65% and more than 50% of them had adverse cytogentic changes. Finally, 55% of patients had secondary AML. Overall, of the 84 patients receiving T + E, 21 (25%) achieved CR, with a median CR duration of 9.8 months. Patients achieving CR had a median age of 77 years and their median survival was 22 months, with 14 (67%) surviving more than 1 year and 9 (43%) still living at 15.5 to 36+ months. These data compare favorably with results achievable after conventional chemotherapy as well as with those reported by using single agent T or T + low-dose ARA-C. Previous study of single agent T had considered a 21-day schedule, which was not feasible in combination with E. In contrast, the same etoposide schedule was tested with the results of this study. 

In the current study, the search of preclinical and experimental clinical trials that avoid unnecessary time to allocate these patients into upfront evaluation and therapy has been successful. It is now possible to treat patients with "weaker" immune response (defined as a change in optical density [OD] or \( \Delta\text{OD} \) of > 1.0 from baseline signal) showed a trend toward earlier onset of PF4/heparin antibodies as compared with patients with "stronger" immune response (\( \Delta\text{OD} < 1.0 \), this did not reach significance. In the subset of patients analyzed for isotype patterns (\( n = 58 \), the authors were unable to document any differences in the timing of onset of the individual immunoglobulins (IgG, IgA, or IgM). The authors provide 2 additional intriguing observations on the trajectory of the HIT immune response during heparin therapy. They note that antibody responses “peak” by day 9 to 10 of UFH/LMWH therapy and decline thereafter, despite continuation of therapy. They also note that in a LMWH extension trial in which investigators were blinded to findings of PF4/heparin serology, 31 seropositive patients (half of whom had platelet activating antibodies) received an additional 9 months of LMWH thromboprophylaxis without developing clinical HIT.

In the companion paper by Warkentin and colleagues,6 daily samples from 48 patients (12 patients who develop HIT and 36 patients who manifest only PF4/heparin antibodies) are analyzed over time to document serologic features associated with HIT. The authors note that although patients with and without HIT have similar time to onset of the immune response (median interval of 4–5 days), the time to onset of IgG isotype (interval onset time of 4.7 days in HIT patients and 6 days in non-HIT patients) and magnitude of the IgG response (median OD, HIT = 1.63 vs non-HIT = 0.94) differs in the 2 groups. Importantly, the authors provide a “timeline” for development of clinical complications relative to seroconversions. They show that

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**THROMBOSIS & HEMOSTASIS**

Comment on Greinacher et al, page 4970, and Warkentin et al, page 4963

**Nothing typical about HIT**

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In this issue of Blood, 2 articles describe the evolution of the PF4/heparin immune response and provide further proof that the HIT immune response is anything but typical.

The immune response to PF4/heparin, which is the serologic basis of heparin-induced thrombocytopenia (HIT), is unlike any other known drug-induced hypersensitivity reaction. Whereas most reactions to drugs are rare, idiosyncratic, and short-lived, the immune response triggered by heparin is fairly common,1 predictable in certain clinical settings,2 and short-lived in most cases.1 The immunologic basis of these atypical features in HIT is unknown.

Animal models of PF4/heparin antibody formation have recently been developed to gain insights into the immune pathogenesis of HIT.4 Modeling the disease in animals, however, has been hampered by a lack of knowledge of the early events related to human PF4/heparin seroconversions. In this issue of Blood, Greinacher and colleagues5 and Warkentin and colleagues6 address some of the critical gaps in our understanding of the HIT immune response. Although both studies employ the same general approach to studying the timeline of seroconversions, the goals of each study differ. The study by Greinacher and colleagues provides a detailed serologic analysis of the PF4/heparin immune response; the study by Warkentin et al focuses on characteristics of seroconversions leading to clinical complications in HIT.

Greinacher et al document the course of PF4/heparin seroconversions in a cohort of 435 patients treated with thromboprophylactic doses of unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH).3 Using serially collected samples from these patients, the authors characterize the temporal course of PF4/heparin antibody formation, isotype patterns, and the trajectory of the immune response during continued heparin therapy. They note that more than 90% of the seroconversions occur within 4 to 14 days of heparin therapy while 6.6% occur before day 4 and another 1.6% occur after day 14. Although patients with a “strong” immune response (defined as a change in optical density [OD] or \( \Delta\text{OD} \) of > 1.0 from baseline signal) showed a trend toward earlier onset of PF4/heparin antibodies as compared with patients with “weaker” immune response (\( \Delta\text{OD} < 1.0 \), this did not reach significance. In the subset of patients analyzed for isotype patterns (\( n = 58 \), the authors were unable to document any differences in the timing of onset of the individual immunoglobulins (IgG, IgA, or IgM). The authors provide 2 additional intriguing observations on the trajectory of the HIT immune response during heparin therapy. They note that antibody responses “peak” by day 9 to 10 of UFH/LMWH therapy and decline thereafter, despite continuation of therapy. They also note that in a LMWH extension trial in which investigators were blinded to findings of PF4/heparin serology, 31 seropositive patients (half of whom had platelet activating antibodies) received an additional 9 months of LMWH thromboprophylaxis without developing clinical HIT.

