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Pillars of Immunology

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Hemophagocytic lymphohistiocytosis (HLH) refers to a group of rare genetic disorders characterized by a massive proinflammatory cytokine storm resulting in coagulopathy, CNS dysfunction, increased hemophagocytosis, pancytopenia, multiorgan failure, and death (1). Defects in cytolytic function are typical in the immune cells of these infants, and without early aggressive immunosuppression and bone marrow transplantation, familial HLH (fHLH) is a uniformly fatal condition that affects 1 in 50,000 live births (2). Even with bone marrow transplantation, 5-y survival rates are ~50% (3). Most of the familial cases of HLH are associated with autosomal recessive disorders. The first of these genes to be identified as a cause of fHLH was perforin (4).

In 1999, Stepp et al. (4) connected two important facts, that defects in cytotoxic function had been regularly associated with fHLH, and that the 10q21–22 locus had been consistently linked to multiple families with fHLH in their pedigrees. They hypothesized that the missing link connecting these two associations was that the gene encoding perforin, a critical effector molecule of cytotoxic function, mapped to the 10q21–22 locus. In their landmark paper (4), they went on to more finely map perforin to the centromeric end of the 10q21 locus, and then showed that eight of eight unrelated fHLH patients studied with 10q21–22 linkage had mutations in their perforin genes. To demonstrate that perforin-mediated cytotoxicity was indeed defective in these patients, they used a Fas-deficient target cell in combination with a Fas-blocking Ab in an anti-CD3 Ab-mediated cell killing assay to eliminate the possibility that Fas–Fas ligand interactions might confound killing measurements. T lymphocytes from patients harboring a nonsense mutation mediated no killing in this assay, and cells from patients with a missense mutation had dramatically reduced killing, suggesting that the genetic alterations in perforin in these patients did indeed result in loss of perforin-mediated killing. The authors then went on to demonstrate loss of perforin protein in these patients by immunofluoresence, showing no, or greatly reduced, perforin staining in granzyme B^+ vesicles. Thus, the genetic lesions in perforin were linked to both protein level and function in these patients, supporting loss of perforin as a functional cause of fHLH in these patients.

The impact of the identification of perforin deficiency as a cause of fHLH can been seen in clinical medicine, as well as in fundamental immunology. Although defective NK cell cytotoxicity had been previously associated with fHLH (5), establishment of perforin deficiency as a causative genetic defect provided a mechanistic rationale for using NK cell cytotoxicity for clinical testing. This knowledge also provided a basis for the identification of other fHLH causative genes by offering clues to search for genes involved in perforin release when other genetic loci associated with disease in perforin-sufficient families were identified (6–9). These perforin-mediated cytolytic pathway gene products include other causes of fHLH (Munc13-4, Syntaxin 11, Munc18-2), as well as some immunodeficiencies (e.g., Rab27a mutation in Griscelli syndrome type 2) (10). Consequently, the perforin-dependent cytolytic pathway employed by $CD8^+$ T cells and NK cells has been further explored via natural mutations in the associated genes.

Armed with the concept that perforin deficiency must be linked with pathogenesis, a multitude of murine studies linked a $CD8^+$ T cell–intrinsic perforin defect with excessive IFN-γ production and disease pathogenesis (11–14). The exploration of a perforin defect in NK cells has also demonstrated their role in HLH, and NK cell cytolytic activity and protection against HLH appear to be IFN-γ–independent (15). Most recently, it has been shown that defects in perforin-mediated killing of target cells by NK cells and $CD8^+$ T cells prolongs the synapse time between the killer and its target cell by 5-fold, and this results in a proinflammatory cytokine storm (16). Hence, there is now a direct link between defective perforin-mediated cell lysis and the resultant cytokine storm associated with clinical HLH.

Recognition that the rheumatic disease–associated macrophage activation syndrome (MAS) was similar in phenotype to fHLH has led to investigations that suggest depressed perforin function may also be found in this disorder (17–21). In addition to perforin deficiency, murine models of HLH have been explored in a variety of other mice deficient in various proteins in the cytolytic pathway (e.g., Munc13-4, Munc18-2). Indeed, there has been a recent suggestion that the degree of cytolytic dysfunction in both mice and humans correlates with the severity of HLH (22). Intriguingly, there appear to be single copy mutations in the gamut of fHLH genes in a substantial percentage of children and adults with the much more common disorders of secondary HLH and MAS (10, 23). This is blurring the genetic distinction between fHLH and both secondary HLH and MAS (10, 24).

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Abbreviations used in this article: fHLH, familial HLH; HLH, hemophagocytic lymphohistiocytosis; MAS, macrophage activation syndrome.

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Lately, there have been some compelling data to suggest that some monoallelic fHLH mutant genes identified in patients with secondary HLH or MAS may even function as dominant-negative cytolytic pathway proteins (10, 25). This may help to explain the much more frequently observed occurrence of secondary HLH and MAS relative to fHLH (26). The presence of a monoallelic cytolytic pathway gene defect in combination with a hyperinflammatory state from any of a variety of potential triggers is consistent with a two-hit or multihit model of HLH disease development. Indeed, we are perhaps only beginning to recognize the tip of the iceberg in terms of triggers of secondary HLH and MAS. Everything from influenza (27) to dengue hemorrhagic fever (28), from systemic lupus erythematosus (29) to systemic juvenile idiopathic arthritis (30), and from T cell leukemia (31) to non–Hodgkin lymphoma (32) has been reported in association with secondary HLH and MAS. Whether cytolytic defects are associated with all of these associations, however, currently remains unclear.

Conceptually, the fact that cytotoxic function is not only important for effector immune function but also for resolution of inflammation has provided an important model for understanding the complex regulatory networks that characterize an immune response (33). More recently, comparing and contrasting perforin-dependent and -independent mechanisms that lead to hemophagocytic syndrome has expanded our view of how other regulatory networks may be important in preventing immune responses from spiraling out of control (34–36). Based on the knowledge gained from basic science, anti-cytokine therapy has been attempted for various forms of secondary HLH and MAS, and these therapies have been reported in retrospective series to save the lives of those afflicted by what appears to be a much more common disorder than previously considered (37, 38). Excitingly, we are now entering an era where industry-sponsored trials of cytokine neutralization are being initiated for HLH. The study of HLH/MAS has thus evolved from the early recognition of mutations in perforin contributing to fHLH (4) to the current state of the art, in which randomized placebo-controlled clinical trials of anti-cytokine therapy for MAS are just beginning (39).

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Disclosures

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