THE FOUR PROBLEMS that still limit the overall success of allogeneic bone marrow transplantation (BMT) are regimen-related toxicity, recurrent leukemia, graft-versus-host disease (GVHD), and cytomegalovirus (CMV) infection. The last 5 years have seen considerable progress in improving the treatment and prevention of GVHD and decreasing leukemic relapse. Until recently, cytomegalovirus was a major cause of morbidity and mortality after allogeneic BMT and, among patients who were seropositive, approximately 15% to 20% of the deaths occurring after transplant could be attributed to CMV disease of the lung. The recent substantial progress in the approach to CMV infection and disease after allo- genic BMT and, among patients who were seropositive, approximately 7 to 10% of the deaths occurring after transplant could be attributed to CMV disease of the lung. The recent substantial progress in the approach to CMV involves not only the treatment but also the prevention of CMV-associated pneumonitis (CMV-IP). Nevertheless, despite these improvements in patient management, the control of CMV remains less than optimal. This review summarizes the progress to date and describes future strategies for prevention of CMV-related complications of BMT.

CLINICAL DESCRIPTION OF CMV-ASSOCIATED PNEUMONITIS

CMV-IP in the allogeneic marrow recipient develops approximately 7 to 10 weeks after BMT, with a median onset of approximately 50 days and with most episodes occurring in the first 100 days. Patients usually present with the sudden onset of nonproductive cough, tachypnea, and occasionally chest pain. Hypoxia is the major physiologic abnormality observed and radiologic evaluation suggests interstitial pneumonitis. The infiltrates become diffuse, although there can be considerable variation in the initial radiologic pattern. The histopathology of CMV-IP involves thickening of the interalveolar membranes with a cellular infiltrate and edema. Several studies have indicated that the quantitative assessment of infectious CMV, either in a lavage or in tissue, cannot distinguish patients in terms of disease severity, and virus burden is not predictive of outcome. In fact, before improved anti-CMV therapy, nearly all patients progressed to respiratory failure with only rare examples of recovery.

RISK FACTORS

Although it would appear to be obvious, the most significant risk factor for the occurrence of CMV-associated disease after allogeneic BMT is the development of CMV infection. This is important to note because it is a strong confirmation that the heterogeneous syndromes which can present during CMV infection are, in fact, caused by this infection and that CMV is not merely present and masking another pathologic process. In addition, the virologic risk allows us to focus on management of patients by recognizing the factors that predict for CMV infection and disease. Thus, an important predisposing factor is the serologic status of the patient and the donor. In a seronegative donor who has a seropositive recipient, the donor marrow itself, as evidenced by studies that eliminate CMV-positive blood support as a component of virus transmission after BMT. However, in a CMV seropositive recipient, the donor status is probably not relevant to the subsequent development of infection, although the donor marrow immune response could influence subsequent disease occurrence.

A second major risk factor for CMV-IP is the alloreactivity of the developing donor graft. This is clear from the observation that the occurrence of GVHD is associated with the development of CMV pneumonitis and that patients with syngeneic transplantation who have approximately the same rate of CMV infection show only a low incidence of CMV-IP. Similarly, patients undergoing autologous BMT, despite similar preparatory regimens including total body irradiation (TBI), have a relatively low incidence of CMV-associated pneumonia.

Thirdly, after BMT, the development of a virus-specific immune response to CMV is an important determinant in limiting virus infection and disease after BMT and should be considered an additional risk factor. The development of HLA-restricted cytotoxic T-lymphocyte (CTL) function is the most important immune response for protection of the host from severe disease and mortality after BMT. Quinna et al initially demonstrated that CMV-specific CTL function was associated with survival from CMV-IP in this patient population. The role of virus-specific CTLs in protecting the patient was confirmed by an analysis of CTL development in allograft recipients with and without CMV-IP. Patients who developed a CTL response early after BMT did not develop CMV disease as frequently as those patients whose response was either low or delayed. Ironically, then, it appears that immunologic responses of recipients of allogeneic marrow transplantation can be either a condition for the development of CMV-IP or an essential component of host resistance needed to limit infection and prevent disease. Patients with GVHD are at high risk for the development of IP and have impaired development of CMV-specific CTL function. Patients who have unimpaired development of CMV-specific CTL function are able to recover from CMV infection after BMT.

