

Sepsis as a severe but rare complication of BCG-instillation therapy occurs in 0.4% of treated patients.¹ We show for the first time that, once in systemic circulation, BCG systemically activates and enhances NK- and CTL-cell-mediated cytotoxicity and, therefore, might contribute to the development of multiple organ failure and, hence, to fatal outcome.

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To the editor:

Increased prevalence of CMV gB3 in marrow of patients with aplastic anemia

Various infectious agents have been implicated in causing aplastic anemia (AA), either by direct lytic infection or by inducing a pathophysiologic host immune response.¹ But little attention has been given to cytomegalovirus (CMV), even though the myelosuppressive potential of this virus, in vivo as well as in vitro, is well established.²⁻⁶ Undoubtedly, the relatively high prevalence of this virus has made it an unlikely agent for AA, which is a very rare disease. But CMV has a broad spectrum of pathogenicities and sites of infection. Mechanisms responsible for this heterogeneity are not defined but are hypothesized to include both host and viral differences.

Our past studies indicate that genetically distinct strains of CMV, identified by variations in the gene encoding envelope glycoprotein B (gB), occur at variable frequencies and can be associated with different clinical outcomes.⁵⁻⁸ CMV gB types 1 and 2 were shown to be more frequently associated with survival following marrow transplantation than were types 3 and 4.⁷ In a second study, types 3 and 4 were specifically associated with death due to persistent neutropenia.⁵

Given the strong statistical association between CMV gB3/4 with posttransplantation myelosuppression, we hypothesized that these strains may also contribute to the pathogenesis of AA and, if so, that the virus would be detected more frequently in AA marrow than in marrow from patients with other hematologic diseases and, further, that gB types 3 and/or 4 would be overrepresented.

To test this hypothesis, we measured the incidence of CMV-infected marrow and the distribution of gB types in AA patients compared to patients with other hematologic diseases. Experimental samples consisted of fresh-frozen marrow biopsies obtained

from 100 CMV-seropositive AA patients before transplantation. Controls consisted of marrow aspirates from 151 CMV-seropositive non-AA patients harvested at day 28 after allogeneic marrow transplantation. This control population was chosen because it has an increased risk of CMV exposure, reactivation, and disease, thereby raising the background of CMV in the control samples and making our estimate of differences between AA patients and controls more conservative. Patient groups were similar for gender and ethnic background but differed in regard to age, with the AA patient group being much younger. For this reason, the logistic regression analysis was adjusted for age. CMV genotyping was based on sequence variations in the gene encoding gB as detected by restriction analysis of polymerase chain reaction (PCR)-amplified gB DNA.⁵ Table 1 shows that the frequency distribution of CMV gB types differs between AA and control patients, with the control group being comparable to previously reported results. Results shown in Table 2 indicate that the odds of possessing CMV in the marrow, particularly gB type 3, are significantly increased among AA patients. This association, together with previous reports, makes it reasonable to hypothesize a role for CMV in the pathogenesis of aplastic anemia in some patients.

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Table 1. Frequency distribution of CMV genotypes gB 1 through 4

	Total CMV ⁺ patients	gB type 1	gB type 2	gB type 3	gB type 4	Total strains detected*
AA patient marrow (n = 100)	33	8 (21%)	9 (23%)	19 (50%)	2 (5%)	38
Control patient marrow (n = 151)	19	8 (40%)	6 (30%)	5 (25%)	1 (5%)	20
Clinical isolates all sites (n = 281)†	281	137 (46%)	49 (18%)	76 (26%)	26 (9%)	288

*Among the 33 CMV⁺ AA patients, 4 had a gB 2/3 mix, 1 had a gB 3/4 mix. Among the 19 CMV⁺ control patients, 1 had a gB 2/3 mix. Among the 281 clinical isolates cultured from CMV⁺ patients, 3 had a gB 2/3 mix, 3 had a gB 3/4 mix, and 1 had a gB 1/3 mix.

†Previously reported.⁵ gB frequency among viral isolates cultured from infected patients.

