et al. In gene frequency studies such as this, a larger patient cohort yields a more robust statistical analysis.

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References

Response:

PTLD risk and IFNG polymorphisms

First, we would like to thank Thomas and colleagues for their interest in our work, their provision of additional data, and their commentary regarding genotyping studies and clinical posttransplantation lymphoproliferative disorder (PTLD). A central component of our prospective preclinical study using the human peripheral-blood leukocyte severe combined immunodeficient (hu PBL-SCID) mouse model of Epstein-Barr virus (EBV) lymphoproliferative disease was the demonstration of a statistically significant association of the adenosine (A/A) interferon-γ (IFNG) genotype with the spontaneous development of EBV lymphoproliferative disease. As part of our discussion, we mentioned that our prospective preclinical studies were in line with our retrospective clinical observation in a group of renal transplant patients showing that the same A/A IFN-γ genotype was statistically more prevalent in renal transplant patients who developed PTLD (n = 12) when compared with renal transplant patients who did not develop PTLD (n = 135, P = .02). This suggests that the A/A IFNG genotype may be a risk factor for the development of PTLD in renal transplant patients.

Thomas and colleagues did not observe an association of IFNG genotype with the development of PTLD in their transplant patients. However, as pointed out by Professor Thomas, our PTLD patients were all renal transplant recipients, while their cohort consisted of a heterogeneous group who had undergone renal, lung, heart, hematopoietic cell, and liver transplantations, the latter with or without small-bowel transplantations. Further, the control population in our retrospective analysis consisted entirely of renal-transplant patients who did not develop PTLD. It is unclear if the transplant-recipient controls in the Thomas et al study were matched for the organ transplanted, immunosuppression, race, or ethnicity. Assuming that the variables were not matched, the results from our analysis are not comparable with the data from Thomas et al. If the Thomas et al study was properly controlled and the results were as reported, it is conceivable that our hypothesis holds true for renal-transplant patients but not for other solid-organ transplant recipients who receive different regimens of immune suppression and have a different incidence of PTLD.

The hypothesis that an IFNG genotype is associated with the development of clinical PTLD is an active area of investigation. We do agree with Thomas and colleagues that a larger patient cohort is needed to properly assess this association in a statistically robust fashion. Most transplant centers have only a few PTLD patients each year, often of different organ types, making it difficult to perform well-controlled analyses. For this reason, we have joined with several medical centers to compare transplant patients with PTLD to transplant patients without PTLD that are matched for age, ethnicity, immune suppression, and for the transplanted organ. By our calculation, this analysis will require 65 PTLD organ transplant patients and 130 matched non-PTLD organ transplant patients to achieve 80% power. This study was recently funded by the US National Cancer Institute, and we would welcome the participation of other centers to assist us in determining if the IFNG genotype is a risk factor for the development of clinical PTLD.

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To the editor:

Dose-response relationship and pharmacogenetics of anti-RhD monoclonal antibodies

In their paper, Miescher et al describe the effect of MonoRho, a recombinant anti–Rhesus D (RhD) human immunoglobulin G1 (IgG1)/kappa monoclonal antibody (mAb), on RhD red blood cell (RBC) elimination, and the association between this elimination and both FCGR2A and FCGR3A gene polymorphisms. They conclude that (1) there is a correlation between dose and mAb serum concentrations and an absence of correlation between mAb dose and RBC elimination, and (2) FCGR2A and FCGR3A genotypes influence this elimination.
A pharmacokinetic analysis using the measured mAb concentrations would have been useful to confirm the first assertion. However, Figure 1 of their paper clearly shows that concentrations do not increase linearly with the dose. Therefore, the analysis of the relationship between mAb dose and RBC elimination should have been based on mAb concentrations rather than dose, and on the calculation of RBC elimination rate over time rather than on estimation of global RBC half-life. In addition to studying the dose-response relationship, the authors should have taken into account individual genotypes as a covariable.

Miescher et al also report that FCGR3A-158VF and FCGR2A-131H/R genotypes are independently associated with RBC elimination. The influence of the FCGR3A polymorphism confirms our previously published results on the response of patients with non-Hodgkin lymphoma to rituximab, a chimeric IgG1 mAb. We showed that FCGR3A-158VV natural killer (NK) cells display a higher affinity for rituximab than FCGR3A-158FF, and that rituximab leads to higher antibody-dependent cellular cytotoxicity (ADCC) with FCGR3A-158VV than with FCGR3A-158FF NK cells. The influence of the FCGR3A genotype may be explained by a similar mechanism of action of these mAbs (ie, ADCC). The FCGR2A polymorphism is known to influence human IgG2, but not human IgG1, binding to the Fcγ receptor IIa (FcγRIIa). Therefore, the influence of the FCGR2A polymorphism is unexpected since the anti-RhD mAb under study was an IgG1. The reported influence of the FCGR2A genotype on RBC half-life was at the limit of significance (P = .05). In addition, the doses received by the subjects cannot be considered similar since their means were 975, 780, and 1440 μg for RR, HR, and HH patients, respectively (Kruskall-Wallis test, P = .098). The influence of the FCGR2A polymorphism observed by the authors can also be explained by the well-known linkage disequilibrium between FCGR3A-158 and FCGR2A-131 polymorphisms in white individuals. Indeed, both genes are physically very close together (about 25 kilobase pairs apart). We have confirmed this by analyzing the sequence of a bacterial artificial chromosome (BAC) clone available in public databases: the intergenic distance is only 24 863 base pairs (Figure 1). Despite the relatively small size of the population studied by Miescher et al, a nonrandom distribution of FCGR2A and FCGR3A genotypes can be detected when pooling FCGR2A-R carriers and FCGR3A-F carriers (Fisher exact test, P = .024).