HISTORY OF TREATMENT OPTIONS

In the decade of the 1980s, considerable advances were made in the development of antiviral agents. Attempts to

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treat CMV-IP with these new drugs was uniformly unsuccessful despite their ability in certain instances to exert a prompt antiviral effect. For example, use of vidarabine, interferon-gamma, interferon-alpha, and foscarnet did not produce a significant improvement in the outcome of CMV-IP. It was particularly disturbing that ganciclovir and foscarnet, which showed clinical efficacy for the treatment of CMV retinitis in acquired immunodeficiency syndrome (AIDS), failed to alter the clinical course of CMV-IP in the marrow recipient. Attempts were also made to treat CMV-IP with CMV-antibody–enriched intravenous immune globulin (CMVIG), ending with mixed results.

This led to the suggestion, because of the allogeneic nature of the marrow grafts, that the combination of immune modification and viral inhibition could be an approach to this disease. The initial use of combined treatment was reported by Reed et al., who described the unsuccessful use of ganciclovir plus methylprednisolone in six marrow recipients.

Animal studies deserve note because, simultaneously, they provided results relevant to the approach of combined use of ganciclovir and immune globulin. In a mouse model of CMV-IP, Shanley and Pesanti reported that ganciclovir, while decreasing the amount of murine CMV infection in mouse lung, failed to prevent interstitial pneumonia. However, Wilson et al. demonstrated that the combination of ganciclovir and mouse immune serum would protect from a lethal challenge with murine CMV. In this study, neither ganciclovir nor immune serum alone provided protection.

These studies set the stage for regimens that combined ganciclovir and intravenous immune globulin (ganciclovir/IVIG) for the treatment of CMV-IP. The initial reports of combined use of ganciclovir/IVIG and improved outcome of CMV-IP were by Bratanow et al. and by Reed et al. Subsequently, several centers published results using this treatment. This approach produced the first consistent, successful reversal of CMV pneumonitis in recipients of BM allografts. Thus, it was not until ganciclovir was combined with IVIG that an improvement in the outcome of this disease was observed. Although these results were derived from uncontrolled studies, ganciclovir/IVIG has become the recommended treatment for CMV-IP in the BMT recipient.

Despite the improvement in outcome with this treatment, it is less than satisfying for several reasons. The mechanism of action is not known, and, as noted, there have been no controlled trials to confirm these results. In addition, some patients do not respond at all, and, among those who do respond to treatment, the long-term follow-up of these patients indicates that only approximately one third survive for 6 months. Therefore, although this regimen has become the standard of care in BMT centers, the outcome remains poor, and there is no established rationale for its use. Clearly there is a need for a new and better method for approaching the problem of CMV-IP in the marrow recipient.

PREVENTION OF CMV INTERSTITIAL PNEUMONIA

The most successful means of preventing CMV pneumonia in a patient is to prevent exposure to the virus itself. Because most patients coming to marrow transplantation are seropositive, this strategy has only limited application. Nevertheless, it is recognized that the presence of leukocytes in blood products increases the transmission of CMV, and the use of CMV-seronegative blood products in CMV-seronegative recipients decreases the incidence of primary CMV infection. Because no such effect occurs in CMV-seropositive patients, the use of screened blood products should be reserved for CMV-seronegative recipients. This approach should probably be used also in patients with either autologous or syngeneic grafts. Although the risk of CMV disease is lower, patients can develop disease, which in some cases can be fatal and avoidance of CMV exposure from transfusion support would likely avoid this complication. There are difficulties for the blood bank in providing for patients having large platelet and red blood cell requirements, and therefore techniques are being developed and tested for leukocyte depletion of blood products. The use of blood filters provides a mechanism to produce leukocyte-poor blood products, and these have been shown to reduce the risk of CMV transmission. Such products have been studied for prevention of transfusion-acquired CMV infection in patients undergoing marrow transplantation.

The role of prophylactic IVIG for prevention of CMV infection remains an inadequately understood aspect of BMT management. There have been many clinical studies evaluating both IVIG and CMVIG with mixed results. Although there is a licensed indication for CMVIG in the prevention of CMV-related complications in renal transplantation, the optimum use of either IVIG or CMVIG after BMT remains controversial. The largest controlled study of prophylactic IVIG suggests that the beneficial effects derive from an effect on GVHD and not to a direct inhibition of CMV infection, and this is consistent with earlier observations. Because of this, the use of prophylactic IVIG has become a standard of care in many BMT centers. This is controversial because the rationale has not been proven for what is clearly an expensive therapy. It has been suggested that the active substance in IVIG might be an unknown contaminating material, possibly related to T-cell surface proteins or to HLA antibodies. Because of the extensive use of IVIG in BMT, the mechanism of its apparent beneficial action needs to be determined. In regard to CMVIG in marrow transplantation, a controlled study showed an effect on CMV infection but did not alter the incidence of disease, and at present there is no justification for use of CMVIG in BMT.