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changes in platelet counts follow seroconversions by approximately 2 days for the initial decline and approximately 4 days for a platelet count decline of more than 50%. Thrombotic complications, on the other hand, do not follow a particular pattern relative to development of thrombocytopenia; thromboses were shown to occur either before or after thrombocytopenia. The most intriguing finding in this study is the observation that PF4/heparin antibody responses are at or near maximal at the time of the fall in platelet count.

These studies offer new insights for clinicians and researchers alike. For clinicians, both studies provide a critical “timeline” of events related to PF4/heparin seroconversions. These studies show that IgG seroconversions can occur rapidly (within 4 days of heparin exposure), and the majority of immune responses occur within 14 days of exposure. It is also evident that seroconversions precede development of thrombocytopenia and/or thrombosis, and antibody levels are near maximal intensity by the time clinical complications develop in HIT. This latter observation necessarily implies that a platelet count decline and/or thrombosis preceding a seroconversion is unlikely to be “incipient” HIT. Finally, the observation that some patients with circulating platelet-activating PF4/heparin antibodies can be exposed to LMWH must be noted, but viewed with caution. Because circulating PF4/heparin antibodies are known to be associated with “rapid-onset” HIT, care must be exercised in knowingly exposing seropositive patients to heparin in the absence of additional studies or compelling clinical indications.

For researchers, these studies furnish new avenues for investigation. The finding that IgG seroconversions can occur rapidly and unambiguously without IgM precedence suggests that the immune response to PF4/heparin may be primed by prior exposure to PF4 and endogenous glycosaminoglycans. Alternatively, as suggested by the authors, lack of a “classic” pattern of isotype switching in HIT could also be explained by mechanisms, such as independent signaling pathways, induction of peripheral tolerance mechanisms, or unique immunostimulatory signals elicited by antigen binding to a particular class of antigen-presenting cells. The observation that the immune response “peaks” during sustained heparin therapy is also intriguing and suggests an active down-regulation of the immune response, further lending support for the role of peripheral tolerance mechanisms in HIT. Last and most importantly, both studies reinforce the notion that not all PF4/heparin antibodies act equally. The ultimate challenge to investigators in this field is to understand what additional serologic or host risk factors predispose PF4/heparin antibodies to be pathogenic in some patients but asymptomatic in others.

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REFERENCES

A new role for P^k: finding the 1 in a million

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Blood group antigens can have critical functions beyond the red blood cell. In this issue of Blood, Lund and colleagues demonstrate a role for P^k in HIV entry, providing biologic insight, identifying potential therapeutic target motifs and expanding the intersections of hematology, transfusion medicine, and infectious disease.

It is said that there are more than 400 blood group antigens on the red blood cell and of these, about 40 have known clinical significance. Significance in this context refers to transfusion-related concerns, typically meaning the ability of an antibody, when in proximity to the cognate antigen, to cause hemolysis—intravascularly, extravascularly or in utero (as in transfusion reactions or hemolytic disease of the newborn). More recently using precise molecular methods, investigators have been able to ascertain and assign biologic functionality and thus clinical significance to a number of blood group antigens, now including P^k.

P^k or Gb3 is a member of the GLOB collection which includes P, P^a, and LKE. P^k is related to the P blood group system discovered by Landsteiner and Levine in 1927 and has its name “as it was the first letter following M, N and O, which had already been used.”1^p76 Biochemically, P^k is Galα(1-4)Galβ(1-4)GlcCER (synthesized from lactosylceramide or Galβ(1-4)GlcCER) by the sequential addition of galactose via α(1-4) galactosyltransferase encoded in the P^k gene), which when acted upon by the enzyme encoded by the presence of the P gene becomes P.1^ Alternately, lactosylceramide can be modified to P1 via less understood reactions. In addition to the presence of P^k on erythrocytes, this antigen is also found on epithelial cells, monocytes, and B cells where it takes on the designation of CD77. Importantly, while P1 is found in approximately 80% of whites, P^k is found in only about 1 in a million.

Previously, this group has shown: (1) an accumulation of P^k, in patients with Fabry disease due to reduced α-galactosidase conferred resistance to R5 HIV-1,2 and (2) a soluble analog of P^k inhibits HIV infection in vitro.3 The current report also builds on the report of Fantini et al, demonstrating that HIV-infected mononuclear cells have increased P^k expression.4

It is upon this background that Lund et al assessed the susceptibility to HIV–1 of peripheral blood mononuclear cells (PBMCs) from blood donors high in P^k (the P^1 phenotype) compared with those with essentially no P^k expression (the
Nothing typical about HIT

Gowthami M. Arepally