Comment on De Matteis et al, page 262

HLH: birds of a feather flock together

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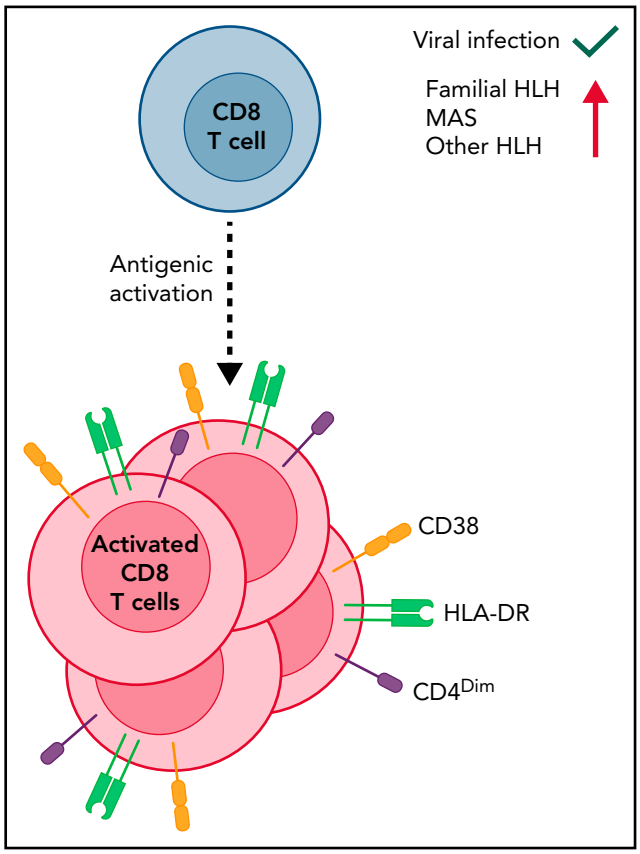
In this issue of *Blood*, De Matteis et al[1] describe the expansion of recently activated CD8 T cells in patients with macrophage activation syndrome (MAS) or secondary hemophagocytic lymphohistiocytosis (HLH), demonstrating important immunologic similarities to familial HLH. They also identified a potentially useful new marker of severe disease.

Many hematologists would agree that patients with HLH are among the most memorable ones they have cared for. Although HLH is an inflammatory syndrome with diverse etiologies, is there a distinctive underlying immune profile shared among these patients? Building on prior reports, the data presented by De Matteis et al in this issue clearly say yes, there is a common immune profile among genetic and some secondary forms of HLH. They found that patients with MAS (mostly developing as a complication of juvenile idiopathic arthritis), as well as other secondary forms of HLH, have a clear peripheral blood profile of acute CD8 T-cell activation. This study builds on prior studies involving familial (primary) HLH in 2 important ways. First, it describes increased CD38^{bright}, HLA-DR^{+} CD8 T cells in patients with MAS, which are essentially identical to those seen in familial HLH.[2-4] Similar to familial HLH, the finding of even relatively low frequencies of these cells can efficiently distinguish patients with MAS from those with the same underlying condition without MAS, in this case juvenile idiopathic arthritis. Second, they identify dim expression of CD4 on a fraction of activated (CD38^{bright}, HLA-DR^{+}) CD8^{+} T cells as a potentially useful marker of MAS disease severity.



CD8 T-cell activation as a key measure in various forms of HLH, including MAS. When CD8 T cells are activated by encounter with cognate antigens, they divide and differentiate. Some cell surface markers, including high levels of CD38, HLA-DR, and low levels of CD4, have been demonstrated in humans to signify recent antigenic activation. Although this process is a normal part of T-cell responses, experimental models of HLH demonstrate that CD8 T-cell activation is greatly heightened and drives disease pathogenesis. Similarly, patients with familial HLH display distinctive and high levels of recently activated CD8 T cells. De Matteis et al have now extended this finding to patients with MAS and other secondary HLH and identify dim CD4 expression as correlating with MAS severity.

What do these findings tell us more generally about HLH? First, they demonstrate a unified immune profile among both familial forms of HLH and some forms of secondary HLH in humans. Of note, the series described by De Matteis et al included 30 patients with MAS or other secondary HLH, but only 2 had malignancies and none with iatrogenic forms of HLH (eg, recipients of CAR T cells or T-cell–activating agents). However, despite the limited scope of the cohort, this finding suggests that similar immune pathogenesis among diverse patient groups underlies their clinical similarities.

