Infections remain a major cause of morbidity and mortality in patients with chronic lymphocytic leukemia (CLL). The pathogenesis of these complications is related to immune defects inherent to the primary disease as well as to therapy-related immunosuppression. The spectrum of infections seen has evolved with the therapeutic use of purine analogs, which induce specific cellular immune defects, as well as the monoclonal antibodies alemtuzumab and rituximab. Although bacterial infections are most common, fungal and herpesvirus infections are also seen with use of these agents. This overview will summarize the pathogenesis of infection in patients with CLL as well as the spectrum of infection and approaches to the prophylactic and therapeutic management of these complications.

Pathogenesis

The pathogenesis of infection in patients with chronic lymphocytic leukemia (CLL) is multifactorial. The major risk factors for infection in these patients are immune defects that are inherent to the primary disease process, and therapy-related immunosuppression. Disease-related defects include hypogammaglobulinemia, as well as perturbations in cell-mediated immunity, complement activity, and neutrophil function (Table 1).1,2

Hypogammaglobulinemia, which occurs in virtually all patients with CLL, may be profound and correlates with disease duration and stage. This defect may be related to functional abnormalities of both T- and non-clonal CD5+ B-cells, and primarily affects the level of immunoglobulin subtypes IgG3 and IgG4. Even with a therapeutic response, there is no improvement in the underlying defect. Hypogammaglobulinemia has been associated with the frequency of infections and survival in these patients, although the correlation between a specific immunoglobulin class deficiency and infection risk is not as clear.1,3,4 The increased frequency of respiratory tract infections in patients with CLL of all stages may be related to deficiencies in serum IgA and IgG4, as well as defects in mucosal immune function.4

The impact of immunoglobulin VH mutation (i.e., unmutated versus mutated) status on humoral immunity and infectious complications has recently been examined.5,6 In the series of Sinisalo et al, no significant differences in immunoglobulin levels, mannan-binding lectin, or immune responses to Haemophilus influenzae B vaccination were found between two groups of patients that differed in the mutational status of the B-cell clone.5 Likewise, no difference in infection rate was found among the mutated and unmutated patients in this series. In a retrospective analysis of 280 patients with CLL, those with an unmutated immunoglobulin VH gene status had a significantly shorter time to first infection and higher infection-related mortality than those patients with immunoglobulin VH gene mutations, despite immunoglobulin levels being comparable among the two groups.5 However, unmutated gene status had no impact on the occurrence of recurrent infections. Patients with FISH-detectable genetic abnormalities such as p53 and CD-38+ cells also had a shorter time to first infection. ZAP-70 expression levels had no impact on risk of infection.

Limited data exist regarding the integrity of mucosal immunity in patients with CLL and the relationship between systemic immune dysfunction and mucosal immune defects. In a preliminary report, levels of serum and mucosal (salivary) IgA, IgG, and IgM were measured in a se-

Table 1. Inherent immune defects in patients with chronic lymphocytic leukemia (CLL).

- Hypogammaglobulinemia
- Inhibition in B-cell proliferation
- Cell-mediated immune defects
  - Functional abnormalities of T-lymphocytes, nonclonal CD5+ B-lymphocytes
  - Abnormalities in T-cell subsets, with a decreased CD4/CD8 ratio
  - Excessive T-suppressor and deficient T-helper cell function
  - Downregulated T-cell function
  - Defects in NK-cell, lymphocyte-activated killer cell activity
  - Reduced T-cell colony-forming capacity
  - Defective antibody-dependent cytotoxicity
  - Defective delayed hypersensitivity responses
- Defects in complement activity
  - Reduction in complement component levels
  - Defects in complement activation and binding
- Neutrophil defects
  - Defects in neutrophil function (phagocytic, bactericidal activity, chemotaxis)
  - Reduced absolute neutrophil count
- Monocyte defects (deficiencies in β-glucuronidase, lysozyme, myeloperoxidase)
- Potential mucosal immune defects
aries of patients with CLL. With hypogammaglobulinemia, salivary IgM levels were profoundly decreased in the CLL patients, but no differences were found in salivary IgG or IgA levels among patients with CLL and controls. No association was found between salivary immunoglobulin levels and infections. It is not known whether mucosal immunity is regulated independently of systemic immune function in patients with CLL, and whether the mucosal B cells are part of the malignant B-cell clone.

