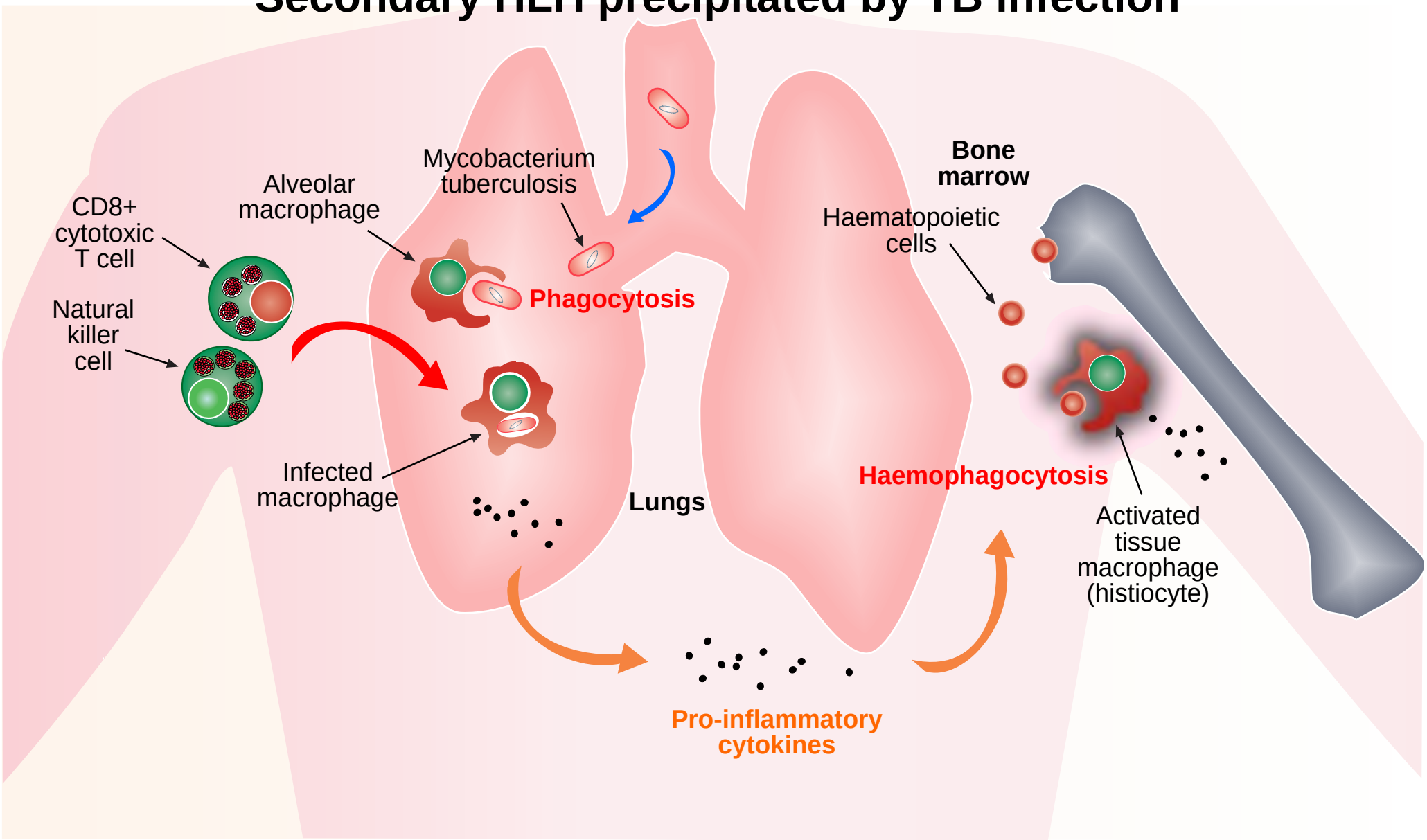
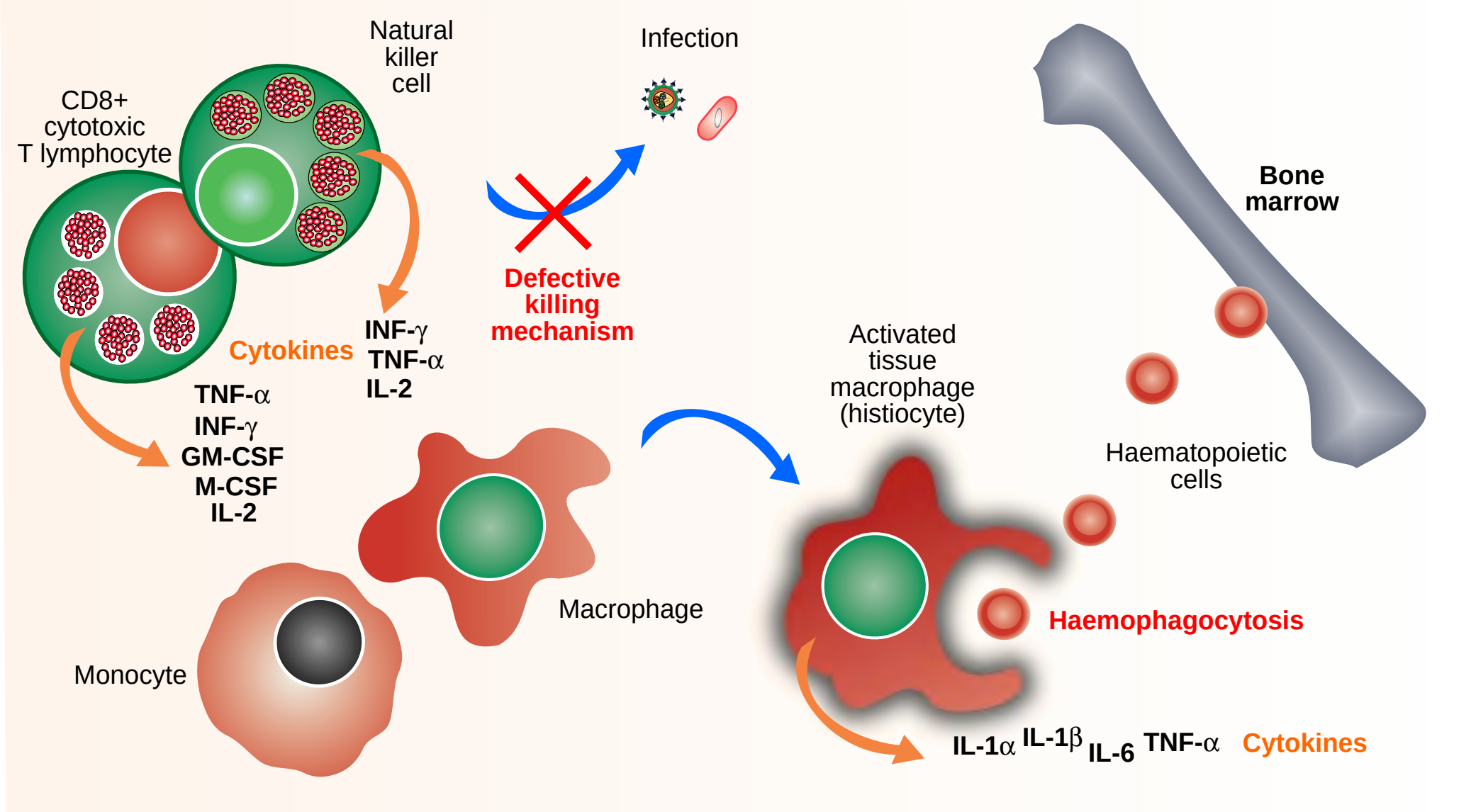
Secondary HLH precipitated by TB infection