Preemptive versus prophylactic therapy for prevention of CMV-IP. The choice of optimal method of CMV prevention is also controversial. The question is whether to try to prevent the reactivation of latent infection or to wait until reactivation occurs and then attempt to modify the course of viral infection. Both methods have been used, and both have benefits and deficiencies. Acyclovir, ganciclovir, and foscarnet have been used in regard to prevention of virus reactivation. Acyclovir, which is used before marrow engraftment because of a lack of marrow toxicity, has been reported in two studies to prolong the time to reactivation of virus and to reduce the overall probability of CMV infection and mortality. Ganciclovir has been used either be-
before BMT and/or at the time of engraftment and has significantly reduced virus reactivation and associated disease.\textsuperscript{34-38}

The problem with this approach is that all patients are treated irrespective of risk for disease, and therefore, the risks and costs of treatment must be borne by all. Foscarnet has been used in a phase I/II trial, and, although it appeared to prevent CMV infection, nephrotoxicity prevents further evaluation in the early period after BMT.\textsuperscript{70}

Can this prophylactic approach be applied more selectively? Studies have shown that the presence of CMV in blood\textsuperscript{69} or in lungs,\textsuperscript{61} but not in urine or in the throat, are the best predictors for subsequent serious disease. Thus, there is an approach that relies on the detection of infection before disease followed by "preemptive" treatment before disease onset. Using this strategy, it has been shown that ganciclovir therapy significantly reduces subsequent infection and disease and spares the use of this agent in approximately half of the patients.\textsuperscript{61,62} However, this method is not without flaws because it must rely on aggressive detection of infection and because reactivation of CMV and onset of disease can occur nearly simultaneously in certain individuals. In fact, in the published applications of the preemptive approach, failure has been due to inability of detection of CMV in prospective bronchoscopy\textsuperscript{60} or to concurrence of virus detection and clinical disease.\textsuperscript{62}

Thus, at the present time, the choices facing the BMT physician regarding CMV prevention in at-risk persons are one of three: (1) the use of acyclovir in all allogeneic recipients, (2) the use of ganciclovir for universal prophylaxis or for preemptive treatment, or (3) the sequential use of both agents. Although acyclovir clearly has a role in the prevention of herpes simplex virus infection, because of its cost and the availability of a more active anti-CMV agent, the use of high-dose acyclovir for prevention of CMV should be reconsidered. Acyclovir should be reserved for the prevention of reactivation of herpes simplex virus during the immediate posttransplant period. Ganciclovir has a much better effect in control of CMV infection and is, for the time being, the preferred agent in a CMV prevention plan.

It is realized that the use of ganciclovir is not without problems. The major complications associated with ganciclovir are neutropenia and creatinine elevation.\textsuperscript{57,61,62} Neutropenia occurs in approximately 30\% of patients and, based on the experience of Goodrich et al.\textsuperscript{59,60} lasts for a median of 12 days. Importantly, ganciclovir-associated neutropenia is significantly associated with bacterial complications.\textsuperscript{57} Its effect on renal function often requires dose adjustment of cyclosporin, and overall transfusion requirements are increased during these early stages of marrow development. Therefore, to maximize the antiviral effect and minimize marrow suppression, the optimal regimen would use this drug in those BMT recipients at highest risk for CMV-IP. Studies are now in progress to determine whether the newer methods of virus diagnosis by CMV antigen detection in blood or by polymerase chain reaction (PCR) analysis of blood for CMV DNA can be used in the determination not only of when to start therapy but also when to stop treatment.\textsuperscript{53-60} In this regard, the optimal ganciclovir dosing frequency and duration remain to be determined. The drug should be administered at least for the period of risk and at doses sufficient to prevent continued CMV infection.

**METHODS OF DETECTION**

Thus, it is fundamental to optimum management of the BMT recipient that one have available rapid CMV diagnosis. The conventional method for detection of CMV has relied on observing cytopathic effect in tissue culture or in tissue biopsy. This method is inefficient because of assay time and variable sensitivity. Today, our ability to intervene early in the management of CMV infection is due to improved methods of detection of CMV infection using rapid analysis of clinical specimens. The methods preferred are the shell-vial assay for infectious virus,\textsuperscript{67-69} the pp65 antigen assay in blood,\textsuperscript{70,71} and the PCR for CMV DNA.\textsuperscript{72-74} Based on the concerns regarding risk factors for CMV-IP, it appears that detection of CMV alone is not a sufficient determinant for subsequent disease, but, as noted above, detection of disseminated CMV infection in blood and lungs is an important risk factor.\textsuperscript{60-62} Although preemptive therapy strategies are based on the use of the shell-vial assay for detection of disseminated virus, recent results suggests that the antigen assay\textsuperscript{60} and the PCR method\textsuperscript{66,68} will have important uses here. Recently, the detection of CMV in plasma by PCR shows promise in simplifying early detection.\textsuperscript{75}

