generation in HIT came from studies by Pouplard et al and Arepally et al, indicating that monocytes express tissue factor (TF) when stimulated by the HIT immune complex, consisting of macromolecular heparin/PF4 in combination with anti-heparin/PF4 IgG antibodies. Now Kasthuri et al confirm and extend these findings, discovering that monocytes express TF and release TF/microparticles (MPs) on stimulation by HIT immune complexes.

The authors are to be commended for validating that both human HIT IgG and the HIT-like monoclonal antibody KKO act the same. As per the figure, they nicely demonstrate that the CD64 FcγRI receptor on monocytes, and not FcγRIIA (CD32a), transduces the signal to TF generation. Furthermore, they demonstrate that MEK and erk1/2 are involved. One more interesting note from these careful studies: their data are consistent with the localization of the stimulatory HIT immune complex on the neighboring cell surface, in keeping with the observations by Rauova and colleagues.

The generation of TF and TF+ MPs by monocytes likely plays a substantial role in the thrombosis of HIT. We can hope that this new knowledge of the effectors in this process enables targeted approaches that minimize hemorrhagic side effects in treating HIT.

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SNPs and GVHD prediction: where to next?

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The article by Chien et al in this issue of Blood uses a novel approach to assess the role of single nucleotide polymorphisms (SNPs) in acute graft-versus-host disease (GVHD). Using a genome-wide association study (GWAS) employing an Affymetrix GeneChip Genome-Wide Human 500 000 SNP array, they screened 1298 allogeneic hematopoietic stem cell transplant donors and recipients and tested whether the results from 40 previously reported candidate SNPs could be replicated. They also used a novel approach to impute data using IMPUTE software (http://nathgen.stats-ox.ac.uk/impute/impute.html) where the genotyping data were not available.

Since the first publication describing a potential role of TNF and IL10 polymorphisms in predicting GVHD, a large number of reports have described a wide range of SNPs that are often also associated with inflammatory disease or autoimmune disease. Among the most studied but also with variable results have been IL10, TNF, and NOD2.

Of the 40 published candidates in the study by Chien et al, only 16 had usable data (genotype or imputed) for analysis. Interestingly, of all the SNPs analyzed, IL6 was the most consistent. The IL6G74 genotype has also often been consistently associated with increased GVHD and increased serum levels of IL-6 that have also correlated with C reactive protein levels. Of the other SNPs that could be assessed by genotyping or imputation were those of CTLA4, MTHFR (5,10-methylenetetrahydrofolate reductase), and

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IL2, all of which were over the $P < .05$ threshold. In total, 6 SNPs could be geno-
typed and 10 could be imputed with therefore
only 16 of 40, or 40%, available for analysis.
The results from the imputed *IL10* SNPs (rs
1800871 and rs 1800872) replicated previous
studies and this method of analysis could well
be useful in future studies. However, imputed
data are not without drawbacks and are limited
by the quality of the original SNP data.