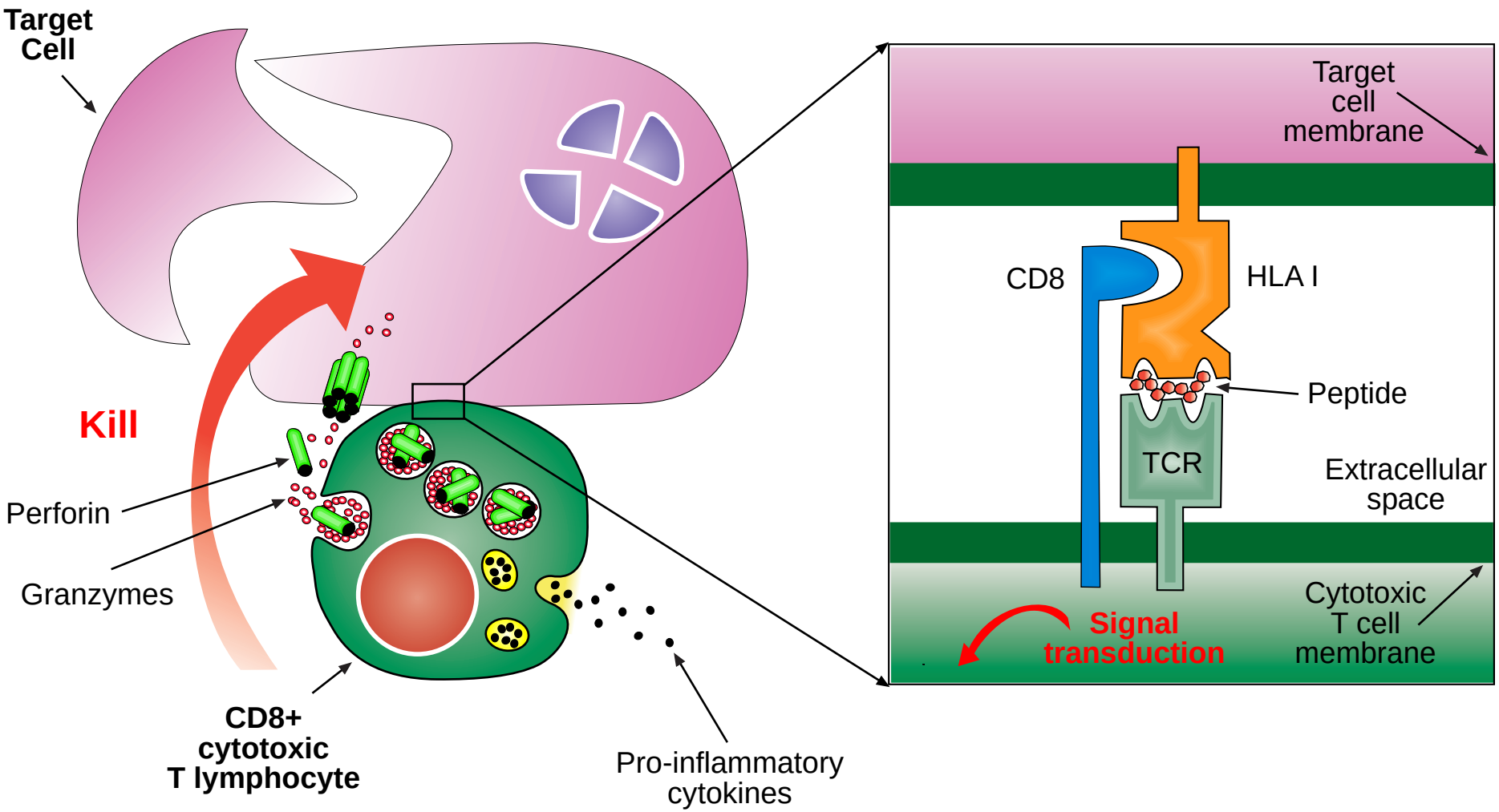
Secondary HLH occurs mostly in adults and is usually associated with bacterial or viral infection. Familial HLH, that normally occurs in early childhood, has 5 defective genes identified, whereas, no genetic abnormalities are known to cause secondary HLH. However, there may be subtle genetic defects that only manifest during overwhelming infections. Dysfunctional cytotoxic cells (CD8+ cytotoxic T cells and natural killer cells), unable to control infection, such as Mycobacterium tuberculosis, which is an intracellular pathogen, promotes excessive production of pro-inflammatory cytokines. High systemic cytokine levels hyperstimulate tissue macrophages (histiocytes) which in severe cases may phagocytose haematopoietic cells in bone marrow (also spleen and lymph node) causing cytopaenia. Pro-inflammatory cytokines secreted by activated macrophages mediate fever, rash (increased vascular permeability) and multiple organ infiltration by immune cells.

