Published in final edited form as:

Transfusion. 2016 October ; 56(10): 2648–2649. doi:10.1111/trf.13746.

Immune Destruction of Human Platelets in The NOD/Scid Mouse

Daniel W Bougie, Ph.D.,

Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI

Dhirendra Nayak, and

Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI

Richard H Aster, M.D.

Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI

Abstract

Recently Fuhrmann et al published in Transfusion an article on optimization of the NOD/scid mouse model for studying survival kinetics and immune clearance of human platelets *in vivo* (Transfusion, Apr 18, 2016). An additional unresolved issue concerning this model is whether cross-species incompatibility between human IgG Fc and mouse Fc receptors (FcR) adversely affects antibody-induced platelet clearance. If so, it might be necessary to use mice transgenic for human FcR to optimize the model for study of immune destruction of human blood cells. We used the IgG1 mouse monoclonal 7E3, specific for human GPIIb/IIIa, a humanized variant of 7E3 (c7E3) and c7E3 Fab fragments to show that the inter-species FcR difference impairs clearance of human platelets sensitized with Ig1 antibodies only slightly and cite *in vitro* studies suggesting that the same is likely to be true of IgG3 antibodies, the second most common pathogenic antibody isotype. Findings made validate use of the NOD/scid mouse model for the study of antibody-mediated clearance of human blood cells.

The NOD/scid mouse lacks xenoantibodies that would normally cause rapid destruction of transfused human cells and appears to provide an excellent tool for studying antibody-mediated clearance of human platelets^{1,2}. In the April 18 issue of Transfusion, Fuhrmann et. al. provide evidence that reproducibility of measuring post-transfusion recovery and survival of platelets in the mouse can be improved by injecting platelets via the tail vein route and by fixing platelets in harvested blood samples before to subjecting them to analysis³. Clearance of sensitized platelets in this model depends on recognition of antibody Fc domains by phagocytic cells. An unresolved issue is the extent to which cross-species incompatibility between human IgG Fc and mouse Fc receptors (FcR) impairs the clearance of cells coated with human antibodies. This shortcoming could, in theory, be corrected by introducing human FcRs into the animal by transgenic means. Here, we describe studies to determine whether this should be considered.

The IgG1 monoclonal antibody (mAb) 7E3, specific for platelet membrane glycoprotein GPIIb/IIIa, is the source material for abciximab, widely used to reduce thrombotic

complications in patients undergoing percutaneous transluminal coronary angioplasty⁴. To reduce immunogenicity in patients, 7E3 variable regions that confer specificity for GPIIb/IIIa were incorporated into a human IgG1 framework and this chimeric version of the molecule was used to produce Fab fragments that make up abciximab. Since 7E3, chimeric 7E3 and abciximab all recognize the same epitope on GPIIb/IIIa and differ only in having constant regions comprised of mouse Fc, human Fc and no Fc domain, respectively, these reagents (kindly supplied by Robert Jordan of Johnson and Johnson Inc, New Brunswick, NJ) are ideal for studies to determine whether Fc-dependent cell clearance mechanisms in the mouse distinguish between human and mouse Fc domains.

Experimental procedures have been previously described². NOD/scid mice were transfused with 4×10^8 human platelets. After allowing 30 minutes for platelets to stabilize, a baseline tail blood sample was taken and 2 μ g of 7E3, chimeric 7E3 or abciximab was injected into the peritoneum. Residual human platelets were quantified by flow cytometry in blood samples taken 5 and 24 hours later and residual human platelet levels were compared to the baseline value. As shown in Figure 1, 7E3 and chimeric 7E3 cleared about 75% and 30% of the transfused cells, respectively ($p < 0.01$) at 5 hours and 100% at 24 hours. As expected, the Fab fragment, abciximab, was ineffective in promoting platelet clearance.

The findings indicate that recognition and clearance by the mouse of platelets coated with IgG1 antibodies possessing human constant regions is only slightly less efficient than clearance of cells coated with mouse IgG1. These observations validate the mouse as a model for demonstrating the ability of human IgG1 antibodies, the isotype that is most often pathogenic, to promote blood cell destruction *in vivo*. *Ex vivo* studies performed by Overdijk et al with recombinant mouse FcRs suggest that human IgG3 antibodies will behave similarly⁵.

References

1. Newman PJ, Aster R, Boylan B. Human platelets circulating in mice: applications for interrogating platelet function and survival, the efficacy of antiplatelet therapeutics, and the molecular basis of platelet immunological disorders. *J Thromb Haemost.* 2007; 5 Suppl 1:305–9.
2. Bougie DW, Nayak D, Boylan B, et al. Drug-dependent clearance of human platelets in the NOD/scid mouse by antibodies from patients with drug-induced immune thrombocytopenia. *Blood.* 2010; 116:3033–8. [PubMed: 20587782]
3. Fuhrmann J, Jouni R, Alex J. Assessment of human platelet survival in the NOD/SCID mouse model: technical considerations. *Transfusion.* 2016 Apr 18. Epub ahead of print.
4. Knight DM, Wagner C, Jordan R, et al. The immunogenicity of the 7E3 murine monoclonal Fab antibody fragment variable region is dramatically reduced in humans by substitution of human for murine constant regions. *Mol Immunol.* 1995; 32:1271–81. [PubMed: 8559151]
5. Overdijk MB, Verploegen S, Ortiz Buijsse A, et al. Crosstalk between human IgG isotypes and murine effector cells. *Journal of immunology.* 2012; 189:3430–8.

Figure 1. Clearance of human platelets in NOD/scid mice following injection of murine monoclonal 7E3, chimeric 7E3 and abciximab

Platelets (4×10^8) suspended in normal plasma were injected into the retro-orbital plexus of NOD/scid mice. After 30 minutes, a baseline blood sample was collected and 2 μ g of 7E3 (mouse IgG1), chimeric 7E3 or abciximab (Fab fragment of chimeric 7E3) were injected intraperitoneally.

Residual human platelets were measured in tail blood samples collected at 5 and 24h.

Percent human platelet survival (relative to baseline) is shown on the ordinate. Abcissa denotes times were taken after antibody injection. Values shown are the averages of results obtained in three mice $\pm$ 1.0 S.D. Mean values obtained with 7E3 and chimeric 7E3 at 5 hours were significantly differently different ($p < 0.01$, **).