

an open question. But even if it were, such correlations could be indirect because these complications may induce a massive expansion of lymphocytes with a subsequent impact on the average size of the telomeres in the patient's leukocytes.

In conclusion, we think it unlikely that the telomere shortening observed after allogeneic SCT is substantial enough to cause replicative exhaustion that would lead to subsequent complications. Importantly, correlations between short telomeres and a less beneficial outcome may be found even when telomere shortening and replicative exhaustion are not the cause of such complications. In recipients of autografts, this can be the case because patients with a poor prognosis often receive transplants of lower numbers of stem cells. In allograft recipients, correlations between short telomeres and a less beneficial outcome may be found in patients who develop GVHD or severe viral infections. In both situations a

substantial expansion of T lymphocytes may occur that could be accompanied by significant telomere shortening.

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**To the editor:**

**Bacillus Calmette-Guérin sepsis: shift of an intended local toward a detrimental systemic cytotoxic immune response**

A 56-year-old male patient was admitted to our intensive care unit (ICU) because of sepsis with multiple organ failure. Twelve days before admission, the patient underwent adjuvant renal instillation of bacillus Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis*, after subtotal resection of the right renal pelvis due to papillary transitional cell carcinoma. Bone marrow aspiration revealed multiple epitheloid-cell granulomas with central necrosis, and a positive polymerase chain reaction (PCR) for *Mycobacterium tuberculosis* complex was found in the tracheobronchial secretion. Diagnosis of BCG sepsis was made. Despite extensive and adequate therapy, multiple organ failure persisted and the patient died after 35 days in the ICU. On autopsy, multiple epitheloid-cell granulomas with central necrosis were found in the spleen, bone marrow, and lungs.

Serial blood samples were collected during the first 5 days in the ICU, in order to evaluate levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon  $\gamma$  (IFN- $\gamma$ ), interleukin-6 (IL-6), IL-8, IL-10, and granzyme A (GrA). Results are given in Table 1.

Instillation of BCG is used to enhance the cytotoxic activity by natural killer (NK) cells and cytotoxic T (CTL) cells against transitional cancer cells.<sup>1,2</sup> Upon stimulation with BCG, macrophages produce IL-12 and TNF- $\alpha$ , which stimulate NK cells to produce IFN- $\gamma$  and T cells to differentiate into the T helper type 1 (TH1) subset, then generating IL-2 and IFN- $\gamma$ . The released IFN- $\gamma$  potentiates macrophages' microbicidal activity, phagocytosis, oxidative burst capability, and IL-12 production (positive feedback), but in synergy with IL-2 it also activates and enhances cytotoxic properties of NK and CTL cells.<sup>3</sup>

In our patient, elevated plasma levels of IL-12, INF- $\gamma$ , and TNF- $\alpha$  revealed a cytokine pattern typical for activated macrophages and TH1 cells. The concomitant rise in granzyme A in plasma, a specific marker for CTL- and NK-cell activation, strongly suggested enhanced cell-mediated cytotoxicity. The appearance of BCG in the circulation led to a systemic inflammatory response, as reflected by the elevated proinflammatory cytokines IL-6 and IL-8. The anti-inflammatory cytokine IL-10, however, which was reported to play a role in down-regulation of IL-12 production, was hardly detectable in our patient.<sup>3</sup>

Upon microbial stimulation, patients with a IL-12 receptor deficiency are unable to generate sufficient amounts of IFN- $\gamma$ .<sup>4</sup> Patients with a complete IFN- $\gamma$  receptor deficiency were reported to suffer from spontaneous recurrent disseminated mycobacterial infections, thereby showing characteristic immature leprosislike granulomas in tissue.<sup>5</sup> In contrast, disseminated mature granulomas found in our patient's tissue and elevated levels of IL-12, IFN- $\gamma$ , TNF- $\alpha$ , and GrA suggest the local cell-mediated cytotoxicity exhibited by macrophages, NK cells, and CTL cells to be sufficient. But systemic release of IL-12, IFN- $\gamma$ , and TNF- $\alpha$  due to BCG appearance in circulation might have led to a systemic activation and enhancement of cytotoxicity by NK and CTL cells, as reflected by elevated GrA levels. At least in animal models, there is strong evidence that activation of NK cells contributes to the development of multiple organ failure.<sup>6</sup> Hence, due to systemic release of IL-12, TNF- $\alpha$ , and IFN- $\gamma$ , enhanced cytotoxicity elaborated by NK and CTL cells might have been a key step in the development of multiple organ failure in our patient.

**Table 1. Cytokine and granzyme levels**

Time (h)	TNF- $\alpha$ (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IL-12 (pg/mL)	IFN- $\gamma$ (pg/mL)	GrA (pg/mL)
0	33	31	339	44	6 623	137	170
24	188	594	1 435	58	8 691	225	206
48	128	107	1 066	47	9 447	91	301
72	100	235	1 199	44	11 498	131	251
96	97	284	1 442	54	4 445	128	272
(Normal levels)	(< 5)	(< 10)	(< 20)	(< 50)	(50-150)	(< 50)	(< 60)

Sepsis as a severe but rare complication of BCG-instillation therapy occurs in 0.4% of treated patients.<sup>1</sup> 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.<sup>1</sup> 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.<sup>2-6</sup> 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.<sup>5-8</sup> CMV gB types 1 and 2 were shown to be more frequently associated with survival following marrow transplantation than were types 3 and 4.<sup>7</sup> In a second study, types 3 and 4 were specifically associated with death due to persistent neutropenia.<sup>5</sup>

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.<sup>5</sup> 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 <sup>+</sup> 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<sup>+</sup> AA patients, 4 had a gB 2/3 mix, 1 had a gB 3/4 mix. Among the 19 CMV<sup>+</sup> control patients, 1 had a gB 2/3 mix. Among the 281 clinical isolates cultured from CMV<sup>+</sup> 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.<sup>5</sup> gB frequency among viral isolates cultured from infected patients.