To the editor:

Does telomere shortening count?

Telomeres are specialized chromosomal end structures of eukaryotic chromosomes that consist of 2- to 15-kb repeat sequences of TTAGGG in humans. Since telomeres shorten with subsequent cell divisions and decline with age by 50-100 bp per year, they reflect the proliferative history of cells and serve as a mitotic clock on our way to senescence.\(^1,3\) The extent of telomere loss has been subject to extensive in vitro and in vivo analyses, since short telomeres limit the remaining replicative capacity of cells and may be responsible for subsequent changes and potential deteriorating effects, such as acquisition of secondary myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML) after life-saving therapies such as stem cell transplantation (SCT).\(^1,4\)

We and others have shown that hematopoietic cells lose telomeres after extensive proliferative stress despite activation of telomerase, which is insufficient to prevent telomere shortening upon massive cell expansion and proliferation in vitro.\(^1\) Moreover, we have demonstrated considerable telomere loss after repetitive chemotherapy cycles or high-dose chemotherapy followed by autologous or allogeneic SCT\(^2,3,5,6\); this loss was higher after extensive chemotherapy cycles (defined as 6 or more cycles) than after a lower number of cycles (fewer than 6),\(^5\) correlated with chemotherapy-dose intensity with a 3- to 4-fold-higher telomere loss after high-dose chemotherapy (autologous SCT, n = 17) than after standard-dose (n = 13) chemotherapy, and was comparable after autologous (n=17) and allogeneic (n=11) SCT with an approximately 1- to 2-kb telomere restriction fragment (TRF) loss in mononuclear cells (MNCs) and granulocytes (GRs).\(^6\)

The finding that telomeres shorten after chemotherapy and autologous SCT is not very surprising since extensive cell renewal and reconstitution occur after nonablative and myeloablative therapies. A telomere loss of 1-2 kb after autologous transplantations will account for 20-40 years of premature aging in the autologous recipient, since patients receive their own stem cell support instead of donor stem cell support. This leaves a 60-year-old with 6 kb prior to SCT and with 4.5 kb after SCT, thereby closely reaching the Hayflick limit (disregarding the effect of prior chemotherapy, subsequent infections, or other factors). This is very likely to effect the observed subsequent changes, such as altered colony-forming unit (CFU) or long-term colony-initiating cell (LTC-IC) efficiency, as well as the potential development of secondary MDS/AML in some patients. With allogeneic transplantations, the situation is somewhat different, since the patient’s malignant hematopoietic system is replaced by the healthy donor’s. Telomere loss has been observed, nevertheless, due to the extensive cell regeneration after reinfusion of approximately 1% of donor stem cells necessary to fully reconstitute the patient’s blood count. With an observed telomere loss of 1 kb and a telomere loss of 50 kb per population doubling (PD), hematopoietic cells have to undergo 20 PDs after allogeneic transplantations.\(^5,6\)

In a recent paper, Rufer et al measured telomeres of peripheral blood monocytes and T lymphocytes in 4 patients and their respective donors by flow cytometry after T-cell–depleted HLA-identical allogeneic SCT.\(^5\) In the first year after transplantation, they found an accelerated telomere loss that was later comparable to the loss in healthy donors and controls. Although this work fits with our\(^1,3,5,6\) and other\(^7-10\) reports demonstrating a telomere loss immediately after strong proliferative stress such as SCT, whereas later this slows down significantly in most patients or may in rare patients even show a telomere elongation\(^2,3,5,6\); (either due to telomere regenerative mechanisms such as telomerase activation or repopulation of genuine pluripotent stem cells that have not undergone extensive telomere shortening\(^11\)), some critical points of Rufer et al’s paper may need to be pointed out:

First, the number of patients and donors tested (although at various time points and with long-term follow-up) are fairly low (n = 4). Both in this study\(^8\) and a previous study by this group,\(^7\) telomeres were studied under standard transplantation conditions, here after cyclophosphamide and total body irradiation (TBI) conditioning, graft-versus-host disease (GVHD) prophylaxis with cyclosporine A alone, and no acute or chronic GVHD development in any patient. Before concluding that telomere shortening is relatively insignificant after SCT, as Rufer et al have done,\(^8\) it would be interesting to study telomere loss within a larger patient cohort, focusing especially on patients and donors with respect to their age, possible differences between peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT), possible differences between non- and HLA-identical transplants, possible differences between standard and reduced conditioning regimens, the effects of acute and chronic GVHD, and the stem cell dose.

Second, mean or median values of telomere losses in patients and donors would have been more important than individual numbers alone, especially since the telomere loss in Figures 1 and 2 seems fairly unintelligible to nonexperts. Nevertheless, from their figures it is conceivable that the telomere loss during the first year after SCT is substantial and decreases considerably thereafter, which is consistent with our analyses showing a loss of 0.9 kb in MNCs and 0.5 kb in GRs immediately (1-2 months) after autologous SCT and of 0.4 kb in both after allogeneic SCT (after 6 months, 0.6 kb in MNCs and 0.5 kb in GRs).\(^6\) This might, however, increase in HLA-mismatched SCT, with acute or chronic GVHD, after cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infections as recently shown,\(^12\) low cell numbers reinfused,\(^9,10\) and repetitive conditioning such as tandem transplantations or extensive previous chemotherapy regimens.\(^2,3,5,6\)

Finally, their conclusion that “the transplantation-induced loss of telomeres represents only 20% of the ‘telomere reserve’ . . . should . . . have little consequence for the function of different hematopoietic lineages”\(^8(p577)\) stands in contrast to several observations of potential detrimental effects after SCT, such as the significant decrease of both CFU and LTC-IC capacity\(^13,14\) and development of secondary MDS/AML that in a series of 552 autologous transplant recipients showed an estimated incidence at 10 years of 19.8% and continues to be a major concern for patients who survive long-term.\(^7\) These adverse events seem less worrisome.
after allogeneic transplants than after autologous transplants since a new hematopoietic system is established. Nevertheless, this must undergo extensive proliferation and is further challenged with immunosuppressive therapy, acquisition of infections, GVHD, and so forth.

