macrophages, CEP was also identified as a prothrombotic and a proinflammatory factor, respectively.[6,7]

In their article, Yakubenko et al report on the role of CEP in leukocyte trafficking during sterile inflammation. They showed in the inflamed peritoneal cavity of mice (after stimulation of the peritoneal cavity with thioglycollate over 72 hours) a strong upregulation of CEP expression compared with that in untreated tissue. Using CEP-blocking antibodies, they demonstrated that early neutrophil extravasation was not affected by blocking CEP, but subsequent macrophage infiltration was significantly reduced. From these results, the authors concluded that infiltrated neutrophils might be involved in the generation of CEP within the peritoneal cavity, which in turn might help support the second wave of cell infiltration by circulating monocytes as well as macrophages from the local environment. To demonstrate that neutrophils are indeed responsible for the generation of CEP in inflamed tissue, the authors set up a 3-dimensional (3D) fibrin gel assay in the Boyden chamber. Using this setup, they found that fMLF-dependent neutrophil migration in the gel led to CEP generation within the gel. Interestingly, CEP production could be prevented in part by blocking neutrophil-derived myeloperoxidase (MPO), which suggests that MPO is contributing to the generation of CEP. The role of neutrophil-derived MPO in CEP generation was further confirmed in a mouse peritonitis model in which neutrophil but not monocyte/macrophage depletion led to a strong reduction in peritoneal CEP expression. Furthermore, CEP expression was strongly reduced in a wound-healing assay in MPO-deficient mice compared with wild-type mice. Finally, coincubation of fibrinogen, DHA, and MPO in vitro generated CEP adducts on fibrinogen. Having elaborated the molecular mechanisms of generating CEP in the inflamed peritoneal cavity, the authors then investigated how CEP regulates monocyte/macrophage recruitment. Although CEP did not exert any chemoattractant or integrin-activating function, immobilized CEP was shown to bind to macrophage-expressed β_2 integrins in a macrophage static adhesion assay. Using HEK293 cells transfected with different β_2 integrins, the authors found that $\alpha_M\beta_2$ (Mac-1) and $\alpha_D\beta_2$ integrins bound CEP, but $\alpha_L\beta_2$ (LFA-1) did not interact with CEP. These results were further confirmed in biochemical assays using α_L-, α_M-, and α_D-I-domain-containing integrin fragments. Finally, the authors again used the Boyden chamber and a 3D fibrin gel matrix to investigate migration of isolated peritoneal macrophages in the presence or absence of CEP. Intriguingly, they found that the presence of CEP strongly supported β_2 integrin-dependent migration of macrophages under 3D conditions, but neutrophils did not change their migration behavior in the presence of CEP.

In summary, Yakubenko and colleagues have revealed a new and interesting mechanism of indirect crosstalk between neutrophils and macrophages during sterile inflammation. After neutrophils have arrived in inflamed tissue, they release MPO into the local environment, which leads to the formation of CEP-decorated extracellular matrix components that serve as guidance cues for macrophage migration through binding to macrophage-expressed $\alpha_M\beta_2$ and $\alpha_D\beta_2$ integrins. Furthermore, interactions between CEP and β_2 integrin could also trigger additional macrophage recruitment, stimulated by CEP-mediated outside-in signaling events. Taking into consideration the differential modulation of macrophage subtypes by CEP, as reported recently,[7,8] inflamed tissue-expressed CEP might turn out to play an important modulating role in fine-tuning the transition from the initial proinflammatory phase to the subsequent resolution phase of inflammation.

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Chinn et al, page 89

HLH: genomics illuminates pathophysiological diversity

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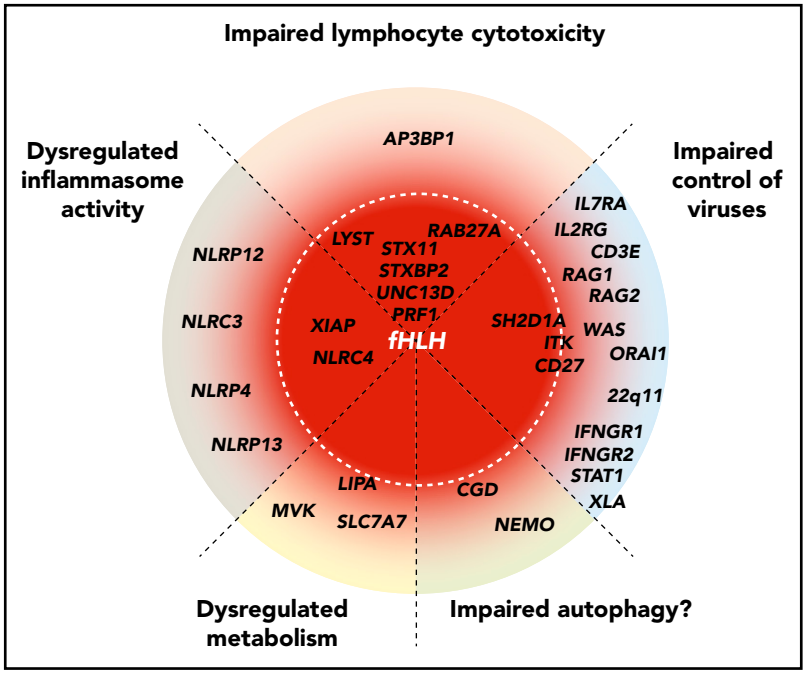
In this issue of *Blood*, Chinn et al report genetic findings in a large cohort of children with hemophagocytic lymphohistiocytosis (HLH), indicating that many cases may be explained by mutations in genes other than those required for lymphocyte cytotoxicity.[1]