Although many of the genes associated
with HSCT outcome have also been implic-
ated in autoimmune disease, few have re-
tained significance after GWAS. *CTLA4* is the
exception; it has been implicated in au-
toimmune thyroid disease, rheumatoid arthritis,
and type 1 diabetes. In previous studies in the
HLA identical sibling HSCT setting, the
presence of the donor CT60 polymorphism
(G allele; rs 3087243) in the *CTLA4* gene re-
duced overall survival (increased relapse) and
increased the development of GVHD in the
presence of the AA genotype. The opposite of
these results was observed by Chien et al.1
Further studies in a matched unrelated donor
cohort by Vannucchi et al could not confirm all
of these findings, although the donor AA ge-
totype predicted both grade III-IV acute and
extensive chronic GVHD. The T-cell cyto-
toxic *CTLA-4* antigen is involved in T-cell
activation and HLA binding via the antigen-
deleted signaling molecules involving the
B7/CD80/CD86/CD28 interleukin path-
way. *CTLA-4* antigen is homologous to the
CD28 molecules and competes for B7 binding,
which results in overall down-regulation of the
T-cell response and may be involved in failure of
T-cell tolerance. This mechanism of action of
*CTLA-4* is important in the development of
autoimmune disease, presenting a potential
new target for therapeutic intervention, but is
also relevant to hematopoietic stem cell cell
transplantation. *CTLA* is expressed on Tregs and
induces indoleamine2, 3 dioxygenase (IDO),
which suppresses IL-6 production in dendritic
cells while IL-10 production is increased.
Other cytokines such as IFN$\gamma$ are potent in-
ducers of IDO, which has recently been shown
to have an important role in clinical GVHD.7
High levels of IL-6 can also make effector
T cells refractory to T regulatory immunosup-
pression. The GWAS results of Chien et al
identifying a limited but important number of
key immunoregulatory SNPs may therefore
be linked and play a role in Treg immuno-
biology (see figure), the development of
GVHD, and possible reduction in graft-
versus-leukemia (GVL) effects involving al-
erations in IL-2 production as well as metabo-
ism of methotrexate via MTHFR.

Despite more than 10 years of research
using the candidate gene approach to identify-
ing polymorphisms associated with HSCT
outcome, there have been relatively few genes
that consistently predict either GVHD,
transplant-related mortality (TRM), or sur-
产学. This is due to a number of factors that
are beyond the individual researcher’s control.
One is the size and heterogeneity of the trans-
plant cohort, including age at transplant, type
of conditioning, type of GVHD prophylaxis,
and type of donor. Transplant protocols have
also changed dramatically over the past
10 years with the instigation of reduced inten-
sity conditioning, increased use of peripheral
blood stem cells, and increasing age of the
patient at time of transplant, and in reduced
numbers of chronic myeloid leukemia (CML)
patients within cohorts because of successful
drug trials using inabim (a tyrosine kinase
inhibitor targeting the deletion in CML) and
its derivatives. Validation of the results is a
critical issue.

HSCT GWAS studies were initiated by
Chien et al where they identified a number of
SNP genotypes associated with gram negative
bacteremia and bronchiolitis obliterans using a
large cohort of more than 3000 patient-donor
pairs for discovery and validation studies.9
A follow-up replication study further assessed
65 SNPs associated with 12 HSCT pheno-
types including those associated with acute
and chronic GVHD/tolerance, acute lung or
kidney injury, bronchiolitis obliterans, gram
negative bacteremia, CMV activation, overall
survival, transplant-related mortality, and
relapse. Of the 9 SNPs described by Chien
et al associated with gram negative bacteremia,
only 1 was successfully replicated. In conclu-
sion, the authors suggest a fuller increase in
cohort size to increase the power and odds
ratio to nearer 2.0.

Other studies have failed to replicate re-
results identifying immunomodulatory SNPs
(*IL10, IL1 receptor antagonist (IL1RN)*) and
TNF receptor II (*TNFRSF1B*) in a large
transplant cohort of CML patients.11 This was
likely due to significant clinical differences
between the 2 cohorts.

These types of results illustrate the ques-
tionable clinical relevance of studies reporting
low odds ratios and the importance of confir-
matory studies. However, low odds ratios can
still identify the encoded proteins as poten-
tially novel drugs. This has been demon-
strated in type 1 diabetes where peroxisome
proliferator-activator receptor $\gamma$ (*PPARG*)
and *KCNJ11* genes (encoding for the ATP-
sensitive potassium channel subunit Kir 6.2)
are now major drug targets.12

GWAS and SNP studies also reveal impor-
tant disease pathways that identify therapeutic
targets and potential biomarkers that can be
used for monitoring the disease and response
to therapy. It is in this latter role that SNP
analysis finds its usefulness. Transplant outcomes (especially for survival) can be done using a number of risk indices\(^1\)). GWAS and SNP studies are used to indicate directions for therapy; for example, via T regulatory cells or pathways that indicate early events that could then be used to target novel treatment in the future.

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