Autopsy Case Reports

Editorial

Hemophagocytic lymphohistiocytosis: a rare diagnosis, an even rarer opportunity to appraise our understanding of the immune system

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The syndrome identified as hemophagocytic lymphohistiocytosis (HLH) poses a rather difficult challenge to the physician. HLH was initially described as a familial disorder,[1] which was later associated with various genetic defects that affect the cytotoxic machinery of CD8+ T and NK cells,[2] but it also occurs sporadically, usually prompted by infections, rheumatic disorders, or neoplasia.[3] Although its diagnostic criteria are established, its clinical presentation overlaps with many different conditions, requiring an enhanced awareness of the attending physician in order to reach the diagnosis and to initiate treatment early enough.[4]

While the diagnostic criteria include either the presence of a known genetic disorder or five of a list of eight signs and symptoms,[3] regardless of its presentation, the predominant pathophysiological feature of HLH is an overactive immune system. This can be inferred by the name-giving feature of the syndrome: hemophagocytosis, reflecting a hyperactive mononuclear phagocytic system, as a result of what can be classified as a cytokine storm.[5] Thus, one could be satisfied by a general view that this syndrome is another one of those where the immune system becomes rogue and turns against the body. Nonetheless, it is worth looking deeper into the known HLH immunopathological features and checking the believed pathway of its development against our models and assumptions about the physiology of the immune system.

In a sense, all data seem to point to an immune dysfunction that fits perfectly with what one expects. The genetic disorders associated with the familial forms of HLH are those that affect the cytotoxic machinery of NK and T lymphocytes, compromising the cytotoxic granule contents themselves or the molecules involved in granule exocytosis, so that in any case, the cytotoxic function of these cells is faulty. Furthermore, even in sporadic cases, the frequent precipitation of this syndrome by viral infections or its association with NK and T-cell lymphomas indicates that there, too, defective cell cytotoxicity may be the culprit.

Actually, after the role of perforin deficiency was described in familial cases of HLH,[6] an experimental model that reproduces the human syndrome was developed.[7] Perforin-deficient mice, when challenged with the lymphocytic choriomeningitis virus (LCMV), develop a progressive disease that leads rapidly to death if not treated. The mice present a disease that includes the major clinical, laboratory, histopathological, and immune features of the human HLH.[7] Adding to the resemblance between the human disease, both in its familial and sporadic forms, and the

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experimental mouse disease, is the fact that both are viral infection-triggered and rapidly progressive. From these data, an initial hypothesis about the pathophysiology of HLH is that it is a disease caused by an uncontrolled viral infection, which, maintaining a chronic stimulation of the immune system, would precipitate its derailing and, hence, the disease. This view is further supported by the observation that, in another model, mice lacking the orthologue gene of the human *Munc13-4* (a gene involved in the cytotoxic granule exocytosis in NK and CD8+ cytotoxic T cells) develop a very similar disease, when infected by LCMV.[8] In humans, it is relevant to note that the Epstein–Barr virus (EBV) infection is frequently the trigger of HLH, both in familial and sporadic cases[9] and, likewise, is a disease where cytotoxic CD8+ T cells have a definite role (already noticeable by their identification in blood smears as the characteristic "atypical lymphocytes"). So, experimental models data confirm that what could be inferred from clinical observation: defective cell cytotoxicity indeed plays a very significant role in HLH.

However, a simple explanation—lack of or defective cell cytotoxicity, allowing an unchecked viral expansion—does not explain all observations. It is true that in the perforin-deficiency model, an LCMV infection triggers the HLH-like syndrome and is associated with a very high viral load. However, this insufficient clearance of viruses is not enough to cause the disease. First, in the *Munc13-4* orthologue gene deficiency, although the animals have poor cell cytotoxicity and are especially susceptible to the murine cytomegalovirus infection (MCMV), this latter disease does not trigger the HLH-like disease; only the LCMV infection does.[8] Second, in the perforin deficient mice, treating the mice with antibodies against CD8, and thus causing the complete depletion of CD8+ T cells, protects the animals from the HLH-like syndrome,[7] but not from a very high viremia. Indeed, when the kinetics of the disease in these mice was investigated, it became clear that it was not the viral load that prompted the immunological imbalance, but rather the excessive activation of the system.[10]