Response:

Pharmacogenetics of monoclonal anti–Rhesus D antibody (MonoRho): effect of FCGR polymorphisms

In our study, we tested a monoclonal anti–Rhesus D (RhD) antibody (MonoRho) in RhD-negative volunteers challenged with RhD-positive erythrocytes. Elimination of RhD-positive erythrocytes was not dose dependent, but rather influenced by polymorphisms in the Fcγ receptor IIa (FCGR2A) and FCGR3A genes. Ternant and colleagues question the influence of the FCGR2A polymorphism and suggest additional evaluations, which we have now performed.

A possible effect of dose on the elimination of RhD-positive erythrocytes was investigated in an analysis of variance (ANOVA) with the following factors: dose, FCGR2A, FCGR3A, and FCGR3B. The dose effect was not statistically significant (P = .07), and there was no trend for increasing elimination speed with increasing dose levels. On the other hand, the P values for FCGR2A and FCGR3A became even more clearly significant (P = .02 for both versus P = .05 for both when dose was not entered in the model). The effect of FCGR3B remained nonsignificant (P = .64).

We repeated the ANOVA with the covariate serum concentration (area under the curve [AUC] from 1 to 48 h) and the factors FCGR2A, FCGR3A, and FCGR3B. This analysis showed no significant effect of the serum concentration (P = .13), while the effects of the FCGR2A and FCGR3A, but not of FCGR3B, were significant (P = .02, P = .04, and P = .97, respectively). The lack

References

of a concentration effect is not unexpected, as the analytical method used measures only the antibody that has not bound to the target epitope. Additionally, saturation of anti-RhD binding sites was observed at all MonoRho doses except 300 μg.

Analyzing elimination rates rather than half-lives generates the same results, when both variables are log transformed.

The results of the ANOVA that we have performed rule out the possibility that the observed effect of the FCGR2A polymorphism was due to a linkage disequilibrium between FCGR2A and FCGR3A polymorphisms.

Thus, in our study the FCGR2A polymorphism had a statistically significant influence on the clearance of RhD-positive erythrocytes mediated by the monoclonal immunoglobulin G1 (IgG1) antibody MonoRho. However, there was no effect on the clinically more important end point of RhD sensitization. An effect of FCGR2A polymorphism on the clinical response to another IgG1 monoclonal antibody, rituximab, has been reported, while others have not seen such an effect.

These observations are consistent with an independent effect of FCGR2A polymorphisms on antibody-mediated effector mechanisms. This effect may vary according to the monoclonal antibody, the effector mechanisms involved, the disease, and the end point of the study. Nevertheless, the possibility of a linkage disequilibrium with genes other than FCGR3A cannot be ruled out.

Further investigations are needed to clarify the effect of FcγR polymorphisms on the response to antibody therapies. In clinical trials, statistical tests such as analysis of variance or logistic regression should be used to test for effects of both polymorphisms, taking linkage disequilibrium into account. Clinical trials should be complemented with laboratory studies, which may help to unravel the different mechanisms and have important implications for the treatment of many diseases with monoclonal antibodies.

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To the editor:

Up against the wall

In the January issue of Blood, Day and colleagues published an article claiming that macrovascular thrombosis is driven by tissue factor (TF) derived primarily from the blood vessel wall. In this study, the authors compared TF activity measurable in a homogenized carotid vessel with relatively unperturbed, intact, isolated blood fractions and concluded that the vessel wall “drives” thrombosis. As TF within the vessel wall is spatially separated from the lumen, it is likely that such, if not all, of the activity being measured by the authors is due to a “vessel-wall fraction” never comes into contact with flowing blood during a thrombotic episode. Hence, the thrombus-driving TF activity coming from the vessel wall is likely grossly overestimated. Using this rationale, the authors should have concluded that the brain (very rich in TF) drives thrombosis, and that the blood vessel is irrelevant. Moreover, the use of isolated blood fractions is likely to lead to underestimation of the ability of these fractions to either recruit or expose decrypted TF. Whereas the authors do recognize that they are measuring active TF, they apparently ignore the commonly held view that circulating TF is likely cryptic. Comparisons of vessel-wall activity and blood activity using equal volumes ignore the ability of flow to convectively deliver TF to the injured site from a larger pool. Thus, the comparisons of vessel-wall TF activity to TF activities from the blood are improper, and the conclusion that vessel-wall TF “drives” thrombosis is unwarranted.

Furthermore, the in vivo experiments, using a harsh combination of laser and Rose Bengal to induce continuous injury for 60 minutes (based on cited reference no. 31), do not support the conclusion that vessel-wall TF “drives” thrombosis. It is clear that vessel-wall TF has a role in the initiation of thrombosis. Thus, it may be more difficult to initiate a successful vessel-wall injury in a mouse that has low levels of TF in the vessel wall. Moreover, the long duration of the injury makes it difficult to conceive what events may be occurring in the blood over the 30 to 45 minutes prior to occlusion.

In their introduction, Day and colleagues summarized a report by Giesen et al and stated that “a small amount of TF is detectable in the blood and is capable of supporting clot formation in vitro.” The cited article, however, actually measured ex vivo thrombus formation, not in vitro clot formation. In their discussion, the authors stated that the experiments of Himber et al did not establish a cause-and-effect relationship between leukocyte-TF expression and thrombus growth. However, Himber et al observed that the addition of an anti-TF antibody abrogated thrombus growth as measured by fibrin accretion in a silastic jugular-jugular shunt.

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