**IMMUNOLOGIC STRATEGIES**

The observation, in the preganciclovir days, that patients who were able to develop CMV-specific CTLs recovered from infection, suggests that strategies to provide a more effective immune response earlier after marrow transplantation might limit infection and subsequent disease. The role of specific CMV proteins in the induction of CTL function in humans was first described by Borysiewicz et al.\textsuperscript{35} who showed that nonstructural CMV proteins, as well as the virus envelope glycoprotein, gB, could induce CTL immunity. Later, it was shown that a cellular immune response exists to other surface proteins\textsuperscript{37} as well as to the internal matrix protein, pp65.\textsuperscript{70-81} Cellular immunity specific for CMV appears to be transplanted from the donor\textsuperscript{82} but sometimes requires extended time for the development after BMT to be effective in controlling infection.\textsuperscript{72-83} If virus-specific CTL function can derive from the donor, which is unclear at this point, then, depending on the rapidity of reconstitution of these donor-derived CTL responses, the patient may be at more or less risk for developing disease. This concept of donor-derived, adoptive cellular immunotherapy has been studied with the ex vivo expansion of CMV-specific CTLs to restore or augment viral immunity early after BMT and provide protection from disease.\textsuperscript{84} The infusion of these cloned T cells into the patient after BMT leads to restoration of viral immunity and appears to have an antiviral effect.

The concept of adoptive immunotherapy would obviously be difficult to apply to all BMT recipients, yet it might be possible to accomplish the same result by stimulation of the CMV-specific immunity of the donor pre-BMT followed by expansion of these immune cell in the recipient. If active immunization of the donor is attempted, what vaccine would
be used? At present a live, attenuated CMV vaccine and recombinant viral vaccines are in various stages of development. It is unlikely that live CMV or recombinant vaccinia virus would be used in the setting of the immunocompromised recipient, although recombinant genetic vaccines engineered to contain a suicide gene that would permit control of vector-derived complications could be studied in BMT. Alternatively, a subunit vaccine could eventually be tested as a means for boosting donor immunity. At the present time, several recombinant CMV antigens could be considered. There is already evidence that purified CMV-gA/gB glycoprotein envelope induces immunity in human volunteers. There is evidence from isolated CTL clones from BMT donors and recipients that a dominant immune response to CMV internal antigens pp65 and pp150 occurs (S. Riddell and P. Greenberg, personal communication, July 1993). Based on the evidence that CMV-pp65 can interact in the induction of class I and class II immune functions, this protein or its peptide epitopes deserve evaluation in candidate CMV vaccines. Whatever the eventual vehicle for active immunization to CMV, clearly the time has come for more immunologic expertise to be applied and tested in this important area.

CONCLUSION

The expectation, of course, is that an antiviral chemotherapy regimen will be developed to control CMV. The ideal anti-CMV agent(s) would be effective both in an oral and in an intravenous formulation, would suppress CMV reactivation, and would not be toxic to kidneys and BM. At the present time there is no such agent. A nucleoside analog, (S-1-(3)-hydroxy-2-(phosphonomethoxy)-propyl cytosine (HPMPC), is currently beginning clinical studies that will define the toxicity of this agent. In addition, cyclobutyl G, an orally bioavailable agent, is also entering initial clinical evaluation. Nevertheless, it needs to be recognized that any chemotherapeutic control of latent virus infection is limited and must face the possibility of resistance, breakthrough, or toxicity. Until an optimal agent is available, the use of preemptive ganciclovir therapy in the BMT recipient with documented blood or asymptomatic lung infection remains the treatment of choice.

The use of adoptive immunologic approaches involving transfer of CTLs fulfills a rationale which, if technical problems and resource-utilization limitations are overcome, could control CMV without the toxicity and cost of chemotherapeutic antiviral agents. The chemotherapeutic agents could then be reserved for patients whose disease cannot be prevented immunologically. Eventually, it may become possible to augment CMV-specific immune responses in the donor marrow that would then be transferred to the recipient at the time of BMT to finally produce a solution to the problem of CMV infection after BMT.

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