anemia developed GVHD of the skin that initially manifested as inflammatory dermatitis and then caused fissuring and ulcerations on the back and arms[3]. One year after transplant, these ulcerations were covered by full- and split-thickness skin grafts from the marrow donor. The skin grafts healed well and remained viable with no recurrence of ulcerations even though the surrounding recipient skin continued to show evidence of GVHD. A skin graft from an unrelated donor, placed at the same time, was rejected in 17 days. If GVHD in this patient was generated and maintained by cytokines, the marrow donor's skin grafts should have developed the same lesions affecting the surrounding host skin. The observation that the grafts remained healthy was consistent with the hypothesis that GVHD in this patient was mediated by donor lymphocytes cytotoxic to disparate mHAg expressed on host skin epithelial cells.

Third, in randomized and controlled trials, neither the TNF-α antagonist pentoxifylline or an IL-1 receptor antagonist affected the incidence or severity of acute GVHD after hematopoietic stem cell transplantation from HLA-matched or single HLA antigen–mismatched donors, both related and unrelated[4,5].

We conclude that HLA disparity between donor and recipient must be present in both recipient APCs and recipient epithelial cells, and that nonspecific inflammatory cytokine activity is not sufficient for the induction of acute GVHD in prototypical epithelial target organs of random-bred large animals and human patients. Thus, mechanisms of GVHD induction that are operative in highly inbred strains of mice cannot necessarily be extrapolated to random-bred species.

Competing interests statement
The authors declare that they have no competing financial interests.

MARCO MIELCAREK[1,2], BEVERLY TOROK-STORB[1] & RAINER STORB[1,2]
[1]*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA*
[2]*Department of Medicine, University of Washington, Seattle, Washington, USA*
Email: mmielcar@fhcrc.org

Teshima et al. reply—The issues raised by Mielcarek et al. are important and help to clarify our study's conclusions[1], which we believe are consistent with their examples. Our first conclusion, that host APCs are needed for both the activation and effector phases of acute GVHD, is supported by additional data published in the dog study[2]. After infusion of naive donor lymphocytes, none of the nine chimeras developed significant clinical GVHD, and none developed histopathologic lesions in the skin. Thus, alloantigen on target epithelium alone did not elicit acute GVHD.

Our second conclusion was that alloantigen expression on target epithelium is not always required for GVHD damage. This is especially true for GVHD mediated by donor $CD4^+$ T cells. We also showed that when acute GVHD is mediated by donor $CD8^+$ T cells (as is the case for most GVHD elicited by mHAg), alloantigen on target epithelium increases GVHD mortality but does not account for all of it. The dog study demonstrated that GVHD occurred after donor lymphocyte infusion only when donors had been previously sensitized to the host. Still, only two of twelve animals showed pathologic changes of GVHD in the skin, and only one showed severe changes[2]. We therefore agree that donor cells, especially when primed, can sometimes attack GVHD target epithelium directly, a conclusion recently confirmed by a mouse GVHD model using donor $CD8^+$ T cells activated by mHAg[6].

The example of the patient with chronic GVHD who rejected a third-party skin graft but not a donor skin graft[3] is consistent with a donor response to alloantigen on APCs but not necessarily on the epithelium, where damage could be mediated by cytokines, cytotoxic T lymphocytes or both. The protection of the donor skin graft in this example emphasizes the importance of local APCs for the induction of GVHD in that target tissue, a feature not stressed in our study but shown for hepatic GVHD in a mouse model[7]. Such APC geography is probably a factor in the unusual target organ distribution of GVHD, although other variables such as chemokines and trafficking molecules may also play a role.

Our third conclusion was that inflammatory cytokines can mediate mortality and target destruction, because extended blockade of both IL-1 and TNF-α prevented both of these outcomes. We agree that inflammatory cytokine activity alone is not sufficient to induce acute GVHD because cytokine infusion does not recreate the full disease spectrum, even in the mouse. Two randomized trials of single cytokine antagonists did not show any benefit in preventing GVHD[4,5]. Pentoxyfylline was poorly tolerated, and no cytokine data were recorded[4]. IL-1 receptor antagonist was only administered until day 10 and its administration did not change TNF receptor levels[5]. Prevention of clinical GVHD may require blockade of both cytokines, a longer blockade or both. Although we do not agree with the authors' conclusions that both recipient APCs and epithelial cells must express alloantigen for the induction of acute GVHD, we concur that insights from all animals models (both inbred and outbred) must be extrapolated to human patients with caution and that the effectiveness of any agent or approach must be verified first in well designed, carefully controlled clinical trials.

JAMES L.M. FERRARA[1] & TAKANORI TESHIMA[2]
[1]*Departments of Internal Medicine and Pediatrics*
University of Michigan Medical School
Ann Arbor, Michigan, USA
[2]*Department of Medicine II*
Okayama University
Graduate School of Medicine and Dentistry
Okayama, Japan
Email: ferrara@umich.edu

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