Haemophagocytic Lymphohistiocytosis mechanism



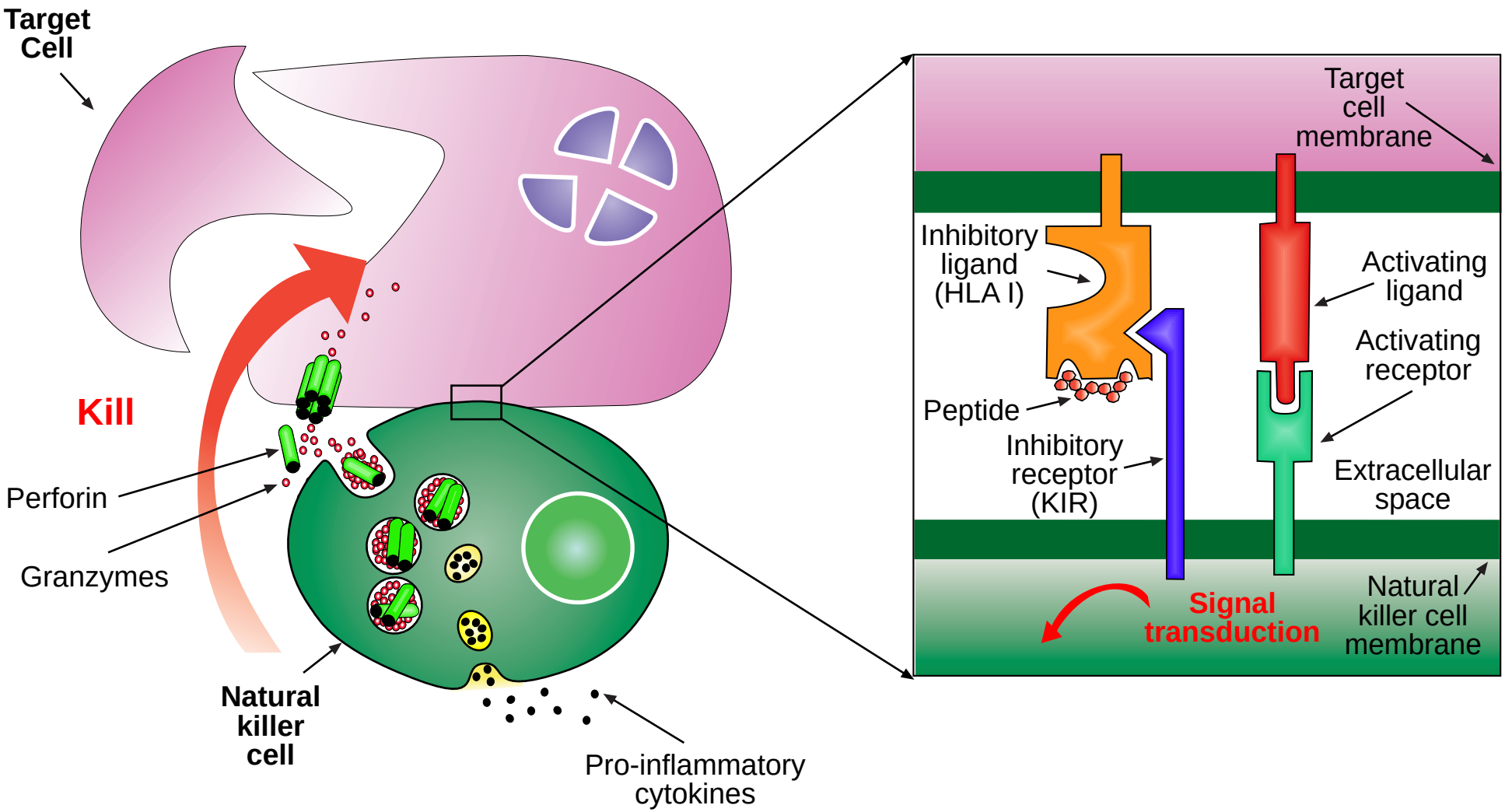
Gene defects are not known for secondary HLH, however, in familial HLH, several genes involved in the cell cytotoxicity mechanism of CD8+ cytotoxic T cells and natural killer cells have been identified. The failure of cytotoxic cells to resolve infection coupled to increased systemic levels of pro-inflammatory cytokines, particularly **INF-γ** and **TNF-α**, promotes excessive activation of macrophages. The enhanced phagocytic ability of activated macrophages can lead to destruction of haematopoietic cells or their precursors in bone marrow (also spleen and lymph node) causing cytopaenia. Activated macrophages also secrete pro-inflammatory cytokines, such as **IL-1**, **IL-6** and **TNF-α** , that are associated with some of the symptoms of HLH such as fever, rash (increased vascular permeability) and multiple organ infiltration by immune cells.

