also has a profound influence on the magnitude of major lymphoid subsets within healthy individuals,50 and drives the accumulation of oligoclonal CD4+ and CD8+ T-cell expansions.51-53 Several lines of evidence now suggest that T cells in patients with CLL have been driven strongly through many cell cycles of replication. In particular, a range of inhibitory receptors, such as PD-1, Tim3, Lag-3, and CTLA-4, have been identified on human T-cell subsets and are induced after sustained T-cell engagement, possibly as a mechanism to control the magnitude and effector function of immune responses.54 The reduced effector function of T cells that express these molecules has often been taken as a representation of T-cell "exhaustion."55 Increased levels of PD1, CD160, and CD244 expression are observed on both CD4+ and CD8+ T cells in patients with CLL, and there is now considerable interest in understanding the mechanisms that drive this
Role of CLL cells in mediating immune suppression: the tumor as a regulatory B cell

A clear observation is that, in untreated patients, the severity of immune suppression in CLL increases with time from diagnosis and that this does not necessarily associate with tumor progression. As such, the policy of “watch and wait” before treatment is also acceptance of...
CD5 Promotes STAT3/NFAT2-mediated IL-10 secretion (24).

CD23 Associates with regulatory B-cell phenotype (24).

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CD25 Reduces T-cell proliferative capacity, inhibits Th1 differentiation, and skews peripheral T cells toward Th2 response in tissue by IL2 deprivation (63).

CD27 Enhances survival of Tregs; induces hypogammaglobulinemia by interacting with CD70 on plasma cells (64,65).

CD39/CD73 Ectoenzymes producing immune suppressive adenosine to inhibit T-cell proliferation (66,67).

CD60/CD86 Induced on activated CLL cells; induce T-cell tolerance (14,68).

CD152 (CTLA-4) Acts as an "off" switch signal via B7.1/B7.2 (CD80/CD86) to control T-cell responses (69).

CD178 (FasL) Induces hypogammaglobulinemia by CD95(Fas)-mediated suppression of primary plasma cell function (70).

CD200 Induces impaired actin synapse formation of T cells; inhibits IL2 and IFN-γ production by CD4+ T cells; promotes Treg differentiation of CD4+ T cells; inhibits antigen-presenting cells (14,71).

CD269 (BCMA) Favors plasma cell death and hypogammaglobulinemia by BAFF/APRIL deprivation (72,73).

CD270 Induces impaired actin synapse formation of T cells (14).

CD274 (PD-L1) Induces impaired actin synapse formation of T cells; enhances susceptibility to apoptosis on CD4+ T cells at late stages of disease; induces hypogammaglobulinemia by plasma cell apoptosis (14,74,75).

CD276 (B7-H3) Induces impaired actin synapse formation of T cells (14).

CD279 (PD-1) Inhibits IFN-γ production by CD8+ T cells (76).

NAMPT Expressed on activated CLL cells, polarizes monocytes differentiation toward M2 macrophages (23).

HLA-G Associates with lower serum IgG, higher serum IL-10, and lower CD4+ T cells (77).

Table 1. Regulatory molecules of CLL cells

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APRIL, a proliferation-inducing ligand; BCMA, B-cell maturation antigen; BAFF, B-cell activating factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IFN-γ, interferon-gamma; NAMPT, nicotinamide phosphoribosyltransferase; NFAT2, nuclear factor of activated T-cells 2; PD-1, programmed cell death protein 1; STAT3, signal transducer and activator of transcription 3.

Improving the management of CLL: immunology comes center stage

The current backbone of CLL therapy relies on the use of chemotherapy agents, such as alkylating drugs or fludarabine, combined with anti-CD20 monoclonal antibodies. Although they have proven efficacy in symptomatic control of disease, these strategies do have a negative impact on immune function. As such, the consequences of these treatments on overall immune function in the patient with CLL will depend on the balance between the improvement that is gained from reduction in tumor burden and with the side effects of therapy. Overall there is a net accumulation of infectious episodes over time and a failure to reverse the underlying features of immune suppression.

Lenalidomide was perhaps the first example of how the immune system may be harnessed in the management of CLL. The drug has several immunomodulatory activities and its use is often associated with an acute inflammatory reaction. The features of this “tumor flare” include upregulation of CD40 and reduced expression of PD-L1 on the tumor cell, T-cell activation, and improvement in immune synapse formation and tumor lysis.
The subsequent introduction of novel targeted therapies with inhibitors of BCR-associated kinases now offers an opportunity for a reappraisal of the consequences of treatment on immune function. Phosphoinositide 3-kinase δ (PI3Kδ) can be targeted by the highly effective oral inhibitor idelalisib. PI3Kδ is expressed in several lymphoid subsets and its inhibition might therefore be expected to negatively affect immune competence. Instead, no overt increase in infection rate has been observed during treatment, and there may be some improvement in immune parameters. Despite these observations, immune dysregulation may be a concern and it is likely that the side effect of colitis reflects the consequence of impaired gut immunity and tolerance to commensal microbiota.

Another key enzyme is Bruton’s tyrosine kinase (BTK), which plays an essential role in BCR signaling in normal and CLL B cells, and which is targeted by ibrutinib. Therapeutic efficacy is obtained initially by the death of tumor cells within lymph node and bone marrow, although a significant proportion of tumor cells egress and persist as nondividing cells in the circulation up to several months during treatment. Current studies of patients taking ibrutinib suggest that there is recovery of humoral immunity, with an increase in serum immunoglobulin levels, predominantly of the IgA isotype, and a gradual significant decrease in infection rate, even when the circulating CLL count remains elevated. This may reflect the ability of bone marrow plasma cells to recover function at least partially, when relieved of suppressive activity of the CLL clone. However, BTK is important in immune tolerance and the potential development of autoimmune antibodies is something that should be monitored in future studies, particularly in light of the observation that ibrutinib may induce immunogenic cell death. In addition, it should be noted that congenital BTK deficiency leads to marked impairment of B-cell development, hypogammaglobulinemia, and recurrent infection, and the effect of chronic drug inhibition on the adult humoral immune system remains unclear. Ibrutinib also targets interleukin-2–inducible kinase within T cells, suggesting possible off-target effects that could contribute to disruption of immune homeostasis in the longer term.

Conclusions

Perturbation of the immune system is a dominant feature of CLL, but the complex mechanisms that mediate this are still largely unknown. Immune suppression is revealed by predisposition to infection. Profound alterations are observed within the immune repertoire of CLL patients and it will be important to determine to what extent this may reflect an attempt of the tumor cells to evade immune control. Importantly, CLL cells resemble natural Breg cells in many ways, and attention is now focusing on the direct immunosuppressive properties of the tumor compartment. Regulatory activity may be increased after local activation at proliferation centers within secondary lymphoid tissue, and future
efforts should focus on the importance of tissue microenvironment in relation to local immune response. Finally, the new oral inhibitors of B-cell signaling appear to offer the potential for some recovery of endogenous immune function, although their use will require careful monitoring of the consequences of chronic suppression of natural B-cell function.

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Authorship

Contribution: F.F. and P.M. designed research, reviewed literature, shared data, and wrote the manuscript.

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Correspondence: Francesco Forconi, Cancer Sciences Unit, University of Southampton, Cancer Research UK Centre, Somers Cancer Research Building, MPR24, Southampton General Hospital, Southampton, SO16 6YD, United Kingdom; e-mail: f.forconi@soton.ac.uk.

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