Table 2. Logistic regression analyses for outcome of being CMV positive (CMV⁺) in marrow and for outcome of possessing gB3 among patients who were CMV⁺ in marrow

	Odds ratio	95% CI	P
Group (CMV ⁺ /total)			
Controls (19/151)	1	—	—
AA (33/100)	3.45	1.70-7.00	.0006
Group (gB3 ⁺ /total CMV ⁺)			
Controls (5/19)	1	—	—
AA (19/33)	7.83	1.72-35.79	.008

Models were adjusted for age. To compensate for a potential overrepresentation of stroma in AA biopsies compared with control aspirates, the aspirated marrow was also cultured to expand the stromal cells. Both aspirated marrow cells and expanded stromal cells were then evaluated by PCR. Combining both tests did not change the frequency of CMV⁺ samples in the control group.

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To the editor:

Treatment of extensive chronic sclerodermatous graft-versus-host disease with high-dose immunosuppressive therapy and CD34⁺ autologous stem cell rescue

Beyond many complications of allogeneic hematopoietic stem cell (HSC) transplantation, chronic graft-versus-host disease (cGVHD) still remains one of the most important causes of impaired quality of life. Sclerodermatous cGVHD (SC-GVHD), one of the most disabling forms, resembles systemic sclerosis clinically and histopathologically, in a somewhat different initial location and morphologic appearance of collagen fibers.^{1,2} The skin sclerosis in SC-GVHD might be considered a form of cutaneous fibrosis with features of excessive tissue repair related to an immunologic reaction between lymphocytes of the graft and tissue host cells.³

The updated treatment approaches in progressive cGVHD were recently summarized by Gazie⁴ and Vogelsang⁵; agents like cyclosporine (CsA), corticosteroids, antilymphocyte globulin, mycophenolate mofetil, extracorporeal photopheresis, and monoclonal antibodies are recommended at the expense of unavoidable morbidity and mortality. Recent investigations have suggested that the pathogenesis of cGVHD is more similar clinically to an autoimmune disease than to acute GVHD.^{6,7} Treatment of autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and so forth by autologous stem cell rescue and high-dose immunotherapy is a rapidly growing and encouraging approach that led us to use this strategy in a progressive SC-GVHD patient, who was a 29-year-old male, Ph⁺ CML patient in first chronic phase.^{8,9} Within 2 years of diagnosis, he underwent transplantation from his HLA-identical female sibling donor. After a successful engraftment, on day 1 after transplantation, the patient developed ichthyosis superimposed upon an early stage of de novo cGVHD. The patient was in complete chimeric status and in complete molecular remission. Chronic GVHD progressed, with complaints of edema of the skin on the extremities, xerophthalmia, xerostomia, and hepatic involvement, and the skin biopsy confirmed SC-GVHD. Dysphagia and dyspnea progressed despite administration of CsA, oral corticosteroids, and mycophenolate mofetil, and his performance status deteriorated, but without thrombocytopenia. After receiving a written consent, we mobilized

the stem cells with cyclophosphamide (CY) and granulocyte colony-stimulating factor, an immunomagnetic positive selection was performed on Isolex 300i (Nexell, Irvine, CA), and 4.53×10^6 /kg CD34⁺ cells were reinfused after conditioning with CY (50 mg/kg/day intravenously for 4 days) and antithymocyte globulin (30 mg/kg/day intravenously for 3 days). The hematopoietic recovery was rapid, and no major transplantation-related complications were encountered. The improvement in the patient and resolution of cGVHD were monitored by joint flexibility index, measurement of skin thickness (by ultrasonography), skin biopsies, and quality of life. After a 15-month follow-up, the patient was still on low-dose prednisone but showed marked improvement in joint-movement indexes (50%-70% increase), skin thickness (20%-30% decrease), and quality of life (30%-40% increase). The improvement was more pronounced on the face and upper extremities than on the lower extremities. High-dose immunotherapy (HDIT) did not result in progression of his native disease or a change in complete donor chimeric status. The observation of marked improvement in our patient with life-threatening refractory SC-GVHD after HDIT with autologous peripheral blood stem cell (PBSC) rescue led us to comment about this approach in the light of the recently published review concerning therapy for cGVHD.⁵ Although our patient showed a considerable improvement with HDIT and autologous stem cell support, the underlying mechanism needs to be elucidated. A possible indication and optimal timing of HDIT and autologous transplantation in extensive SC-GVHD should be further analyzed in the context of recent therapeutic options such as extracorporeal photoimmunotherapy, etretinate, thalidomide, and monoclonal antibodies against inflammatory cytokines.

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