Haemophagocytic lymphohistiocytosis (HLH) is a multi-system inflammatory disorder caused by prolonged and excessive activation of immune cells, in particular natural killer (NK) cells and CD8+ cytotoxic T cells (CTL) and antigen-presenting cells. It arises when there is an ineffective cell-mediated immune response to an immune stimulus or failure of regulatory pathways responsible for contraction or termination of the specific immune response. Common triggers for HLH include infections (by intracellular pathogens), malignancies, autoimmune disease or metabolic conditions. In our case the inciting immune stimulus is believed to have been pulmonary Tuberculosis. In such a scenario, dysfunctional CTL and NK cells are believed to be ineffective in controlling the infection. As a result of underlying genetic defects the cytotoxic function of these cells is compromised. This leads to overproduction of pro-inflammatory cytokines by CTL and NK cells, which in turn hyperstimulates tissue macrophages (histiocytes). The hypercytokinaemia is responsible for the cardinal clinical features of this syndrome many of which were present in our patient, namely persistent, hectic fever, hepatosplenomegaly, seizures, ataxia, rash, acute respiratory distress syndrome (ARDS) and progressive organ dysfunction. The cytopaenias are caused by widespread phagocytosis of haematopoietic precursors by infiltrating histiocytes in the bone marrow (haemophagocytosis). Importantly, haemophagocytosis may not always be obvious on the marrow biopsy, especially early during the clinical course. This was the case in our patient. Untreated the disease is usually fatal.

Both hereditary and secondary forms of this disease are recognised, but the distinction between the two has lately become blurred as it has become clear that genetic forms can present quite late in life. It may be possible that all so-called secondary forms of this condition are due to underlying (undetected) genetic disorders. In familial HLH, a growing number of autosomal recessive defects have been identified in genes that encode proteins involved in cell cytotoxic killing function (NK cells and CTLs) and activation-induced cell death (necessary for contraction of the immune response following antigen clearance). These include defects in perforin formation/function and also in key proteins required in the degranulation process following CTL/NK cell activation.
The failure of cytotoxic cells to resolve infection coupled to ongoing production of pro-inflammatory cytokines, particularly gamma-interferon (IFN-g) and tumour necrosis factor-alpha (TNF-a), mediates the hyperstimulation of macrophages. In severe cases, the enhanced phagocytic activity of these cells leads to destruction of haematopoietic cells or their precursors in bone marrow (also observed in spleen and lymph node) that results in cytopaenia. In addition, increased synthesis of pro-inflammatory cytokines by hyperstimulated macrophages, such as IL-1, IL-6, TNF-a and IL-8 is responsible for some of the clinical symptoms such as fever, rash (increased vascular permeability) and multiple organ dysfunction due to infiltration of immune cells.

**Let us look at CD8+ cytotoxic T cells in more detail**

CD8+ cytotoxic T cells (CTL) become activated by engagement of the T cell receptor with antigenic peptide loaded onto HLA class I molecules on the surface of the target cell. This receptor-ligand engagement results in intracellular signal transduction that is mediated by the intracellular domains of the T cell receptor. This promotes a cascade of events in the cytoplasm of the CTL that facilitates the formation and degranulation of exocytic vesicles containing perforin and granzymes into the intracellular space between the CTL and target cell (which we call the immunological synapse). Perforin forms a permeable pore in the target cell membrane, which allows entry of granzymes (that initiates apoptosis) and the loss of water (that causes osmotic shock), leading to cell death.

**Let us look at natural killer cells in more detail**
Natural killer (NK) cells form the initial defense against intracellular pathogens. NK cells do not recognise specific antigens, rather they detect “an absence of self”. That is, they recognise a potential target cell (such as virus-infected cells) by the fact that surface self HLA class I molecules are absent or diminished. Many intracellular pathogens down regulate expression of HLA class I receptors on the surface of the cells they infect in order to evade recognition by virus-specific CTLs. However, this renders them susceptible to attack by NK cells. Inhibitory killer-cell immunoglobulin-like receptors (KIRs) on the surface of the NK cell inhibit activation when they engage self HLA class I molecules. If inhibitory signals are diminished or absent, stimulatory KIRs and other activating receptors will cause NK cell activation and initiate an attack on the target cell. As with the T cell receptor, activation of the NK cell occurs following signal transduction mediated by intracellular domains when the activating receptors are triggered, which in turn promotes a cascade of events in the cytoplasm of the NK cell that leads to the formation and degranulation of exocytic vesicles containing perforin and granzymes that are released into the immunological synapse. Perforin forms a permeable pore in the cell membrane of the target cell, which allows entry of granzymes (that initiates apoptosis) and the loss of water (that causes osmotic shock) leading to cell death.

