

from the N-terminal cytoplasmic domain of the protein (Figure 1C). The normal intensity of this band suggests that the stability of the mRNA transcribed from this allele is normal.

To definitely exclude the possibility that the identified mutations are polymorphisms present in the general population, 100 unrelated white individuals were genotyped for all 3 mutations by restriction analysis with BstY I (IVS12-1G→T), Rsa I (IVS6 + 1G→A, restriction site introduced with a mutagenic reverse primer) and Tsp509 I (c.1219insT), respectively (New England Biolabs). No instance of any of these mutations was found.

In conclusion, we have identified 2 novel mutations in the *TMEM16F* gene in a patient with Scott syndrome. This finding confirms the recent report² that the product of this gene is required for Ca²⁺-dependent phospholipid scrambling and that loss-of-function mutations in this gene can give rise to Scott syndrome.

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To the editor:

Chimerism levels after stem cell transplantation are primarily determined by the ratio of donor to host stem cells

Ever since the concept of the hematopoietic stem cell (HSC) niche was first proposed in 1978, there has been debate whether toxic ablative conditioning is required before transplantation for creating HSC niche space, or whether engraftment is merely determined by stem cell competition between donor and host stem cells. Two recent studies describe low chimerism levels after transplanting high doses of purified HSCs in unconditioned hosts^{1,2} and attribute this poor engraftment to limited HSC niche spaces. This shows that in more than 30 years of research, the issue of HSC niche availability as a prerequisite for substantial chimerism has not been settled.

We directly addressed this issue in 2 different dose-response experimental models. In a congenic parent-into-F1 model, we transplanted 10×10^6 , 30×10^6 , and 100×10^6 unfractionated C57BL/6 CD45.1 bone marrow cells (BMCs) into unconditioned C57BL/6 CD45.1 \times CD45.2 F1 recipient mice. Peripheral blood granulocytes (Gr-1⁺) 16 weeks after transplantation demonstrated dose-dependent chimerism levels of 4.3% (\pm 1.7%), 8.9% (\pm 2.5%), and 23.2% (\pm 8.5%), respectively. Given that the bone marrow compartment of adult mice contains approximately 300×10^6 BMCs,³ these results are remarkably concordant with the theoretical maximum chimerism of 3%, 9%, and 25%, respectively. In the other type of model, we transplanted 10×10^6 , 30×10^6 , and 100×10^6 unfractionated CD45.2 BMCs into anti-CD40-Ligand treated CD45.1 recipient mice and determined chimerism levels 20 weeks after transplantation in the peripheral blood Gr-1⁺ population and in the c-Kit^{hi}/Lin⁻/Sca-1⁺ (KLS) population in the bone

marrow, which is highly enriched for HSCs. Anti-CD40-ligand treatment served to prevent rejection resulting from CD45 polymorphism. Gr-1⁺ chimerism levels showed a similar linear relation to the number of BMCs transplanted as in the parent-into-F1 model. Moreover, a linear relation was also observed in the bone marrow KLS fraction (Figure 1A). These data show that even after transplantation of very high doses BMCs (100×10^6), the majority of donor HSC indeed engraft. In a subsequent experiment, we assessed donor chimerism in the more primitive KLS/CD34⁻ BMC population, containing the HSC fraction. Twenty weeks after transplanting 100×10^6 unfractionated CD45.2 BMCs into unconditioned CD45.1 mice, we detected similar high levels of donor chimerism within the Gr-1⁺, the KLS BMC population, and the KLS/CD34⁻ BMC population (Figure 1B).

We previously reported similar findings after transplanting high doses of BMCs in a hybrid resistance model⁴ and in a major mismatched setting after immunologic conditioning,⁵ indicating that our findings are not model-dependent.

Our data show that high levels of engraftment can be obtained without myeloablation and importantly show that the availability of niche space is not a limiting factor in HSC engraftment. The linear relationship between engraftment and donor to host stem cells ratios may be lost on the use of enriched or purified populations of stem cells, underscoring the important role of accessory cells. This may explain why this issue remained unresolved for such a long time. We conclude that strategies aimed at enhancing donor

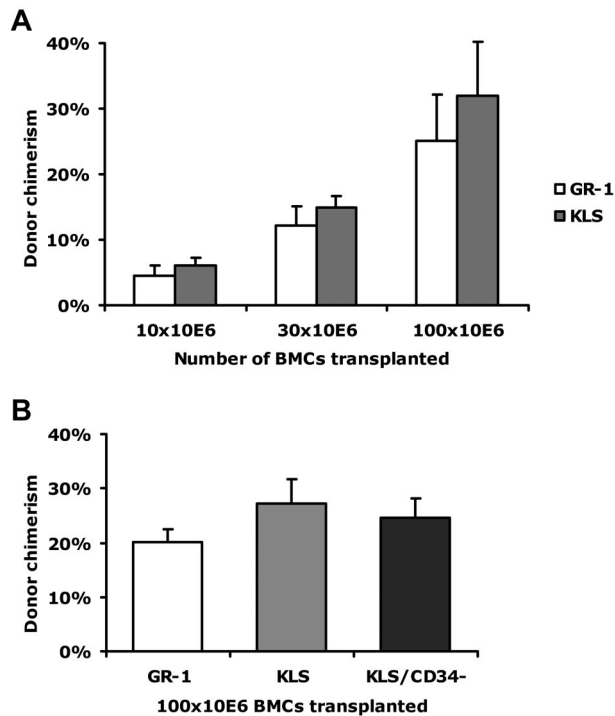


Figure 1. Linear relationship between transplanted HSC dose and chimerism levels. (A) Chimerism levels within the peripheral blood Gr-1⁺ population and the KLS BMC-fraction 20 weeks posttransplantation in anti-CD40Ligand treated C57BL/6 CD45.1 recipients after transplantation of 10×10^6 , 30×10^6 , and 100×10^6 unfractionated C57BL/6 CD45.2 BMC. Data are expressed as means \pm SEM of 2 experiments involving 9 mice per cell dose. (B) Chimerism levels within the peripheral blood Gr-1⁺ population, within the KLS BMCs and within the KLS/CD34⁻ BMCs detected 20 weeks after transplantation in unconditioned C57BL/6 CD45.1 recipients after transplantation of 100×10^6 unfractionated C57BL/6 CD45.2 bone marrow cells. Data are expressed as means \pm SEM (n = 5).

chimerism should focus on increasing the ratio of donor to host HSCs rather than on creating niche space per se.

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