These critical points aside, the essential observation of a TRF shortening of the stem cell compartment is worth reporting. This should alert us, however, that the telomere loss will have a potential adverse effect, especially in older individuals, with older donors and transplantation complications. Therefore, we recommend a more critical and cautious interpretation of the transplantation-induced telomere losses than underestimating their effects.

Monika Engelhardt and Jürgen Finke

Correspondence: Monika Engelhardt, University of Freiburg Medical Center, Hematology/Oncology Department, Hugstetterstr 55, 79106 Freiburg, Germany

References


Response:

Consequences of stem cell transplantation–induced telomere shortening

Engelhardt et al ask the question whether telomere shortening counts or, more precisely, whether the stem cell transplantation (SCT)–induced telomere shortening in hematopoietic cells could be serious enough to be responsible for some of the adverse alterations in the hematologic lineages. This is an important question1 that remains unresolved despite Engelhardt et al’s claims that the conclusions from our study stand in sharp contrast to several observations.

What is the argument about? We found that following allogeneic transplantation, telomere shortening is limited to the first year after transplantation.2 We believe it to be unlikely that the observed telomere shortening per se will result in adverse consequences for the sustenance of long-term hematopoiesis in these patients. The reason for this is simple. If having 1- to 2-kb-shorter telomeres would have considerable consequences for the remaining replicative potential, one would expect to see a much higher heterogeneity in the hematopoiesis of healthy individuals, because already at birth the telomere length in different individuals varies between 4.5 and 14 kb.3 For this reason, we believe that Engelhardt et al’s statement that the transplantation-induced telomere loss “leaves a 60-year-old with 6 kb prior to SCT with 4-5 kb after SCT, thereby closely reaching the Hayflick limit” is misleading. Indeed, previous calculations translating telomere shortening directly into “years of aging” are subject to the same criticism. In general, we believe that one should be extremely cautious explaining posttransplantation complications by a concomitant telomere loss. This might be illustrated best by the example that Engelhardt et al use to support their view. It could indeed be that, in certain patients, replicative exhaustion contributes to the development of secondary myelodysplastic syndrome/acute myelogenous leukemia (MDS/AML).4 But in such patients the number and quality of stem cells that are available for transplantation, engraftment, and sustained hematopoiesis may all have been severely compromised by the underlying disease and/or the prior treatment.

Most of Engelhardt et al’s other comments refer to telomere shortening after allogeneic SCT, where the quality of the graft is not influenced by the clinical status of the patient. Here, their main criticism is that our conclusion “that telomere shortening is relatively insignificant after SCT” is not legitimate because our data come from patients under standard transplantation conditions without complications such as graft-versus-host disease (GVHD) or cytomegalovirus (CMV) infection that could possibly induce further telomere shortening. This critique seems to lack the necessary accuracy. In our study,2 we showed that after 6 months to 1 year of an accelerated rate of telomere loss, patients’ telomeres shortened at a rate comparable to that of their donor. Based on this observation, we concluded that the high rate of telomere loss during the first year is entirely responsible for the difference of 1–2 kb observed through the entire transplantation period. Obviously, we did not say that the loss itself was insignificant. We only concluded that, because the accelerated loss is limited to this initial period, the difference in telomere length of 1–2 kb between the patient and his donor would remain stable and, therefore, would most likely be without severe consequences for the function of the patient’s hematologic lineages. Whether this difference would be more considerable in patients with GVHD or viral infections remains
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 lymphocytes 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 with 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 even when telomere shortening and replicative exhaustion are not the cause of such complications.

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 epithelioid-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 epithelioid-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-α (TNF-α), interferon γ (IFN-γ), 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. Upon stimulation with BCG, macrophages produce IL-12 and TNF-α, which stimulate NK cells to produce IFN-γ and T cells to differentiate into the T helper type 1 (TH1) subset, then generating IL-2 and IFN-γ. The released IFN-γ potentiates macrophages’ microbialic 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.

In our patient, elevated plasma levels of IL-12, INF-γ, and TNF-α 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.

Upon microbial stimulation, patients with a IL-12 receptor deficiency are unable to generate sufficient amounts of IFN-γ. Patients with a complete IFN-γ receptor deficiency were reported to suffer from spontaneous recurrent disseminated mycobacterial infections, thereby showing characteristic immature leprosislike granulomas in tissue. In contrast, disseminated mature granulomas found in our patient’s tissue and elevated levels of IL-12, IFN-γ, TNF-α, 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-γ, and TNF-α 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. Hence, due to systemic release of IL-12, TNF-α, and IFN-γ, 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

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<th>IL-8 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>IL-12 (pg/mL)</th>
<th>IFN-γ (pg/mL)</th>
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