To the editor:

The balance among immune suppression, stem cell ablation, competition, and engraftment

Ito and coworkers describe a model where local irradiation enhances engraftment of CD45 congenic donor bone marrow cells. Their explanation is that the irradiated marrow sites produce cytokines that help proliferation of locally homed stem cells and initiate migration of stem cells to other, nonirradiated sites. This idea sounds appealing because administration of such cytokines would be an ideal opportunity to improve engraftment of transplanted stem cells. However, most of Ito et al’s results can be explained by additional immune suppression and/or additional ablation of the endogenous stem cell reserve. As published and referenced by the authors, an immune response against the CD45 antigen can influence engraftment of transplanted stem cells, especially when mild conditioning regimens are used, as was the case in the study by Ito and coworkers. An alternative transplant model using an intracellular marker (glucose-phosphate-isomerase) showed much higher levels of chimerism suggestive for alloreactivity against CD45. The allotype of CD45 serves as a minor antigen but this alloreactivity is very weak and does not prevent the coexistence of 2 stem cell populations (mixed chimerism), even under conditions of incomplete immune suppression. Ito et al claim that the high marrow dose might overcome immune resistance, but at 25% (5 x 10^7) of the dose they used, resistance was still evident in this model. Mediastinal irradiation (including the thymus) as employed by the authors was shown to be effectively immune-suppressing an alloreponse, which will result in higher levels of chimerism. Although thymectomy did not prevent the increase in chimerism, it seems probable that this procedure already resulted in less alloreactivity. Unfortunately, the authors do not present proper control data on chimerism levels in thymectomized mice without mediastinal irradiation. The alternative method using local irradiation of hind limbs also led to higher levels of chimerism. In that case, approximately 20% of the total marrow mass was irradiated and severely depleted of stem cells. This will most certainly provide additional marrow ablation, allowing engraftment of transplanted cells into these marrow sites. When this extra 20% is added to the 20% already achieved without local irradiation, a level of 40% chimerism might be expected, very close to the levels found in nonirradiated bones. Therefore, although cytokine production by irradiated bone marrow may explain the results presented by Ito and coworkers, alternative explanations such as those discussed here cannot be excluded unless other models without immune reactivity are used. The results show that engraftment depends on a delicate balance among immune suppression, stem cell ablation, and competition.

Ronald van Os and Gerald de Haan

References

Response:

The possible role of alloreactivity in mediating the donor engraftment—promoting effect of local irradiation in an Ly-5 congenic bone marrow transplant combination

van Os and de Haan argue that the results of our study demonstrating a systemic increase in CD45 congenic donor pluripotent hematopoietic stem cell (PHSC) engraftment following local limb or mediastinal irradiation can be explained by immunoblation due to local irradiation. Alternatively, they propose that the effects can be explained by ablation of the endogenous stem cell reserve by local irradiation. We agree with the latter explanation, which is quite consistent with our interpretation of the results. We believe it is likely that local stem and progenitor cell destruction by local irradiation may play an important role in inducing the proliferation of donor stem cells that engraft in those sites. We regret that this was not more explicitly stated in the manuscript, but we thought it was implicit in our discussion. This possibility does not detract from the point of the paper, which is that this local irradiation leads to an early and systemic (ie, including nonirradiated sites) increase in the number of donor-derived PHSCs. As discussed in the paper, these data suggest that irradiation leads to local proliferation of engrafted stem cells in the irradiated sites, and that these stem cells later equilibrate throughout the body. An alternative explanation, that local irradiation increases systemic cytokine levels and hence systemic stem cell engraftment, is less satisfying to us, as it would not explain the improved ability of donor PHSCs to compete with recipient PHSCs at nonirradiated sites. This is discussed in the manuscript.