Defective cell cytotoxicity is the basis of HLH

We explain in a series of easy-to-understand graphics, the events leading to the release of lytic granules (degranulation) of both CTL’s and NK cells. By understanding these events, it is then possible to appreciate the defective proteins that interrupt these events.

- The first event necessary to initiate the degranulation process of cytotoxic cells, such as CTL and NK cells, is cell activation mediated by receptor-ligand signal transduction.
Signal transduction initiates the formation of exocytic vesicles containing perforin and granzymes. These proteins are synthesised in the Golgi body and are sorted into vesicles. Perforin is sorted into a lysosome which fuses with a late endosome containing granzymes to form an exocytic vesicle destined for membrane fusion. Importantly the granzyme-containing endosome is first formed by sorting of granzymes into an early endosome that fuses with recycling endosomes returning from the cell membrane. This brings important proteins such as Munc13-4 and Rab27a together which are required for later membrane docking. In familial HLH type 2, the most common mutations observed are in the perforin gene while mutations in Munc13-4 are known for familial HLH type 3.

The exocytic vesicle containing perforin and granzymes is destined for fusion with the cell membrane of the cytotoxic cell. Fusion must take place at the contact area between the cytotoxic cell and the target cell (at the immunological synapse). The process that directs the exocytic vesicle to the correct position inside the cell is called polarisation and makes use of the microtubule cytoskeleton and important proteins in the vesicle membrane.

- Once the exocytic vesicle has reached the cell membrane, a process called docking takes place which involves proteins in the vesicle membrane and cell membrane that immobilise the vesicle.
and prepare it for membrane fusion. In HLH type 4 and 5, mutations in Syntaxin II and Munc 18-2, respectively, cause failure of the exocytic vesicle to dock at the cell membrane.

- Fusion of the cell membrane with the exocytic vesicle membrane releases the contents (perforin and granzymes) into the extracellular space between the cytotoxic cell and the target cell at the immunological synapse.

Fusion of the cell membrane with the exocytic vesicle membrane releases the contents (perforin and granzymes) into the extracellular space between the cytotoxic cell and the target cell at the immunological synapse. Perforin monomers complex into a permeable pore in the cell membrane of the target cell. Granzymes penetrate the target cell by passing through the pore and initiate programmed cell death (apoptosis). Loss of water through the pore also contributes to cell death by osmotic shock.

In familial HLH a number of genetic defects are known which affect proteins involved in the process of degranulation. Five types of familial HLH have been defined (types 1-5), each associated with a specific gene defect, but additional forms are likely to be described as new gene defects are discovered. (So far all the gene defects that have been defined are autosomal recessive.) In addition HLH syndrome can complicate some other established severe-combined immunodeficiency (SCID) syndromes (see below). In familial HLH type 1 an unknown gene on chromosome 9 is affected. Perforin defects are the most common mutations observed for type 2. In types 3, 4 and 5, respective proteins Munc13-4, Syntaxin II and Munc18-2, which are involved in pre-fusion docking of the exocytic vesicle, are known to be defective.

Active pulmonary TB is likely to have been the immunological trigger in our case study. However, why he was predisposed to respond in this way is not known. No genetic studies were done to determine whether there was an underlying genetic defect to account for the HLH. The absence of previous evidence of hyperimmune reactivity suggests that any genetic defect, if present was subtle. However, it is now recognised that familial forms of HLH can present for the first time in older age (even in the 7th decade of life). It would be interesting to find out whether there was any consanguinity in the family.

**Other protein defects in the degranulation process are known**

Interestingly, haemophagocytic syndrome can also complicate a number of other defined primary immunodeficiency syndromes in which genetic defects also map to proteins important in the degranulation process: In Hermansky-Pudlak syndrome type II mutations in AP3B1 affects sorting of proteins to endosomes and also polarisation of the exocytic vesicle to the cell membrane. Similarly,
Wiskott-Aldrich syndrome affects the WASp protein that is linked to cytoskeleton formation and affects polarisation of exocytic vesicles. Griscelli syndrome type 2 has defects in Rab27a that affects vesicle docking and Griscelli syndrome type 1 has defects in MyoVa which affects polarisation and migration of vesicles. Chediak-Higashi syndrome affects sorting of proteins to endosomes because of defects in LYST protein.

Link to the associated case study

Download combined PDF with all graphics