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THROMBOSIS AND HEMOSTASIS

Comment on Desch et al, page 533

A collapse for venous thromboembolism

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In this issue of *Blood*, Desch et al[1] use gene-based collapsing analysis to identify rare naturally occurring variants in genes that increase the risk for venous thromboembolism (VTE). Functional studies of variants in one of the genes identified shed light on how they contribute to VTE. Collectively, the reported findings improve our understanding of the genetic risk factors for VTE.

Deep vein thrombosis and pulmonary embolism (collectively VTE) represent life-threatening medical conditions with major burden for patients. The risk for VTE is influenced by several risk factors, such as surgery, prolonged hospitalization, older age, and lifestyle.[2,3] Genetic factors play a major role in VTE risk as well. With all the genome-wide association studies performed to date, the most frequently occurring DNA variants in the population, for instance, the Factor V Leiden variant, have been identified.[4] However, currently identified frequently occurring VTE DNA variants do not explain all the genetic predisposition. Independent DNA variants that occur very infrequently in the population are expected to be causal in many complex genetic disorders, including VTE, but most studies are not powered sufficiently to identify rare variants.[5]

Desch et al undertook an elegant approach to identify these rare variants. They selected a cohort of ~400 individuals with VTE without known risk factors to increase the chance of finding genetic factors. All VTE cases were evaluated with whole-exome sequencing. Next, they performed a gene-based collapsing analysis[5] in which, on a per-gene basis, the total number of rare coding variants (with allele frequencies <0.05% in the general population) was compared with those in a large control cohort. In doing so, they identified different rare DNA coding variants in several genes in VTE vs control cases. The success of this approach was underscored by the fact that rare variants observed in 3 of the 4 most significant genes encode anticoagulant proteins, protein C and S, and antithrombin. Naturally, it is expected that the majority of the identified rare DNA variants impair anticoagulant function. This would result in impaired clot resolution and eventually VTE. Functional studies of these rare variants should demonstrate their impact on coagulation, and hence, qualify these as bona fide risk factors. Needless to say, the findings also underscore the importance of rare DNA variants in protein C, S, and antithrombin encoding genes in VTE risk.

A fourth gene, STAB2, was identified that exhibited >4 times more rare coding variants in VTE cases compared with controls. Thus, STAB2 has been identified as a new VTE gene. STAB2 encodes Stabilin-2, an endothelial cell surface scavenger receptor. Desch et al showed that all of the 7 studied rare VTE Stabilin-2 variants exhibited impaired intracellular transport, resulting in lower cell surface expression compared with control Stabilin-2. This is an important finding as Stabilin-2 can clear von Willebrand factor from circulation.[6] von Willebrand factor plays a central role in clot formation, and high plasma levels of this factor may increase the risk of VTE. To substantiate their findings, Desch et al showed in a large independent control cohort that rare STAB2 variants are significantly associated with higher von Willebrand factor plasma levels, consistent with impaired clearance. Thus, rare STAB2 variants that impair intracellular Stabilin-2 transport and surface expression may explain the increased risk of VTE through impaired von Willebrand factor plasma clearance. Apart from the 4 aforementioned genes, several additional genes with lower significance were identified. For the vast majority of these genes, a role in clot formation and VTE remains to be uncovered.

How can we translate these findings into clinical practice? Before individual rare variants can be added in genetic VTE risk classification, functional tests demonstrating their role in clot formation and resolution need to be performed. For Stabilin-2 variants, the effect on von Willebrand factor clearance is important. Here, however, additional work is required as Stabilin-2 recognizes and clears plasma ligands like heparan and keratin sulfate and hyaluronic acid, in addition to von Willebrand factor. It is therefore conceivable that lower Stabilin-2 cell surface expression affects multiple processes and that certain variants may alter the recognition of one or more ligands instead of affecting surface expression.