Despite known defects in cell-mediated immunity and complement activity, no correlations have been made with these defects and the occurrence of infectious complications. It has also not been determined whether these defects improve with disease response to therapy. As with B-cell defects, the T-cell defects become more pronounced with advanced-stage disease and appear to be quite broad (Table 1). Some of this dysfunction may be explained by the fact that the B-CLL cells secrete TGF-β, which inhibits B-cell proliferation, and also release circulating IL-2 receptor, which binds endogenous IL-2, resulting in down-regulation of T-cell function. The CD8+ B-CLL T cells secrete IL-4, which can induce expression of the bcl-2 protein, which may contribute to the pathogenesis and progression of CLL.

Complement levels are reduced in most advanced-stage patients with CLL, and in many with early-stage disease. Deficiencies in at least one component of the complement system have been demonstrated, with the most common deficiency being that of properdin. Defects in complement activation and binding, as well as reduced expression of complement receptors CR1 and CR 2 on B-CLL cells, have also been found.

Quantitative and qualitative defects of neutrophils and monocytes may also be seen in these patients. The absolute neutrophil count is normal to slightly decreased in untreated patients with CLL and may decline further with disease progression, resulting in further marrow infiltration and the use of myelosuppressive therapy. Although neutrophil function is generally normal in untreated patients, it has been found in some series that the sera of CLL patients will inhibit neutrophil phagocytic and bactericidal activity. A decrease in random migration and fMLP and C5a-induced chemotaxis has also been demonstrated. Deficiencies in β-glucuronidase, lysozyme, and myeloperoxidase levels in monocytes have also been reported.

### Spectrum of Infections

#### Spectrum of infection in patients treated with conventional alkylator-based regimens

For decades, chlorambucil, given alone or with corticosteroids, was the mainstay of therapy for patients with CLL. In these patients, the majority of infections are bacterial in origin, caused by common organisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Table 2). Recurrent infections are also commonplace, as are infections originating at mucosal sites. The respiratory tract is, and remains, the most common site of infection in these patients. In contrast, fungal and viral infections occur much less frequently in chlorambucil-treated patients, generally occurring in the setting of therapy-related neutropenia in advanced stage disease patients.

#### Spectrum of infection in patients treated with fludarabine-based regimens

In the past two decades, the purine analogs, specifically fludarabine, have supplanted the alkylators in the first-line therapy of patients with CLL, due to the improved treatment outcome. However, use of this agent has altered the spectrum of infection to include both bacterial infections common to patients with CLL, as well as opportunistic infections (*Listeria*, mycobacterial species, *Nocardia*, *Candida*, *Aspergillus*, *Cryptococcus*, *Pneumocystis*, and herpesviruses [*cytomegalovirus* (CMV), varicella zoster, herpes simplex]). The concomitant use of corticosteroids increases the risk of opportunistic infections and should be avoided.

The pathogenesis of these infections is related to the quantitative and qualitative T-cell abnormalities induced by these agents. A significant decline in the peripheral blood T-cell level occurs early in therapy, via the mechanism of inhibition of cytokine-induced activation of STAT-1 and STAT-1–dependent gene transcription. The impact is greater on CD4+ than on CD8+ or natural killer (NK) cells, and may persist for 1 to 2 years after discontinuation of therapy. Declines in the B-cell and monocyte counts may also occur, with a variable impact on immunoglobulin levels.