CD8+ cytotoxic T cell killing mechanism



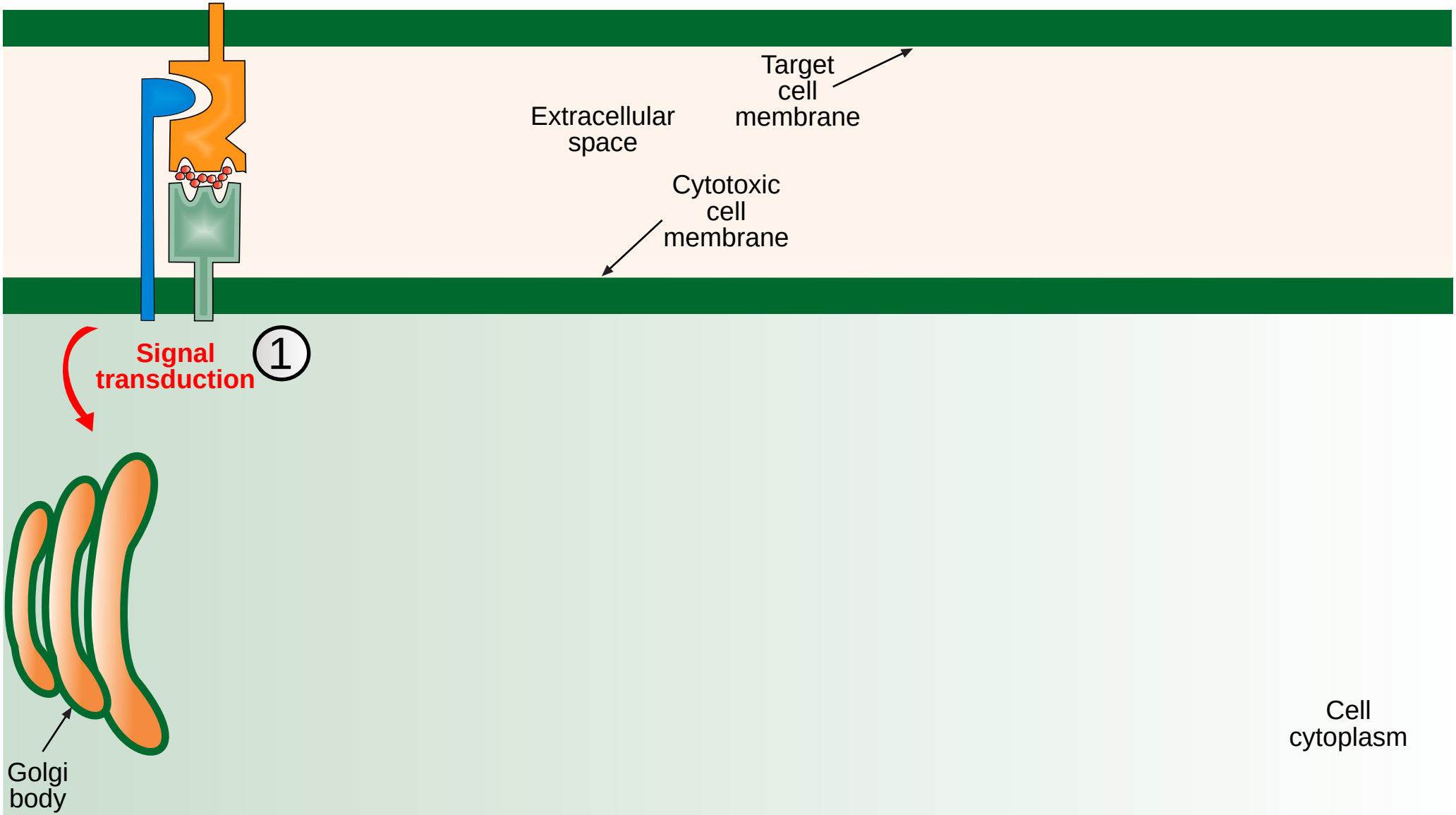
CD8+ cytotoxic T cells are activated by engagement of the T cell receptor with antigenic peptide-loaded HLA class I receptors on the surface of the target cell. Signal transduction mediated by the intracellular domains of the T cell receptor initiates a cascade of cytoplasmic events that promotes the formation and degranulation of exocytic vesicles containing perforin and granzymes at the contact area between the cytotoxic T cell and the target cell (immunological synapse). Perforin forms a permeable pore in the target cell membrane which allows entry of granzymes (that initiates apoptosis) and loss of water (causes osmotic shock) leading to cell death.