Moreover, pointing to a possible explanation for the phenomenon, in the same perforin-deficient animal model, the use of blocking antibodies against IFN-gamma also protects the animals from the HLH-like disease.[7] Thus, the initial hypothesis should be modified: defective CD8+ T cells unable to perform their cytotoxic function effectively, allow the virus to overstimulate CD8+ T cells, which display a deregulated interferon-gamma secretion, thus driving the disease. This modified pathophysiological view is further supported by data showing that, in mice, the continuous injection of IFN-gamma through an osmotic pump is enough to cause acute cytopenias and hemophagocytosis,[11] two distinctive features of HLH. In addition, both LCMV in mice and EBV in humans are infections that cause a vigorous IFN-gamma response, while MCMV infection induces hyporesponsiveness to IFN-gamma in macrophages,[12] providing a possible explanation for the "virus-specificity" triggering of HLH-like disease in immunodeficient mice.[8]

Complementing the hypothesis, data from the perforin-deficient animal model show that, in these mice, the absence of the adaptor molecule, MyD88, protects the animals from the HLH-like disease.[13] The MyD88 molecule is central to the transduction of signals from most of the Toll-like receptors and from the IL-1 receptor;[14] thus, it is clearly involved in the inflammatory process and, more significantly, in the detection of homeostatic disequilibria within tissues. However, it is noteworthy that here the role of this molecule does not seem to be in the initial detection of the disequilibrium, but rather, in the response to an already established immune response; that is, the secretion of IFN-gamma by CD8+ T cells.

With these experimental observations, it is possible to elaborate a more detailed pathophysiological view of the HLH. This disease would be the consequence of an immune dysfunction, where the immune system, confronted by certain IFN-gamma-inducing challenges, fails to control its secretion due to a defective machinery of cytotoxic cells, namely NK and CD8+ T cells. The excessive IFN-gamma secretion, through a MyD88-dependent pathway, would cause macrophages to go rogue and further disrupt an already shaken physiologic balance in the host, prompting the HLH.

However well this proposed model explains the experimental data, one has to ask, does it fit the actual human disease? Initially, at the origin of the model, there is a good correlation between the genetic defects of mice and those found in patients with familial HLH. Furthermore, the explanations for the triggering of the disease in mice and humans coincide. But, when the model gets down to identifying the specific cytokine

responsible for the generation of the disease, the model starts to fail: in humans, an elevated serum IFN-gamma, although sometimes found, is not typical of HLH.[15] Furthermore, in a study where the peripheral blood cells' gene expression profile from healthy controls and from familial HLH patients was studied, IFN-gamma and IFN-gamma-regulated genes did not show up as differentially expressed.[16] One could still argue that cytokine profiles, whether they are established by cytokine serum levels, by intracellular staining, or by gene expression profiles of circulating cells, are still quite distant from the in vivo reality; therefore, one could suggest that the deregulated levels of IFN-gamma, although they do not show up in the studies performed in humans, might still exist within the microenvironment where the disease is initiated.

Then again, one would be missing a real opportunity: to check and even challenge our understanding of the immune system. The immune system is very complex; it was selected to deal specifically with molecularly unknown challenges—the antigens—and it performs its function with exceptional efficacy. Our knowledge of the circuits that control this system is growing rapidly and is becoming so detailed that the whole picture starts to escape from many. In this context, the complexity of the circuits and their interaction requires the construction of (animal) models, where genes can be manipulated at will and hypotheses can be tested with precision. However, diving too quickly into the models may obscure the initial reason for their construction—the understanding of the immune system and how it interacts internally and with its many challenges in nature—and, for the physician, it may also unravel strategies to interfere when the immune system fails and disease ensues due to that failure. Then, reminding us that models are models and diseases are diseases, a syndrome like HLH is invaluable—as are autopsies and case reports—for in these instances, models are often insufficient and our relative ignorance shows up, driving us to challenge the models and deepen our understanding.