HLH is a life-threatening hyperinflammatory syndrome characterized by unremitting fever, cytopenia, and hepatosplenomegaly, which together represent signs of dysregulated immunological homeostasis. Clinically, HLH is diagnosed upon fulfillment of 5 of 8 clinical signs and laboratory parameters, commonly referred to as the HLH-2004 diagnostic criteria.[2] Familial HLH (fHLH) has been associated with autosomal



Schematic representation of the spectrum of HLH-predisposing genetic conditions, representing different pathogenic mechanisms. Genes are grouped by pathogenic mechanisms and radially organized by their associated risk of HLH, where genes at the center are fully penetrant. Mutations in genes required for lymphocyte cytotoxicity invariably lead to HLH in infancy. Mutations in other genes that impair control of common viruses via impaired lymphocyte signaling (eg, *SH2D1A, ITK,* and *CD27*) are often associated with HLH, whereas impaired lymphocyte development and function (genes causative of severe combined immunodeficiency or combined immunodeficiency) may also predispose patients to HLH. Moreover, patients with more general defects in interferon signaling (eg, genes causative of Mendelian susceptibility to mycobacterial diseases) may develop HLH. Constitutive activation or dysregulation of the inflammasome, as observed in patients with activating *NLRC4* mutations or loss-of-function *XIAP* mutations, can cause HLH through primary macrophage activation. This might also be the case for genetic variants in other *DIAP* genes, as revealed by Chinn et al. Similarly, even in metabolic diseases such as Wolman disease (*LIPA*), primary macrophage activation resulting from accumulation of metabolites might constitute the initial trigger for HLH development. In chronic granulomatous disease, impaired autophagy may represent a mechanism for HLH susceptibility.

recessive mutations in genes required for lymphocyte cytotoxicity (ie, *PRF1, UNC13D, STX11, STXBP2, RAB27A,* and *LYST*).[3] A hallmark of these forms of primary HLH, caused by fHLH-associated mutations, is impaired lymphocyte cytotoxicity, which also constitutes 1 of the HLH-2004 diagnostic criteria. Moreover, mutations in *SH2D1A* and *XIAP* cause X-linked forms of fHLH, which exhibit distinct cellular mechanisms. These genes encode proteins involved in lymphocyte signaling as well as regulation of apoptosis and inflammasome activity, respectively.[3] Hematopoietic stem-cell transplantation represents the only curative treatment of primary HLH. However, if defects in lymphocyte cytotoxicity are not present, a less aggressive treatment strategy may be appropriate. Patients lacking fHLH-associated mutations are often described as having secondary HLH. Underlying conditions such as infections, rheumatism, or malignant diseases represent a common feature of secondary HLH, the pathogenesis of which is less well understood. Notably, patients with secondary HLH may harbor other genetic predispositions.[3] For example, metabolic disorders and other primary immunodeficiency diseases (PIDs) occasionally present with HLH, thus falling under the umbrella of secondary HLH.[4,5] However, the degree to which genetic factors predispose patients to secondary HLH has not been comprehensively determined.

Genetic testing for fHLH has been available since 1999, when mutations in *PRF1* were first discovered. Patients have typically been evaluated by a sequential gene sequencing approach, which is labor intensive and time consuming. Targeted sequencing has also been guided by the results of natural killer cell cytotoxicity and exocytosis, achieving the highest diagnostic yields in patients with proven defective lymphocyte cytotoxicity.[6,7] The introduction of high-throughput sequencing in research as well as clinical diagnostics during the last 10 years has radically changed the molecular approach to rare pediatric diseases, including HLH and the broader group of PIDs.[8] Comprehensive sequencing efforts promise a fuller understanding of HLH predisposition and pathogenesis, enabling precision medicine.