Natural killer cell killing mechanism



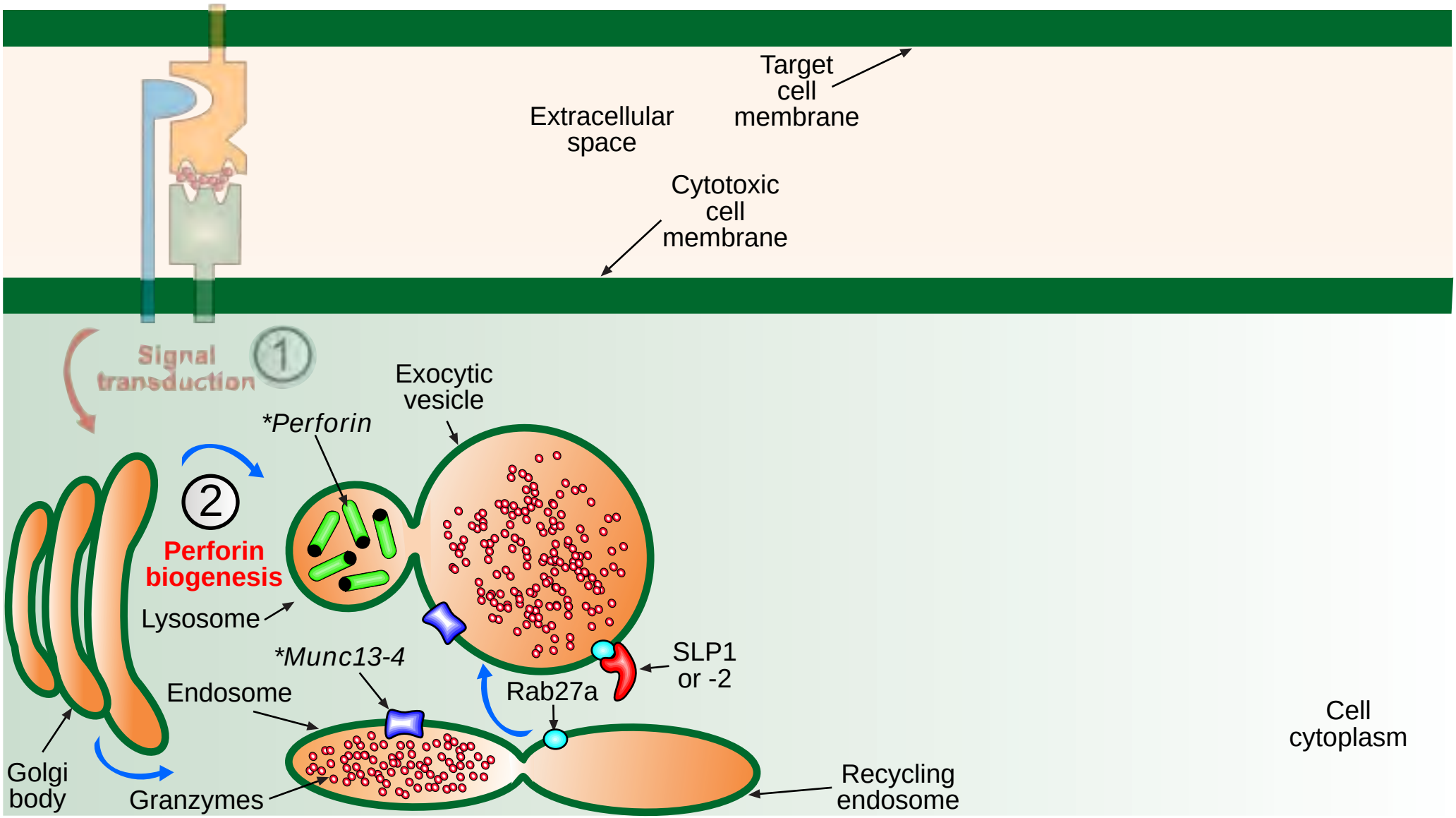
Natural killer cells are activated when stimulatory signals exceed inhibitory signals. Inhibitory killer-cell immunoglobulin-like receptors (KIRs) prevent cell activation by engagement with HLA class I ligands which is peptide-independent. Stimulatory KIRs and other activating receptors activate natural killer cells if inhibitory signals are diminished. Similar to CD8+ cytotoxic T cell activation, signal transduction is mediated by the intracellular domains of activating receptors that initiates a cascade of cytoplasmic events that leads to the formation and degranulation of exocytic vesicles containing perforin and granzymes at the contact area between the natural killer cell and the target cell (immunological synapse). Perforin forms a permeable pore in the target cell membrane which allows entry of granzymes (that initiates apoptosis) and loss of water (causes osmotic shock) leading to cell death.

Degranulation mechanism: signal transduction



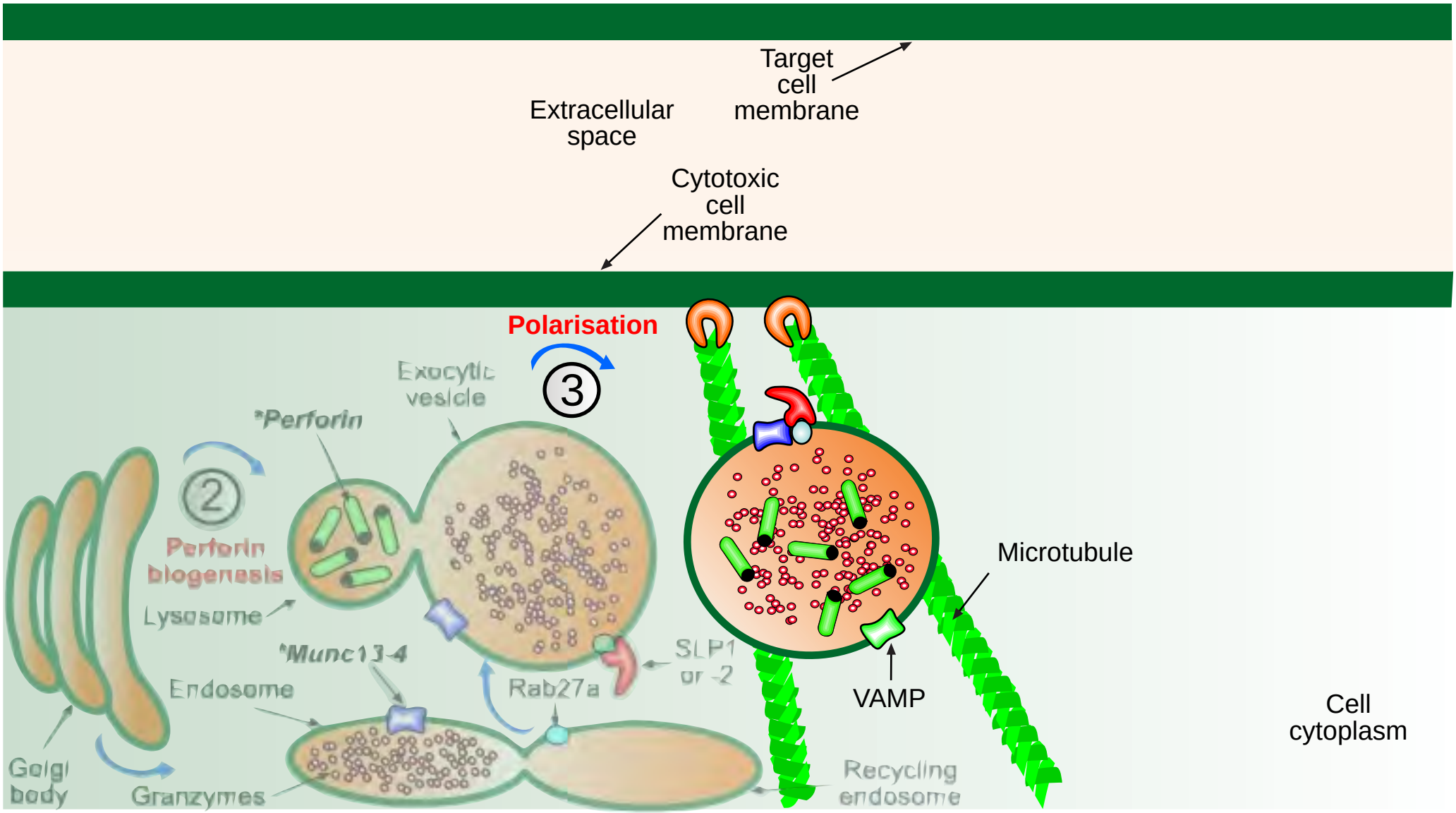
The first event necessary to initiate the degranulation process of cytotoxic cells, such as CD8+ cytotoxic T cells and natural killer cells, is activation of the cell via receptor-ligand signal transduction which takes place at the contact area between the cytotoxic cell and the target cell (immunological synapse). In CD8+ cytotoxic T cells, this is mediated by engagement of the T cell receptor with HLA class I ligands bearing immunogenic peptides. In natural killer cells, activation occurs when stimulatory signals exceed inhibitory signals.

