The authors have provided only scant information regarding the performance of the GM-CSF affinity column used to isolate antibodies. If used repeatedly, it is possible that a proportion of high affinity anti-GM-CSF AAs from PAP sera used as a positive control would be retained on the GM-CSF column and released slowly during subsequent purifications of HC IgG? If PAP anti-GM-CSF IgG contamination did occur, not only would it account for the immunoblot data given in Figure 1a in their paper,1 where radiolabeled GM-CSF was shown to bind to the bound (IgG) fraction from both HC and PAP IgG, but also for the positive results in bioassays. Evidence of absolute clearance of GM-CSF–bound proteins before column reuse would have been useful in eliminating this possibility.

Finally, the functional assays used for GM-CSF, including those used for measuring neutralizing effects, do not appear to have been adequately controlled for specificity. Assays used for measuring GM-CSF activity are not specific as they can respond to a range of cytokines and can be affected by inhibitory components in sera.13 Therefore, if specificity for neutralizing GM-CSF is to be claimed, then it is necessary to show that the affinity-purified antibodies do not neutralize other cytokines, such as interleukin-3, which can be tested using the TF1 cell-proliferation assay.13

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References

To the editor:

Acid sphingomyelinase deficiency does not protect from graft-versus-host disease in transplant recipients with Niemann-Pick disease

Rotolo et al reported1 that ceramide, generated from sphingomyelin by acid sphingomyelinase (ASM) and coalesced into plasma membrane platforms, is necessary for transmembrane relay of cytotoxic T-lymphocyte (CTL) signals, such as Fas-FasL signals, critical in the effector stage of acute graft-versus-host disease (GVHD). Due to defective CTL-mediated lysis of ASM-deficient organs, recipients had reduced GVHD lethality and target organ injury, especially of the gastrointestinal tract and liver. In addition, GVHD amelioration was also associated with increased donor T-cell apoptosis and reduced proinflammatory cytokine responses.

To determine whether the effects of ASM deficiency in mice were translatable to humans, we examined the incidence of acute and chronic GVHD in patients with ASM deficiency, termed Niemann-Pick (NP) disease. Approval was obtained from the University of Minnesota’s Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki. At least 4 types of NP disease exist, 2 of which have either complete (neurovisceral form, NP type A, or NPA) or partial (visceral form, NP type B, or NPB) constitutional deficiency of ASM.2 In both NPA and NPB, ASM substrate (sphingomyelin) accumulates to the same degree in viscera, and leads to progressive multiorgan dysfunction and death. Because phenotypic cross-correction is feasible in ASM-deficient mice,3 hematopoietic cell transplantation (HCT) has been proposed as a therapy for both forms.
Based on published reports\textsuperscript{4-9} and data from the Center for International Blood and Marrow Transplantation Research, 24 NP disease patients who received allogeneic HCT from 1986 to 2007 were available for analysis (Table 1). The median age at HCT was 2 years (range, 0.6-10 years), and median follow-up was 4 years (range, 1-17 years). Nine patients developed GVHD (6 acute and 3 chronic GVHD), and 2 patients had both acute and chronic forms of GVHD. Acute GVHD involved skin in all cases (2 patients with grade 1 GVHD, 1 patient with grade 2 GVHD, and 3 patients with grade 3 GVHD; Glucksberg score), gastrointestinal tract in 3 patients (1 patient with grade 1 GVHD and 2 patients with grade 2 GVHD), and liver in 4 patients (2 patients with grade 1 GVHD, 1 patient with grade 2 GVHD, and 1 patient with grade 3 GVHD). Chronic GVHD was limited in 2 patients and extensive in one.

We found that patients with NP disease had incidence and severity of GVHD comparable with other patients with inborn errors of metabolism (IEM) undergoing transplantation during the same period (Table 1). The expected incidence of clinical GVHD after allogeneic transplantation in a pediatric population with IEM is 34% for acute GVHD and 16% for chronic GVHD, not significantly different from that for NP patients. Therefore, the magnitude of any inhibitory effect of ASM deficiency at the level of GVHD target organs may have been circumvented by the donor T-cell numbers infused, the conditioning regimen type or intensity, GVHD target organ injury or that ASM modulates GVHD pathophysiologic mechanisms.\textsuperscript{10} Given the available data, we find no evidence that NP patients are spared from GVHD target organ injury or that ASM modulates GVHD pathology in humans.

Table 1. GVHD is not diminished in congenital acid sphingomyelinase deficiency, Niemann-Pick A/B disease

<table>
<thead>
<tr>
<th>Graft/disease</th>
<th>N</th>
<th>Acute GVHD, grades 2-4, at day 100 after HCT (95% CI)</th>
<th>Chronic GVHD at 3 y after HCT (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSD BM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick</td>
<td>13</td>
<td>30% (9%-65%)</td>
<td>12% (6%-49%)</td>
</tr>
<tr>
<td>Other IEM*</td>
<td>202</td>
<td>22% (16%-28%)</td>
<td>9% (6%-14%)</td>
</tr>
<tr>
<td>URD BM + CB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick</td>
<td>11</td>
<td>18% (3%-44%)</td>
<td>None†</td>
</tr>
<tr>
<td>Other IEM*</td>
<td>543</td>
<td>39% (34%-43%)</td>
<td>20% (17%-24%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick</td>
<td>24</td>
<td>25% (10%-43%)</td>
<td>13% (3%-30%)</td>
</tr>
<tr>
<td>Other IEM*</td>
<td>745</td>
<td>34% (31%-37%)</td>
<td>16% (13%-19%)</td>
</tr>
</tbody>
</table>

N indicates number of cases; CI, confidence interval; BM, bone marrow; CB, cord blood; IEM, inborn errors of metabolism; MSD, matched sibling donor; and URD, unrelated donor.

Unrelated donor-recipient matching at human leukocyte antigens (HLAs) for BM recipients included 2 cases of 6/6 antigen-matched and 1 case of 5/6 antigen-matched. After typing at the HLA-C locus became available, 1 transplantation was performed that was 6/6 antigen-matched (mismatch at the HLA-B locus, and allele mismatch at the HLA-C locus). For CB transplants, 1 was 6/6, 5 were 5/6, and 1 was 4/6 antigen-matched.

*Similarly aged patients with IEM from the CIBMTR database who underwent transplantation between 1987 and 2007.
†No patient developed chronic GVHD.

References

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