What are the probable reasons for the high rate of gastrointestinal complications and TRM? In contrast to the cohort of Bunjes the majority of our patients suffered from Philadelphia chromosome-positive ALL. Several studies demonstrated a high TRM in patients with this disease. However, this does not explain the high frequency of acute intestinal GvHD. The second obvious difference was the use of unmanipulated grafts or early incremental T-cell add backs. We therefore assume that the combination of anti-CD66 (a, b, c, e) mAb therapy and early exposure to allogeneic T-cells might be the reason for intestinal toxicity. Since anti-CD66 (a, b, c, e) antibodies (clone BW250/183) bind intestinal epithelial cells, radioimmunotherapy might cause tissue damage in the bowel. This might also trigger intestinal GvHD. Moreover, the antigen CD66a is expressed on the surface of small intestinal intraepithelial lymphocytes (iIEL). Via cross fire radiolabeled intraepithelial T-cell binding, mAb might cause additional tissue damage.

In summary, allografting without T-cell depletion or with early T-cell add backs after conditioning regimens including 188Re-labeled anti-CD66 (a, b, c, e) mAb is associated with a high risk of severe intestinal acute GvHD and a high TRM. We therefore suggest that efficient T-cell depletion is strongly recommended for allogeneic hematopoietic cell grafts after a radioimmunotherapy conditioning with anti-CD66 (a, b, c, e) mAb. Furthermore, the anticipated benefit of early T-cell add backs should be weighed out carefully with the risk of severe intestinal GvHD.

Response:

Factors influencing gut GvHD after intensified conditioning with Rhenium 188–labeled anti-CD66 monoclonal antibody

We are grateful to Klein et al for emphasizing one aspect of our study that received comparatively little attention in the original publication, namely the fact that all patients but one received T-cell–depleted grafts. Therefore the conclusions with respect to organ toxicity and the incidence of graft-versus-host disease (GvHD) only apply to T-cell–depleted stem cell transplantations and cannot be transferred to patients receiving conventional GvHD prophylaxis. Klein et al report a high incidence of gut GvHD in a cohort of patients receiving non–T-cell–depleted grafts after intensified conditioning according to our radioimmunotherapy protocol. We cannot entirely exclude the possibility that the specificity of the anti-CD66 antibody used in our study could have contributed to the gut problems observed. However, we have never observed localization of the antibody to the gut in our dosimetry studies, and we have data demonstrating that the labeled antibody per se does not induce a systemic release of cytokines involved in GvHD (interleukin-1, tumor necrosis factor–alpha, gamma-interferon [Buchmann et al, unpublished observations, December 2001]). We have only observed localization of the antibody to the gut in rare patients with significant infectious complications such as severe appendicitis. We therefore feel that this high incidence of intestinal GvHD is due to the use of conventional GvHD prophylaxis, and in particular to the early application of donor lymphocyte transfusions.

Donald Bunjes

To the editor:

Discrepancy between antithrombin activity methods revealed in Antithrombin Stockholm: do factor Xa–based methods overestimate antithrombin activity in some patients?

Antithrombin is a single-chain glycoprotein of 432 amino acids. It is the most important endogenous coagulation inhibitor and inhibits several coagulation factors: IXa, Xa, XIa, XIIa, and thrombin (of which the inhibition of thrombin probably is the most important). In 1992 Blachman et al reported the Antithrombin Stockholm point mutation in codon 392 (Gly to Asp) as a reactive site (RS) type of mutation that results in a type II antithrombin deficiency. It was found in a young woman on oral contraceptives who presented with pulmonary embolism. The authors reported low antithrombin activity measured with thrombin inhibition–based (72%–74%) and factor Xa inhibition–based (53%–56%) methods using chromogenic peptide substrate assays. Several years later, much to our surprise, the proband and her 2 siblings had normal antithrombin activity in a routine factor Xa inhibition–based test. Her 2 siblings had previously presented with low levels but never suffered from any symptoms.

Stefan A. Klein, Stella Hermann, Christoph F. Dietrich, Dieter Hoelzer, and Hans Martin

Correspondence: Stefan A. Klein, Johann Wolfgang Goethe-Universität, Med Klinik III, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany; e-mail: sal.klein@t-online.de

References

Table 1. Antithrombin levels with antigen and activity methods

<table>
<thead>
<tr>
<th></th>
<th>Antigen (Lia test), %</th>
<th>Thrombin-based activity test, %</th>
<th>Factor Xa–based activity test, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband (our results)</td>
<td>93</td>
<td>60-64</td>
<td>110</td>
</tr>
<tr>
<td>Proband (Canadian results)</td>
<td>NA</td>
<td>72-74</td>
<td>53-56</td>
</tr>
<tr>
<td>Sister (our results)</td>
<td>86</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td>Brother (our results)</td>
<td>92</td>
<td>55</td>
<td>89</td>
</tr>
</tbody>
</table>

NA indicates not applicable.