Degranulation mechanism: Perforin biogenesis



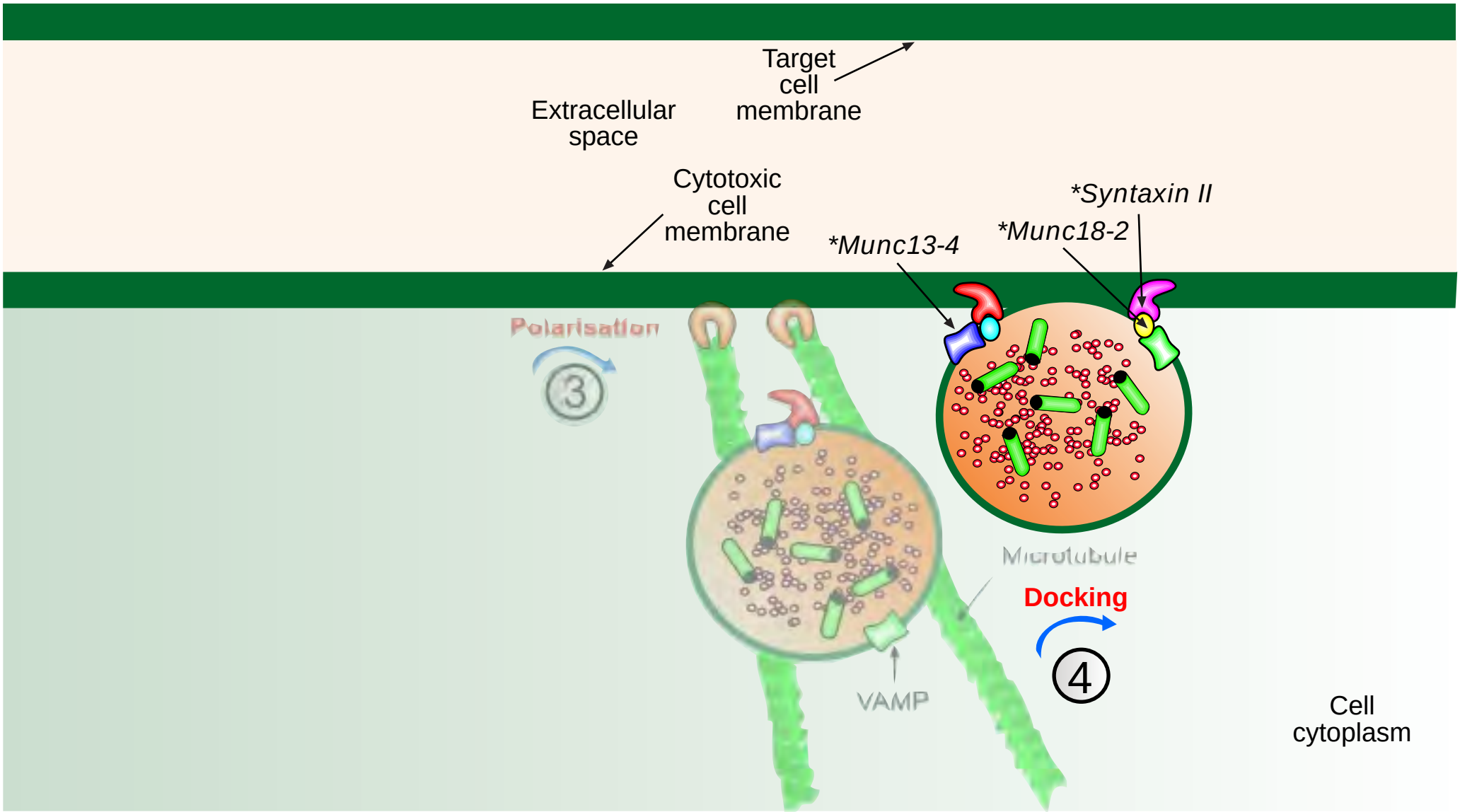
Activation of cytotoxic cells via signal transduction initiates the formation of exocytic vesicles containing perforin and granzymes. These proteins are synthesised in the Golgi body and sorted to vesicles. Perforin is sorted into a lysosome which fuses with a late endosome containing granzymes to form an exocytic vesicle destined for membrane fusion at the immunological synapse. Importantly the granzyme-containing endosome is initially formed by sorting of granzymes into an early endosome that fuses with a recycled endosome returning from the cell membrane. This brings two important membrane proteins, Munc13-4 and Rab27a, together which is necessary for pre-fusion docking of the exocytic vesicle with the cytotoxic cell membrane. In familial HLH type 2, which is the most common form of inherited HLH, mutations are observed in the gene encoding perforin protein, while defects in Munc13-4 are known for familial HLH type 3.