Encompassing >17 years of experience in diagnosing and managing HLH at a large tertiary care center, Chinn et al report their findings in a prospective multiethnic cohort of 122 children fulfilling the HLH-2004 criteria. A vast majority of these children underwent genetic testing for fHLH. Moreover, exome sequencing was performed in 48, representing the largest exome data set hitherto published for HLH. A definitive molecular diagnosis of fHLH was only achieved in a limited number of patients (n = 19; 19%), with fHLH overrepresented in infants diagnosed before 1 year of age (61%). By exome sequencing, a group of patients were diagnosed with other well-known PIDs, such as Omenn syndrome, chronic granulomatous disease, and autoimmune lymphoproliferative syndrome, corroborating previous findings from Bode et al.[4] Taking advantage of a large cohort of individuals undergoing sequencing with the exome platform at the Baylor-Hopkins Center for Mendelian Genomics, the authors were also able to test for enrichment of digenic inheritance in patients fulfilling HLH-2004 criteria compared with nearly 6000 control individuals. In their data, they did not find statistical support for a digenic model of susceptibility to HLH. Instead, they uncovered an association between HLH and genetic variants in a group of genes defined by the authors as dysregulated immune activation or proliferation (DIAP) genes, including significant associations for monoallelic variants in *NLRC4* and *NLRP12* and biallelic variants in *NLRP4*, *NLRC3*, and *NLRP13*. Although heterozygous activating mutations in *NLRC4* have previously been shown to cause severe HLH and macrophage activation syndrome,[9] the associations between HLH and other inflammasome genes are novel. A stratification of clinical features, such as age at onset, survival, and presence of known HLH-associated triggers based on genetic findings, revealed that isolated DIAP-associated variants were in most cases associated with somewhat milder rheumatic disease. By contrast, patients with HLH with infectious or

malignant triggers had the poorest survival. Warranting further investigation, most identified DIAP gene variants are currently of unknown significance.

The findings by Chinn et al strengthen the notion that HLH could be the end result of a variety of pathophysiological mechanisms (see figure), carrying both diagnostic and therapeutic implications. The authors make a case for revising the current diagnostic algorithm for children with HLH. As they point out, cellular assays of perforin expression and exocytosis should still constitute first-line investigations in children with suspected HLH. However, their study highlights the clinical need for genetic analysis beyond the scope of lymphocyte cytotoxicity–related genes, particularly in patients lacking mutations in fHLH genes. Crucially, extended genetic analyses can provide additional molecular diagnoses and reveal new mechanisms of predisposition to HLH, increasing pathophysiological understanding and potentially unraveling new therapeutic avenues. Ultimately, genomics can help stratify secondary HLH into molecularly defined phenotypes, creating the opportunity for better-targeted treatments. As a recent example, individuals with activating *NLRC4* mutations develop a form of HLH that is characterized by high levels of interleukin-18 (IL-18) and (partially) responsive to IL-1 inhibition.[9] Similarly, biological therapy might represent an efficacious approach in targeting additional inflammasome components associated with HLH, as indicated by Chinn et al.

Even after blurring the division between primary and secondary HLH, questions remain regarding the genetic predisposition to HLH. Despite exome sequencing, Chinn et al could not identify a likely genetic explanation in approximately half of the children tested. Additional genetic mutations might have gone undetected. In a cohort of infants with fHLH, >50% of cases were explained by noncoding mutations.[10] Facilitated by the fast-falling cost of sequencing, the future of HLH diagnostics will likely see a rapid implementation of whole-genome sequencing. Because of the opportunity to detect noncoding and structural variants, whole-genome sequencing promises to further close the existing knowledge gaps concerning the pathophysiological mechanisms and potential treatments of HLH.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Sandhu et al, page 101

Phenotyping rare hepcidin deficiency

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In this issue of *Blood*, Sandhu et al[1] comprehensively catalog the individual data from all reported cases of hepcidin-deficient hemochromatosis related to *HAMP*, *HJV*, and *TFR2* mutations to determine their phenotype compared with *HFE* hemochromatosis. Sheldon's *Haemochromatosis* textbook was a landmark in the field, thoroughly reviewing all reported cases of hemochromatosis in 1935.[2] Similarly, this study describes the rare forms of hereditary hemochromatosis that will help interpret results through genotype-phenotype correlations and foster the search for genes involved in the still unexplained forms of non-*HFE* hemochromatosis.

Since the initial description of hemochromatosis, many studies have delineated the regulation of iron metabolism. Because there is no significant mechanism for iron excretion, absorption is tightly regulated; this is where the hepcidin-ferroportin axis plays a central role in iron metabolism. Hepcidin (coded by *HAMP*) is a hormone secreted by the liver, which interacts with the only known iron exporter, ferroportin, at the enterocyte membrane, inducing its degradation that eventually hampers iron egress into the bloodstream.[3] Alteration in the hepatocyte's iron detection platform disconnect hepcidin secretion from body iron stores leading to hepcidin deficiency and thus iron overload. Hepcidin-deficient hemochromatosis is the main type of hereditary hemochromatosis, with *HFE* hemochromatosis its most common.[4] Research progressively identified other genes involved in the detection platform (*HJV*, *TFR2*, *BMP6*), allowing characterization of new subtypes of hepcidin-deficient

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