In conclusion, the presented work provides in important improvements in our understanding of the genetic causes of the risk to develop VTE. Rare DNA variants are important. Smart approaches are required to perform bioinformatic and functional testing of these variants to determine their aggregate impact on the risk of VTE. In the future, their inclusion in genetic testing will improve our options to better predict the risk of VTE at the genetic level.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Carvelli et al, page 542

NK cells: energized yet exhausted in adult HLH

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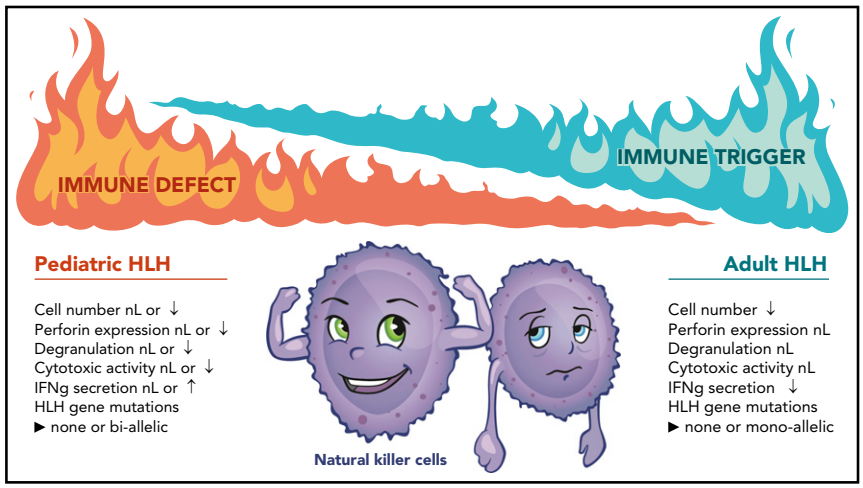
In this issue of *Blood*, Carvelli et al comprehensively analyze the immunologic and genetic features of a large cohort of adults with hemophagocytic lymphohistiocytosis (HLH).[1] The investigators find that natural killer (NK) cells from adults with HLH exhibit an activated phenotype and normal cytotoxic capacity. In contrast, NK cell numbers and interferon-γ (IFN-γ) production are greatly diminished. Genetic studies reveal that 50% of patients harbor ≥1 germline variant of uncertain significance (VUS) in an HLH-associated gene, but none harbor a pathogenic variant.

HLH is a rare disorder of the immune system characterized by the excessive activation of lymphocytes and macrophages that copiously secrete proinflammatory cytokines. Although best known for its occurrence in young children, Scott and Robb-Smith first described HLH in a series of 4 adults who presented with fever, lymphadenopathy, hepatosplenomegaly, peripheral blood cytopenias, and pathologic evidence of cellular proliferation with accumulation of histiocytes, some of whom showed erythrophagocytosis.[2] In 1952, a similar disorder affecting infant siblings was described, suggesting that, in some HLH cases, disease is caused by host genetic factors.[3] Through these and other studies, it has since become clear that HLH occurs as a hereditary (also known as "primary") disorder that is caused by pathogenic germline variants in critical immunoregulatory genes (many of which are involved in perforin-dependent cytotoxicity) or as a nonhereditary "secondary" disorder that is induced by strong immunologic triggers, including infections, malignancies, and autoimmune diseases. In immunocompetent individuals, cytotoxic lymphocytes, such as NK cells and $CD8^+$ T cells, kill virus-infected and activated antigen-presenting cells and, thus, are critical for maintaining immune homeostasis.[4] In contrast, in children with primary HLH, these processes are impaired, leading to a scenario in which even the slightest of triggers can bring on an exaggerated immune response marked by severe and often fatal tissue damage. Because of similarities in the clinical, laboratory, and histologic manifestations between pediatric and adult HLH, it has been questioned whether an analogous pathobiology occurs in both disorders.



Immunologic and genetic differences between pediatric and adult HLH. nL, normal; ↑, increased; ↓, decreased. Joshua Stokes (St. Jude Children's Research Hospital) assisted in the preparation of this figure.

To address this question, Carvelli et al systematically analyzed immune cell number and function in 68 adults with presumed secondary HLH and then correlated these findings with germline genetic information. To place the results from adult HLH patients into context, the investigators compared them with those obtained from a separate cohort of 34 individuals with various inflammatory diseases, as well as healthy volunteers. Importantly, none of the HLH patients or disease controls received immune suppression or other treatments prior to investigation. Therefore, the results obtained should reflect the native state of the immune cells being examined.

Through this study, the investigators make several interesting observations. In contrast with children with primary HLH, who often retain normal numbers of NK cells that display reduced cytotoxic function,[5] most adult HLH patients exhibit a significant lymphopenia that resolves upon recovery from HLH. Despite their reduced numbers, NK cells from