Degranulation mechanism: polarisation



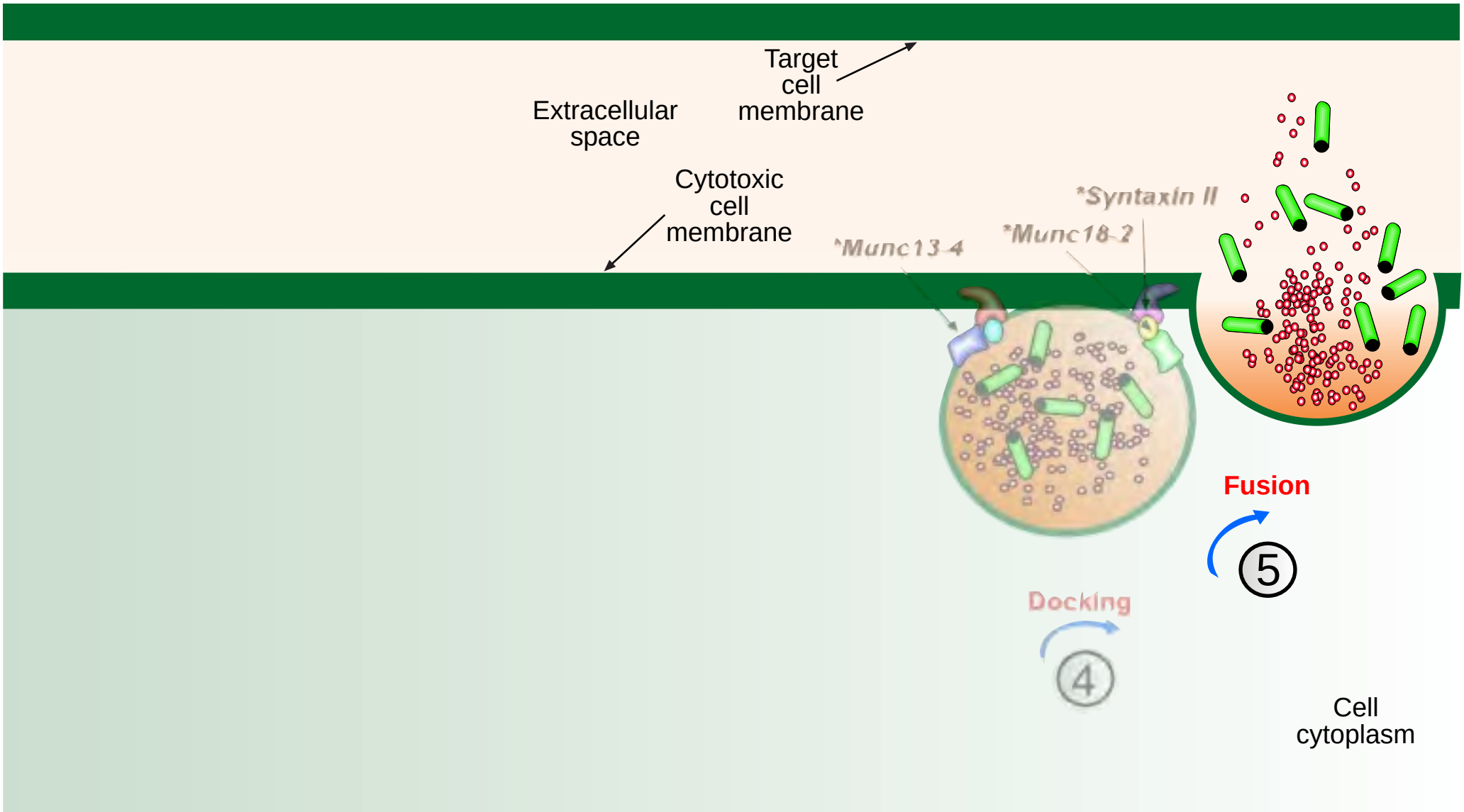
The exocytic vesicle containing perforin and granzymes is destined for fusion with the cell membrane of the cytotoxic cell. Importantly, fusion must take place at the contact area between the cytotoxic cell and the target cell (immunological synapse). The process that mobilises and directs the exocytic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

Degranulation mechanism: docking



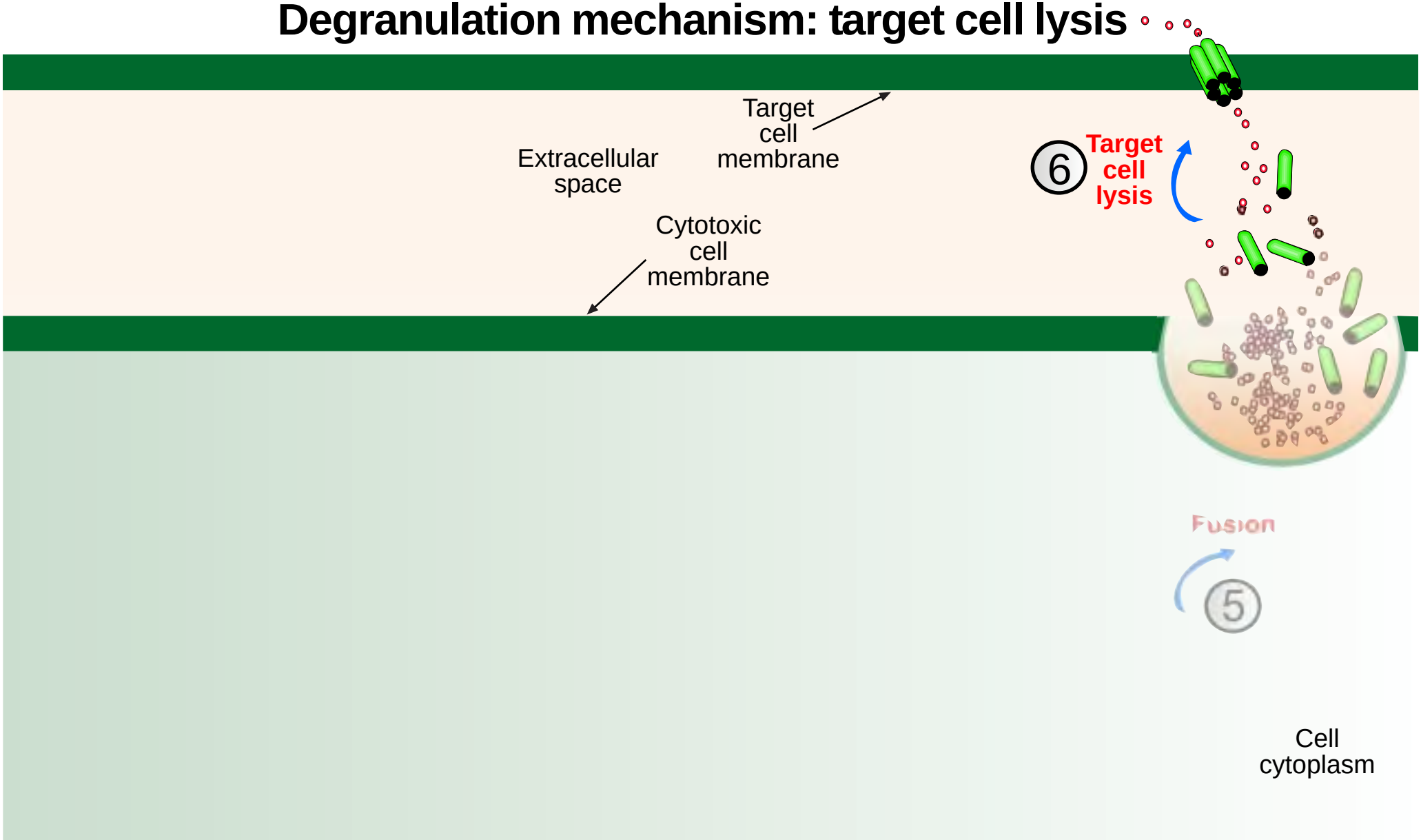
Once the exocytic vesicle has reached the cell membrane, protein interactions immobilise the vesicle and prepare it for membrane fusion called docking. In familial HLH type 3, 4 and 5, defects in Munc13-4, Syntaxin II and Munc18-2 proteins, respectively, cause failure of the exocytic vesicle to dock at the cell membrane, thus preventing membrane fusion from taking place.

