CORRESPONDENCE

A Platelet-Activating Antiglycoprotein Ib Monoclonal Antibody

To the Editor:

In the March 1 issue, Yanabu et al1 reported on a platelet-activating antiglycoprotein Ib monoclonal antibody (MoAb) NNKY5-5. The intact antibody, but not Fab-fragments, caused activation, and (Fab')2-fragments could not be prepared. Inhibition of the platelet FcγII-receptor by the antibody IV.3 did not prevent platelet activation by NNKY5-5. From this the investigators concluded that the action of the antibody on platelets was FcγII-receptor-independent, and hence due to signal transduction via glycoprotein Ib.

We2 previously analyzed MoAb-mediated platelet activation, where we could show that the mechanism of activation clearly depends on the antibody subtype. Activating IgG1-antibodies can do so by cross-linking the FcγII-receptor, Fab- or (Fab')2-fragments are inactive and inhibition is obtained by blocking FcγRII. Activating IgG2-antibodies, on the other hand, essentially cause platelet activation via the complement system, whereas again Fab- or (Fab')2-fragments are inactive. However, FcγRII-inhibition has no effect but leupeptin-treatment or complement inactivation prevents platelet activation. High concentrations of antibody may result in cell lysis and washed platelets are not sensitive or less sensitive.

In their report, Yanabu et al1 mention that only a minimal aggregation response to NNKY5-5 was obtained in washed platelets in contrast to what was seen in platelet-rich plasma. Furthermore, NNKY5-5 is an IgG2-antibody. Both findings may be an indication that complement activation through the Fc-part of the antibody could have been involved in the platelet aggregation response. This possibility was not really excluded.

Only very few MoAbs are able to activate platelets in an Fc-independent manner,1,4 and they indeed represent the most interesting ones for further investigation of receptor involvement in signal transduction. However, a fully detailed characterization of all possible mechanisms of activation is necessary.

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We thank Deckmyn et al for their interest in our study on platelet activation induced by an anti-GP Ib monoclonal antibody (MoAb), NNKY5-5.1 They have raised a possibility that complement activation through the Fc-part of the antibody is involved in platelet aggregation induced by NNKY5-5. Although their points are interesting and deserve attention, we have several lines of evidence to suggest that complement activation cannot totally explain the entire picture of platelet activation induced by NNKY5-5. Several MoAbs belonging to the IgG2-subclass activate the complement system.2 Platelet aggregation induced by antibodies with complement-activating property is extensive and accompanied with cell lysis in platelet-rich plasma.1,4 NNKY5-5, even at high concentrations, showed no sign of cell lysis when added to platelet-rich plasma. In washed cells it induced weak and irreversible aggregation. Although Fab1 fragments of NNKY5-5 failed to induce platelet aggregation, they could induce tyrosine phosphorylation and activation of Syk, a tyrosine kinase, and potentiated platelet aggregation induced by other agonists. These findings clearly indicate that NNKY5-5, independent of the Fc portion, elicit certain intracellular activation signals by interacting with GP Ib. We have no direct evidence to exclude the possibility that complement activation partly contributed to platelet activation observed with platelet-rich plasma, as suggested by Deckmyn et al. We hope to address this in the near future. However, our main finding with NNKY5-5, an anti-GP Ib MoAb, which we described in our previous report in Blood,1 is that an antibody that interacts with GP Ib can mediate platelet activation signals, independent of Fc receptors or Fc portion of the antibody.

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To the Editor:

Although variables relating to disease biology and patient characteristics are strong determinants of outcome after bone marrow transplantation (BMT), the identification of these nonmodifiable factors is often of limited practical use. On the other hand, treatment- and transplant-related factors, which are modifiable, can potentially be manipulated in clinical practice to improve the results of transplantation. The number of nucleated cells infused during transplantation is a variable which is usually controllable. Therefore, it is encouraging to see that increasing this number as much as possible improves survival by reducing transplant-related mortality in patients allografted from unrelated donors.

We have also found in a number of different studies that the nucleated cell dose significantly affects transplant-related mortality, and the speed as well as completeness of hematologic reconstitution after autologous and allogeneic transplantation for hematologic malignancies.

Among 74 patients with acute myeloid leukemia (AML) allografted in first remission after melphalan and single-fraction total-body irradiation (TBI), 7 of 21 patients receiving \( \geq 2 \times 10^8 \) nucleated cells/kg body weight and 7 of 53 patients receiving \( >2 \times 10^8 \) nucleated cells/kg body weight died of treatment-related toxicity (\( P = .047, \chi^2 \) test). In multivariate analysis, patients receiving the higher cell dose had a significantly better disease-free survival (relative risk 2.17, \( P = .045 \)). The higher toxic death rate and poorer disease-free survival in patients receiving \( \geq 2 \times 10^8 \) nucleated cells/kg was only partially due to incomplete or delayed hematopoietic reconstitution with resultant increase in bleeding or infections because the cell dose did not affect the probability or rapidity of engraftment significantly in this group of 74 patients. Because \( 2 \times 10^9 \)/kg cells is our usual target for collection during an autologous marrow harvest, those receiving \( \geq 2 \times 10^9 \)/kg nucleated cells may have represented a group of patients with compromised marrow reserve due to either high-risk disease or previous chemotherapy who subsequently had problems during the transplant.

However, in a study of factors affecting hematologic recovery after unpurged autologous blood or marrow transplantation in 240 patients with acute leukemia (which included the previous 74 patients), a nucleated cell dose of \( <2 \times 10^8 \)/kg was independently associated with slower recovery to \( 0.5 \times 10^9 \)/L neutrophils, and with slower and incomplete recovery to \( 50 \times 10^9 \)/L platelets. There was a strong correlation between neutrophil recovery and five increasing nucleated cell dose levels (\(<1.5, 1.5-2, >2-2.5, >2.5-3.5, \) and \( >3.5 \) \( \times 10^8 \)/kg).

Long-term follow-up of 85 first-remission AML patients allografted from HLA-identical siblings after cyclophosphamide and single-fraction TBI with cyclosporine for graft-versus-host disease (GVHD) prophylaxis showed that infusion of a lower nucleated cell dose (\( <2.6 \times 10^9 \)/kg) was independently predictive of increased transplant-related mortality (relative risk 2.69, \( P = .002 \)). The higher cell dose was associated with better disease-free survival (relative risk 2.01, \( P = .045 \)) in multivariate analysis. Figure 1 shows the effect of the cell dose in this group of patients.

Similarly, in a study of transplant-related mortality in 138 acute leukemia patients allografted after melphalan and TBI with cyclosporine and methotrexate, a high infused marrow cell dose (\( \geq 2.5 \times 10^7 \) total nucleated cells/kg or \( \geq 0.6 \times 10^8 \) mononuclear cells/kg) protected against fatal interstitial pneumonitis compared with lower cell doses (risk ratio 0.47, \( P = .023 \)).

After allogeneic transplantation in 712 patients with hematologic malignancies, a low total leukocyte count in the third week was found to be the most significant predictor of death due to infections, hemorrhage, or graft failure within the first 3 months. A low nucleated cell dose (\(<2.5 \times 10^7 \)/kg) was independently associated with increased risk of death due to these causes (relative risk 2.2, \( P = .025 \)).

![Fig 1. The effect of cell dose on disease-free survival and transplant-related mortality in 85 AML patients allograft in first remission (cell numbers not available for two patients). Higher cell doses are significantly better.](image-url)
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