Risk factors for infection in fludarabine-treated patients have been identified. Disease stage may be predictive for infection. Heavily pretreated patients are also at increased risk for infection, compared with previously untreated patients. Response to therapy may also be a risk factor for infection, despite the fact that response may not correlate with immune reconstitution. Infections are most common in the first several cycles of therapy, and relatively uncommon in responding patients after discontinu-
ation of therapy, with the gradual return of the CD4+ cells.8

In a large single-center series of patients who received fludarabine, alone or with corticosteroids, major infections were more common in previously treated patients than in treatment-naive patients (58% vs 34%; P < .001).13 Risk factors for major infections included advanced stage disease, prior CLL therapy, and an elevated serum creatinine. Among a series of 187 CLL patients treated with various regimens in the purine analogue era, the only risk factor for infection in treatment-naive patients was an elevated lactic dehydrogenase (LDH).18 Risk factors for major infections (those necessitating parenteral therapy and/or hospitalization) in multivariate analysis included the number of prior regimens and hemoglobin lower than 12 g/dL. In this series, age and renal function had no impact on infection rate. The number of prior regimens was the only risk factor for herpetic infections. The impact of prior therapy on infection risk has also identified by others.17 Last, risk factors for infection were identified in the intergroup trial of treatment-naive patients with CLL receiving fludarabine, chlorambucil, or both agents (F+C), including a low baseline serum IgG level.12 Advanced age and a decreased creatinine clearance were risk factors for infection only among patients who received F+C. In contrast to prior series, there was no association between infection risk and either response to therapy or advanced stage disease.

Several studies have addressed the relative risk and spectrum of infections in patients treated with fludarabine as compared to conventional alkylator-based regimens.12,18 In preliminary EORTC report in which previously untreated CLL patients received therapy with either fludarabine or high dose chlorambucil (10 mg/m²/d), moderate to major infections were more common in patients treated with fludarabine (37%) than chlorambucil (37% vs 12%; P = .043).18 The infectious complications among 544 previously untreated patients with CLL randomized to chlorambucil, fludarabine, or F+C on an intergroup trial have also been reported.12 Patients receiving F+C had more infections than patients receiving either single agent (P < .0001). Patients receiving fludarabine had more infections per month of follow-up, major infections and herpesvirus infections compared to those who received chlorambucil (P = .055, .008, .004, respectively). Pneumocystis infections were distinctly unusual, and no Aspergillus infections were seen. In contrast, among previously untreated advanced-stage patients with CLL randomized to therapy in a large French trial with fludarabine or one of two alkylator-based regimens, no opportunistic infections occurred among the fludarabine-treated patients.18 Lastly, in a meta-analysis of single-agent fludarabine therapy trials, it was found that significantly more grade 3/4 infections occurred as compared to alkylator-based therapy.19

Fludarabine plus cyclophosphamide (FC) has been studied in several trials.20-23 In an intergroup trial, previously untreated patients with CLL were randomized FC with filgrastim support and antiviral prophylaxis, or to fludarabine alone.21 All patients received Pneumocystis prophylaxis. Infection rates were less than 10% in both groups. The German CLL Study Group studied FC as first-line therapy, both as a single-arm phase 2 regimen, as well as in a randomized comparison to single-agent fludarabine in younger patients with CLL.22,23 In the latter study, the occurrence of severe and opportunistic infections was comparable, being 33% with single-agent fludarabine and 40% with FC.24 However, dose reductions due to myelosuppression were more common with FC, which may have resulted in a lower risk of infection. In addition, this study included a younger patient population, who may well be at a lesser risk of infection than older patients. The addition of oblimersen, an antisense agent, to the FC regimen does not appear to increase the risk of opportunistic infections.25

The FC regimen has also been used in previously treated patients. In one series, 57% of patients receiving FC had infections or fever of unknown origin, of which 26% were caused by herpesviruses and 7% by fungal pathogens.26 The incidence of these complications in the first 3 cycles of therapy was 74%. Last, the occurrence of Epstein-Barr virus–associated lymphoproliferative disorders has been reported in patients who received FC therapy followed by autologous stem cell transplantation.27