Our present investigation shows that the 3 patients have normal antigen levels measured with Lia antigen test.2 The factor Xa inhibition–activity assay also gave normal values, but decreased antithrombin activity values (about 60%) were found with a thrombin inhibition–based activity test using bovine thrombin (Table 1). Both activity methods were based on chromogenic substrates and were carried out in the presence of excess heparin. Our results differ from the original Canadian report1 especially concerning the factor Xa inhibition–based test.

Chromogenic peptide substrate assays have been used for many years to measure antithrombin activity. These assays are based on either thrombin inhibition1 or factor Xa inhibition.4 For thrombin-based tests bovine thrombin should be preferred because human thrombin also reacts with heparin cofactor II and may lead to overestimation of antithrombin.3,6 In our present study we used bovine thrombin (Sigma Aldrich, St Louis, MO) and substrate S-2238 (Haemochrom Diagnostica, Molndal, Sweden) for the thrombin inhibition assay, which was modified for the Cobas Mira analyzer (Roche Diagnostics, Basle, Switzerland). For factor Xa inhibition we used the Coamatic LR kit (Haemochrom Diagnostica) with S-2772 and the automatic method for Thrombolyzer (Benth Elektronik, Norderstedt, Germany). The Canadian study used human thrombin in the thrombin inhibition assay, which can explain the slight difference compared with our results. Other factors that can contribute to differences in test results are plasma amount, incubation time, and heparin concentration in the assays. These factors however can hardly account for a discrepancy between 55% (Canadian study) and 110% (present study) as in the case of the factor Xa inhibition method (Table 1).

In the present study with the factor Xa–based method normal antithrombin activity was found in the patient with type II deficiency; her siblings also had low levels with the thrombin-based test. We would therefore like to stress that there is a possibility of not detecting all type II deficiencies with a factor Xa inhibition–based test, which is the most widespread routine method. In our investigation the factor Xa–based method overestimated antithrombin activity levels. According to our knowledge this has not previously been described and can cast some doubts about the validity of the use of this test as the test of choice when screening for antithrombin deficiency. Therefore we believe that both the bovine thrombin– and factor Xa inhibition–based tests, together with an antigen method, should be performed in patients with suspected antithrombin deficiency type II.

Johanna S. Ungerstedt, Sam Schulman, Nils Egberg, Jovan Antovic, and Margareta Blombäck

Correspondence: Johanna Ungerstedt, Coagulation Research, Clinical Chemistry Building L25, Karolinska Hospital, S 171 76 Stockholm, Sweden; e-mail: johanna.ungerstedt@ki.se

References

To the editor:

RHCE represents the ancestral RH position, while RHD is the duplicated gene

In 2000, we elucidated the structure of the RH locus1 by showing that it is an example for a gene cluster; RHD and RHCE face each other by their 3’ tail ends, and a third gene, SMP1, was found to be interspersed between the 2 rhesus genes. Two 9 000 base pair (bp) DNA segments, dubbed “rhesus boxes,” of identical orientation fringed the RHD gene (Figure 1, top).

Based on this structure of the RH locus, the RHD gene deletion was parsimoniously explained by an unequal crossing-over event.1 Furthermore, the inverse orientation of the RH genes may facilitate gene conversion among both rhesus genes, which would explain the high frequency of RHD-CE-D or RHCE-D-CE hybrid alleles.3 However, it remained unknown which rhesus gene, if any, represented the ancestral positioning. The close proximity of the RHCE and SMP1 in humans was startling too.

The duplication of the rhesus gene is known to have occurred during primate evolution,3 giving rise to the RHD and RHCE genes in humans. Hence nonprimate mammals, like mice, may reveal the ancient state of the RH locus. In this context an 89 065 bp genomic DNA segment that was recently deposited in public databases (GenBank entry AL611963), which encompassed the mouse RH locus (Figure 1, bottom), was most disclosing. In order to compare the topology in mouse to the human RH locus we assembled a 315 242 bp DNA segment that included the human RH locus.

The assembly of this human genomic DNA was complicated by the fact that the current GenBank entry AL139426 contained sequences representative of RHD, SMP1, both rhesus boxes, and parts of RHCE but did not represent their correct topology. To overcome this limitation we compared the sequence of AL139426 to the sequences of RHD (X63097) and RHCE (M34015) cDNA, of RHD (AB035192) and RHCE (AB035191) intron 3, of RHD (AB035185) and RHCE (AB035184) intron 9, and of the upstream (AJ252311) and downstream (AJ252312) rhesus boxes. We determined multiple misassemblies occurring in long regions between almost identical paralogous sequences (join of RHD exon 3 to
Discrepancy between antithrombin activity methods revealed in Antithrombin Stockholm: do factor Xa–based methods overestimate antithrombin activity in some patients?

Johanna S. Ungerstedt, Sam Schulman, Nils Egberg, Jovan Antovic and Margareta Blombäck