Specifically here, it is not the relative role of one or other cytokine that should cause the impact, but rather the role we ascribe to CD8+ T cells. These cells are identified as antigen-specific, HLA-restricted, cytotoxic cells that perform an essential role in viral infections and in tumor immunity. Evidence from this function comes from both in vitro observation that these cells are, indeed, able to kill specific targets—and this was central to unravel the role of the major histocompatibility antigens in restricting T-cell recognition of antigens,[17] but also from animal models, where their absence allows the uncontrolled progression of viral infections, as happens in the perforin-deficient mouse, which was discussed before.

However, the role of T-cell cytotoxicity in the control of virus infections in humans is not so clear-cut. Since children with immunodeficiencies characterized by the absence of CD8+ T cells are not more susceptible to viral infections than healthy children,[18] one must accept that either cytotoxicity in humans is irrelevant against viruses, or that it is not the CD8+ T cell but other cytotoxic cell that performs this function in these immunodeficient children. Actually, cell cytotoxicity is not an exclusive characteristic of CD8+ T cells—other cells can do it—but CD8+ T cells have the ability to specifically recognize antigens in the context of class I HLA molecules, therefore on the surface of any nucleated cell, and this seems to be unique to these cells. This feature leaves CD8+ T cells equipped to recognize and respond to the presence of a virus or of mutated proteins in "any" cell in the body—a function that would be required, in fact, in cells involved in the control of virus infections and cancer. The relevance of this function could be further stressed by the observation that many viruses hinder the expression of HLA molecules by the infected cells, clearly suggesting the role of these molecules in their control.[19-21] But if cell cytotoxicity is not the pathway through which CD8+ T cells perform their function once they recognize their targets, what would it be? And the answer seems to be in their cytokine secretion. In animal models of cancer,[22] viral infections,[23] and human diseases,[24,25] cytokine secretion by these cells appears to be the decisive, if not the main, effective factor. Thus, after challenging the model of HLH, where IFN-gamma secretion by CD8+ T cells would be the triggering factor, we are back to this hypothesis. Definitely, IFN-gamma (or cytokines) secretion is characteristic of CD8+ T cells and, if exaggerated, could prompt the derailing of the immune system.

But the question remains: how would a cytotoxic defect prompt a deregulated cytokine secretion? We have seen that the viral load, per se, a simple and direct possibility, does not solve this issue; hence, the

answer must be elsewhere. This brings to light another possible role of CD8+ T cells: immunoregulation. In the 1970s and early 1980s, T cells were classified as either helper T cells or suppressor/cytotoxic T cells. The latter population included cells clearly cytotoxic—those that are identified today as CD8+ T cells—and others that were indistinguishable from the cytotoxic cells by the surface markers then available, but whose function seemed to be the specific suppression of immune responses. This putative T-cell population did not resist deeper investigations, and later on, many immune regulatory circuits were described, including the "opposing" T helper subsets and the regulatory T cells, which could explain much of the earlier observations of specific immunosupression. Nevertheless, a "revival" of the regulatory role for the "cytotoxic" T cells seems to offer a solution in the case of HLH.

Thus, the pathophysiology of HLH could be described as an initial challenge to the immune system that drives the activation of CD8+ T cells. Due to a genetic defect (in the familial cases), or to local and/or transient conditions (in sporadic cases), these cells fail to perform an essential (and relatively ignored) role: the control of their own activation. With uncontrolled stimulation, CD8+ T cells cause other cells down the pathway of the immune response, like macrophages, to become further active, to secrete other cytokines, and, thus, trigger the disease. Therefore, in the end, it could seem that the pathophysiology of HLH is solved. Yet, this is not true. It could be enough to remember that it remains to be determined how the cytotoxic machinery of cytotoxic T cells affects immune activation and if it does truly occur in patients with HLH. But the uncertainties go further. The main characteristics of HLH are in the name of the syndrome itself: hemophagocytosis, lymphocytosis, and histiocytosis of tissues. Though a possible explanation for hemophagocytosis can be found on the action of cytokines (IFN-gamma in the model), the tissue infiltration by immune cells is not clearly explained. What drives their movement towards tissues? What keeps them active therein? And if we keep looking at case reports and autopsies of patients that presented HLH, new questions and new challenges will appear—and, hopefully, will drive our investigations towards a more comprehensive view of its pathophysiology and a more effective way to diagnose and treat it.

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