Rituximab, which results in a transient reduction in the B-cell level, has been used as a single agent and in combination with other cytotoxic agents in the treatment of CLL. When used as a single agent, infections have been minimal with no significant opportunistic infections.28 In a randomized phase 2 Cancer and Leukemia Group B (CALGB) trial of 104 patients with previously untreated CLL, rituximab was used either concurrently or sequentially with fludarabine.29 Approximately 20% of patients had grade 3/4 infections. Opportunistic infections were diagnosed in 8 patients receiving concurrent therapy (16%) and in 14 patients who received sequential treatment (26%), with the majority being localized herpesvirus infections and only 2 cases of Pneumocystis pneumonia.

The combination of fludarabine, cyclophosphamide, and rituximab (FCR) has been studied in both treatment-naïve and previously treated patients with CLL.30,31 The majority of these patients received antiviral as well as Pneumocystis prophylaxis for varying intervals. In previously untreated patients, although one-third of patients had infectious complications, only 2.6% had major infections.30 Although 11 of 224 patients (5%) had reactivation of herpes simplex or herpes zoster, none of these occurred in patients on prophylactic antiviral therapy. A small number of opportunistic infections occurred (Pneumocystis, Aspergillus, Candida glabrata, CMV). When FCR was used in 177 relapsed patients, major infections occurred in 16%, including 1 case of CMV pneumonitis.31 Herpesvirus infections occurred in 1% of treatment courses. The incidence of major infections was found to be comparable in fludarabine-sensitive and fludarabine-refractory patients.
Spectrum of infection in patients treated with other purine analogs

As with fludarabine, treatment with 2-chlorodeoxyadenosine (2-CDA) results in quantitative abnormalities in the peripheral blood T-cell subsets. After discontinuation of this drug, although the CD8 counts normalize after several months, significant decreases in the CD4 counts may remain for 1 to 2 years. Risk factors for infection with this agent are similar to those with fludarabine. The incidence of infectious complications was 65% and that of pneumonia/bacteremia was 25% in a small series of fludarabine-refractory patients who received 2-CDA therapy. In patients 55 years and older treated with 2-CDA, prior therapy was a risk factor for infection (45% vs 26%; P < .05).32 These authors reported an infection rate of 16% in another series of elderly patients treated with 2-CDA.33 Grades 3 to 5 infections were reported in 43% of fludarabine-refractory patients who received 2-CDA in a CALGB trial.34 Although bacterial infections were most common, cases of herpes simplex, herpes zoster, cerebral toxoplasmosis, and candidal esophagitis were also reported. Cases of disseminated herpes zoster and pulmonary aspergillosis have been reported from a series of patients with advanced-stage refractory CLL treated with 2-CDA.35 Likewise, in a series of 378 patients with CLL treated with 2-CDA alone or with prednisone, infections/fever of unknown origin and herpesvirus infections occurred more commonly among previously treated patients than in treatment-naïve patients (49% vs 38%; P = .03, and 25% vs 20%, respectively).36 Robak et al reported on a series of treatment-naïve patients with CLL who were randomized to therapy with 2-CDA or chlorambucil, with concurrent prednisone.37 Febrile neutropenia and fever of unknown origin were more common in patients receiving 2-CDA (23% vs 11%; P < .02), as were herpesvirus infections (21% vs 11%).