We respectfully disagree with the first possibility suggested by van Os and de Haan, namely that immune ablation could explain our results. As we acknowledge in the paper, van Os et al have shown that engraftment in a CD45 congenic combination, in the direction opposite from the one we used, can be limited by an...
immune response to minor antigenic differences between the strains. However, this immune response was overcome by increasing the marrow cell dose. While van Os et al observed immune resistance to one fourth of the bone marrow cell numbers administered in our study in nonirradiated mice, they did not examine resistance to the much greater cell dose that we used. Moreover, we have previously shown\(^1\) in the strain combination used in our current studies that minor antigenic differences in the same direction were not present that could cause skin graft rejection, a very sensitive test of histoincompatibility. While we acknowledge that genetic drift could have occurred between these 2 strains since the time of our original study, the argument of van Os et al cannot be gratuitously extended to the reverse strain combination, since it is well-known that minor antigenic differences can exist in a null form in one direction, resulting in a unidirectional immune response. Thus, without specific data implicating T-cell-mediated immune response in the strain combination we utilized, the existing data suggest that there is no such response.

van Os et al argue that our failure to observe a role for the irradiated thymus in the ability of mediastinal irradiation to enhance engraftment cannot be accepted because of the lack of control data on chimerism levels in thymectomized mice not receiving mediastinal irradiation. As shown in Table 1, this control was in fact performed. We observed exactly the same level of chimerism in (and similar multilineage chimerism in adoptive recipients of marrow from; data not shown) nonthymectomized mice not receiving mediastinal irradiation as thymectomized nonirradiated mice, arguing against a significant thymocyte-mediated immune response in the nonthymectomized, nonirradiated mice. In both groups, chimerism was markedly lower in the thymectomized or euthymic mice not receiving mediastinal irradiation than in those that did (not shown). Thus, an immune response from thymocytes cannot be implicated in any of the results that we observed.

van Os and de Haan propose that the reason we saw the same effect of local irradiation to the limbs is completely different than the reason for the effect when we irradiated the mediastinal area. Although our data do not formally rule out this possibility, we believe that the striking similarity between results obtained when local irradiation is given to the mediastinal area and those obtained when it is given to the limb area is most likely explained by a single phenomenon, that is, enhanced donor stem cell proliferation in local sites, followed by systemic circulation and hence increased systemic PHSC engraftment.

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References


To the editor:

Role of KIR ligand incompatibility in hematopoietic stem cell transplantation using unrelated donors

We read with interest the recent report by Giebel et al.,\(^1\) who showed a dramatic effect of KIR ligand incompatibility on survival and transplant-related mortality in a heterogeneous patient population after allogeneic hematopoietic stem cell transplantation using unrelated donors. The authors suggest that these effects might have been mediated by alloreactive donor natural killer (NK) cells. They discuss the use of high-resolution HLA typing and in particular the addition of antithymocyte globulin (ATG) to the conditioning regimen to be responsible for the observed survival benefits that had not been demonstrated in an earlier study.\(^2\)

Because most preclinical work has shown that allogeneic NK cells are active mainly against myeloid malignancies,\(^3,4\) we performed a retrospective study in 118 patients with acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) who had received hematopoietic cells from unrelated donors. In 15 cases, an HLA Cw mismatch predictive for a KIR ligand incompatibility was detected. The additional patient-donor pairs were either fully HLA matched (n = 54) or received a transplant with 1 (n = 31) or 2 (n = 18) allele mismatches. The patient and the transplantation characteristics are summarized in Table 1. Recipients received either ATG Merieux/Sangstat (n = 102) at a dose of 8 mg/kg to 15 mg/kg (median: 10 mg/kg) or ATG Fresenius (n = 16) at a dose of 10 mg/kg to 80 mg/kg (median: 45 mg/kg). Granulocyte colony-stimulating factor (G-CSF) was not routinely administered after transplantation. As shown in Figure 1, we could not detect a significant difference in survival between patients with a KIR ligand incompatibility and those with either fully matched or partially mismatched unrelated donors in this patient cohort. In contrast to the report of Giebel et al, we found a higher probability of relapse in patients with a KIR epitope mismatch (P = .02). In theory, the use of ATG in all transplantations, and peripheral blood stem cells in more than half of the transplantations, should have favored the effects of alloreactive NK cells and resulted in a reduction of relapse rate in patients with a KIR ligand incompatibility. On the other hand, the cumulative

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<th>Table 1. Thymectomy does not affect CD45 congenic marrow engraftment in nonirradiated mice</th>
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Populations are as follows: n = 4 for BMT, no TI group (mean ± SD is shown); and n = 2 for ATX, BMT, no TI group (each row represents an individual animal in this group). For bone marrow transplantation (BMT), 2 × 10\(^6\) CD45.2 B6 bone marrow cells were given to untreated CD45.1 B6 recipients. Ti indicates thymic irradiation; and ATX, adult thymectomy.
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