Second, these findings reinforce that HLH is fundamentally a disorder of unusual and harmful acute CD8 T-cell *activation*. Experimental animal models of HLH have demonstrated that HLH is driven by excessive antigen-driven activation of CD8 T cells, which are otherwise appropriately triggered and directed.[5] This contrasts with the pathology of autoimmune disorders (misdirected toward self), autoinflammatory disorders (inappropriately triggered), or lymphoproliferative disorders (where toxicity of T-cell activation is unclear). However, what do recently activated CD8 T cells look like in humans (see figure)? Miller et al assessed T-cell responses to vaccination and found that dual expression of CD38 and HLA-DR by CD8 T cells is an efficient way to identify antigen-specific T cells, in other words, those T cells that have recently been activated by antigen presentation and are responding to the infection.[6] This CD38/HLA-DR profile is also seen after natural viral infection,[7] so it is not necessarily specific for disease states. However, De Matteis et al and

Chaturvedi et al both showed that it is highly sensitive for MAS or HLH and can be quite specific when attempting to distinguish MAS/HLH from other clinically relevant disorders (active juvenile idiopathic arthritis or sepsis, respectively).[2] Assessment of CD8 T-cell activation with these markers is quite simple for most clinical laboratories and appears to have diagnostic value, although this will require further study.

Third, the current finding of dim CD4 expression on CD8 T cells in patients with MAS or secondary HLH extends the T-cell activation phenotype described in prior studies with familial HLH. Low-level CD4 expression on CD8 T cells has been described as a marker of T-cell activation for decades (at least in vitro[8]) but has not been previously tied to a particular disease state. Its correlation with disease severity deserves further study, especially in comparison with currently used markers, such as sCD25. Although a prior report demonstrated good correlation between T-cell activation and other disease-relevant markers of inflammation,[2] whether CD4 expression (or CD38/HLA-DR) on CD8 T cells has prognostic value in HLH or MAS remains to be determined.

As the previously mysterious syndromes of HLH and MAS reveal their secrets, it is increasingly obvious that they share a common pathophysiology involving T-cell hyperactivation and likely share common therapeutic targets. Revealing this similarity reminds us that, like birds of a feather, they flock together.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Karnes et al, page 274

Novel genotype-phenotype interaction in HIT

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Through genome-wide association studies (GWAS) and fine-mapping, Karnes et al,[1] in this issue of *Blood*, have shown that having platelet factor 4 (PF4)/heparin antibodies in the presence of type O blood predisposes one to heparin-induced thrombocytopenia (HIT).

Unfractionated heparin (UFH) was first isolated in 1916 and entered clinical practice after its structure was determined in the 1930s. It remains one of the oldest drugs still in widespread clinical use.[2] Of all the anticoagulants, heparin's unique pharmacological properties will ensure its continued therapeutic use. However, HIT, first described in 1958, remains a significant iatrogenic complication of heparin use.[3]

HIT is a pathological prothrombotic syndrome caused by an immunoglobulin G (IgG)-mediated immune response to complexes of PF4 bound to heparin. HIT antibodies can activate platelets, resulting in procoagulant platelet membrane changes that enhance thrombin generation.[4] HIT occurs in ~2.4% of patients receiving UFH and low-molecular-weight heparin.[1] The mortality rate of HIT is high, and the prevalence of thromboembolic complications in these patients has been put at 60%.[5] Currently, it is impossible to know before administration of heparin which patients will develop HIT.[6] Furthermore, HIT has similarities to adenoviral vector SARS-CoV-2 vaccine-induced thrombotic thrombocytopenia (VITT); thus, discoveries related to HIT could aid in management of VITT (see figure).

Despite extensive research in the field, molecularly distinguishing pathogenic vs nonpathogenic PF4/heparin antibodies is uncertain. This paper addressed this problem using a genome-wide association study (GWAS) and fine-mapping approach where the phenotype in cases is both a positive heparin-induced platelet activation (HIPA) assay and a PF4/heparin-positive functional assay, and controls are divided into the following 2 groups: (1) antibody-positive (functional assay-negative) with negative HIPA and positive PF4/heparin antibodies; and (2) PF4/heparin antibody-negative controls with both negative functional assay (HIPA) and PF4/heparin antibody-negative results. Because of the experimental design of the control groups, they were importantly able to discover that in the presence of PF4/heparin antibodies, the rs8176719 C deletion encoding the O blood group is a novel risk factor for both platelet reactivity and HIT.

The strength of GWAS and other hypothesis-agnostic genetic approaches lies in their unbiased nature, especially in cases such as HIT where few candidate genes exist. Prior GWAS of patients with HIT were extremely underpowered, lacked appropriate control groups, and had no associations that passed genome-wide