Cellular immune defects induced by deoxycoformycin (pentostatin) therapy also persist for several months after discontinuation of therapy. In a CALGB Phase II trial of pentostatin therapy for CLL patients, some previously treated and others treatment-naïve, infections were seen in over 50% of patients, occurred early in treatment, and were especially common in advanced-stage patients with prior therapy. Opportunistic infections (herpes simplex, herpes zoster, Candida, Pneumocystis) occurred in 26%.38 In an Eastern Cooperative Oncology Group (ECOG) study of pentostatin, chlorambucil, and prednisone therapy for previously untreated patients with CLL, grade 3 infections were seen in 31% of patients, including bacterial, fungal, and Pneumocystis pneumonias, and bacteremia, as well as herpes zoster infections in 20% of patients.39 In a smaller series of previously treated CLL patients, grade 3/4 infections occurred in 9% of patients.40 Results of therapy with pentostatin, cyclophosphamide, and rituximab (PCR) for previously treated patients with CLL have been reported, with grade 3/4 infections occurring in 28% of patients.41 Likewise, results of first-line PCR therapy have recently been reported, with approximately 10% of patients having grade 3/4 infections.42 Pneumocystis and antiviral prophylaxis has been recommended with this regimen.

Spectrum of infection in patients treated with alemtuzumab

In the past decade, the use of the CD52 antibody alemtuzumab has been studied in patients with CLL.43-49 As well as therapy-related neutropenia, this agent results in profound defects in cellular immunity, with significant reductions in B, T, and NK cells, that develop shortly after initiation of therapy and persist for at least 9 months after treatment discontinuation.43 There is no correlation between the cumulative alemtuzumab dose, route of administration, and the severity or length of immunosuppression. Prophylactic antimicrobial therapy for Pneumocystis, fungi, and herpesviruses is generally used, due to the increased risk of these opportunistic infections. CMV reactivation occurs in an estimated 10% to 25% of patients. Likewise, cases of toxoplasmosis, adenoviral infection, and acanthamoebiasis have also been reported.46 These infections may be more common in nonresponding patients than in responders.46,47 In the pivotal trial for this agent in patients who had failed fludarabine therapy, 27% of the 93 patients sustained grade 3/4 infectious complications, including cases of aspergillosis, zygomycosis, candidiasis, Listeria meningitis, Pneumocystis pneumonia, and CMV reactivation.46

Alemtuzumab has also been used in combination regimens and as consolidation therapy for minimal residual disease. When given with rituximab for patients with relapsed/refractory B-cell disorders, 52% of patients developed infections and 24% had CMV antigenemia despite Pneumocystis and antiviral prophylaxis.48 CMV reactivation has been a significant issue in several trials using consolidation therapy with this agent, with early stoppage of one study due to severe infectious complications.42,43 In a preliminary report of a CALGB trial in which previously untreated patients with CLL received 4 cycles of fludarabine followed by 6 weeks of alemtuzumab therapy for those patients with responsive or stable disease, grade 3/4 infectious complications were quite common.49 Due to the common occurrence of CMV reactivation, weekly qualitative PCR testing was implemented. Alemtuzumab therapy was discontinued in patients who developed CMV reactivation and they were treated with ganciclovir. Pre-emptive ganciclovir therapy has also been used by other investigators.45 Preliminary infectious toxicity data from a subsequent CALGB study in which treatment-naïve patients with CLL received 6 cycles of FR followed by alemtuzumab consolidation will be reported at the 2007 meeting of the American Society of Hematology.
Strategies for Prevention and Prophylaxis of Infection

Immunoglobulin therapy
In a randomized placebo-controlled multicenter study of patients with CLL with either hypogammaglobulinemia or prior infections, the benefit of prophylactic intravenous immunoglobulin (IVIG; 400 mg/kg every 3 weeks) was examined. Although patients treated with IVIG had significantly fewer minor or moderate bacterial infections, there was no decrease in major infections or mortality. However, it was found that the benefit related to IVIG administration did not improve quality or length of life and was not cost effective. A subset of these patients continued IVIG in a crossover study, in which it was found that major infections were reduced in the months that IVIG was administered.

Prophylactic low-dose immunoglobulin efficacy has also been examined. Although a reduction in infections has been seen in some of these series, improvement in immunoglobulin levels may not correlate with this improvement. An important aspect is that IVIG infusions do not replace either IgM or IgA, which have been implicated as risk factors for infection in some series. In one of these series, 34 high-risk patients with CLL were randomized to either 250 mg/kg or 500 mg/kg IVIG given every 4 weeks, with no difference in infections between the two doses. Even at these lower doses, cost-effectiveness remains an issue, especially in comparison with prophylactic oral antimicrobial agents. Guidelines for the use of IVIG would be helpful and should address the optimal dose and schedule of IVIG, as well as which patient subsets should receive such therapy.

Oral prophylactic antimicrobial agents
Although there have been no prospective randomized trials to aid in defining the role of prophylactic antimicrobial agents in this patient population, some guidelines have come from a variety of CLL treatment trials and anecdotal reports. As the use of concomitant corticosteroids with fludarabine increases the risk of opportunistic infections caused by Listeria and Pneumocystis, prophylaxis is often used with this therapy. Although varicella zoster and herpes simplex infections were more common among patients who received fludarabine compared with those who received chlorambucil on the intergroup trial, the use of routine antiviral prophylaxis in fludarabine-treated patients should be examined prospectively before firm recommendations for routine antiviral prophylaxis should be made. However, antiviral prophylaxis has been recommended by some in the setting of a low CD4 count. It has also been recommended that elderly patients treated with fludarabine also receive routine antimicrobial prophylaxis due to the increased risk of severe infections in these patients. Although the addition of rituximab to fludarabine therapy does not appear to increase the risk of either bacterial or Pneumocystis infections, and localized herpesvirus infections may be minimally increased, no routine prophylactic antimicrobial therapy is recommended for patients receiving this regimen. Antiviral and Pneumocystis prophylaxis is recommended for therapy with FC as well as with FCR. As the immune defects rendered by purine analog therapy as well as alemtuzumab may persist for several months to a year or two after discontinuation of therapy, prophylaxis in this setting may be continued for some months after treatment is stopped. With alemtuzumab therapy, weekly PCR screening for CMV antigen has been advocated. The institution of ganciclovir therapy has been recommended with positive PCR results, as well as in the setting of fever of unknown origin in these patients. Guidelines for prophylaxis with regard to the treatment regimen used would aid oncologists in the management of such patients. Last, myeloid growth factor support has been used with fludarabine-based combination regimens, both to reduce myelosuppression as well as to allow the delivery of full-dose therapy.

Vaccination strategies
The use of a variety of immunizations, including pneumococcal, influenza, hemophilus, tetanus, typhoid, diphtheria, and mumps, has been examined in the CLL population. Immunization responses are suboptimal due to impaired antibody production as well as defects in antigen presentation. Serologic responses to pneumococcal, hemophilus, and influenza vaccines were enhanced in patients with IgG levels higher than 700 mg/dL in one series. Other authors have concluded that immunization responses may be superior with protein and conjugated vaccines than with polysaccharide vaccines, and that vaccine response may be enhanced with adjuvant ranitidine treatment. Unfortunately, most of these studies are small; thus, formal vaccine recommendations for this patient population are lacking. Response to vaccination may well be different in patients in remission as compared to those patients with active disease.

Issues for the Future
Infectious complications continue to have a significant effect on the clinical course of patients with CLL, despite of or because of our treatment advances in the therapy and outcome of patients with this disorder. As new agents and treatment approaches are developed, not only is the effect of these therapeutic modalities on the traditional disease outcome parameters such as response rate, remission duration, and survival of importance, but also of significance is the influence of these agents on subsequent immune function and infectious complications. Further study of mucosal immune function in these patients will be of interest. Likewise, it will be important to identify discrete patient subsets that are at increased risk for infection, using measures such as baseline demographic and disease characteristics as well as response parameters, so that both prophylactic and therapeutic interventions may be best used to reduce the incidence and impact of infections in this patient population.
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