Degranulation mechanism: fusion



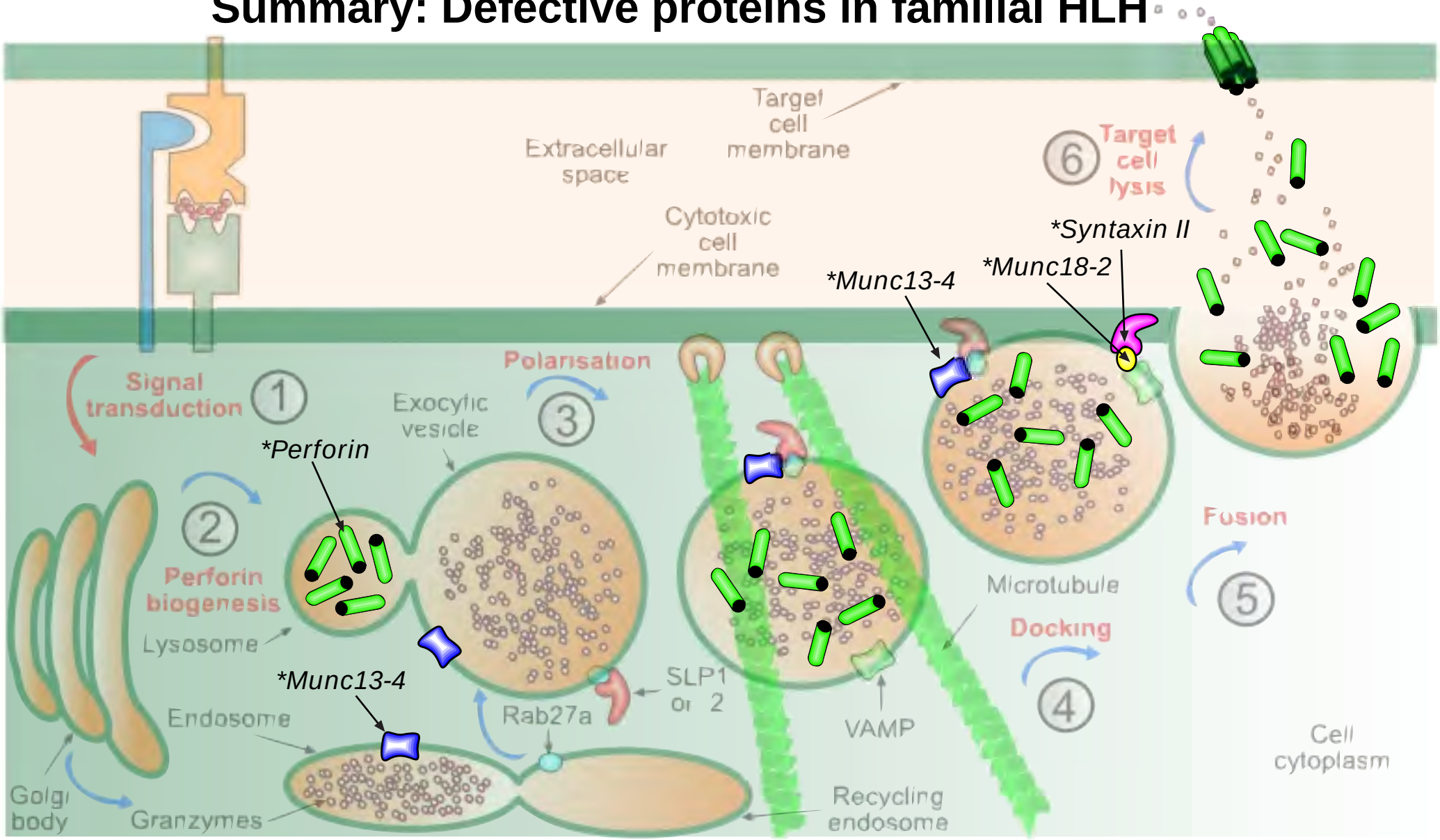
Fusion of the cell and exocytic vesicle membranes facilitates the release of perforin and granzymes into the extracellular space between the cytotoxic cell and the target cell at the immunological synapse.

Degranulation mechanism: target cell lysis



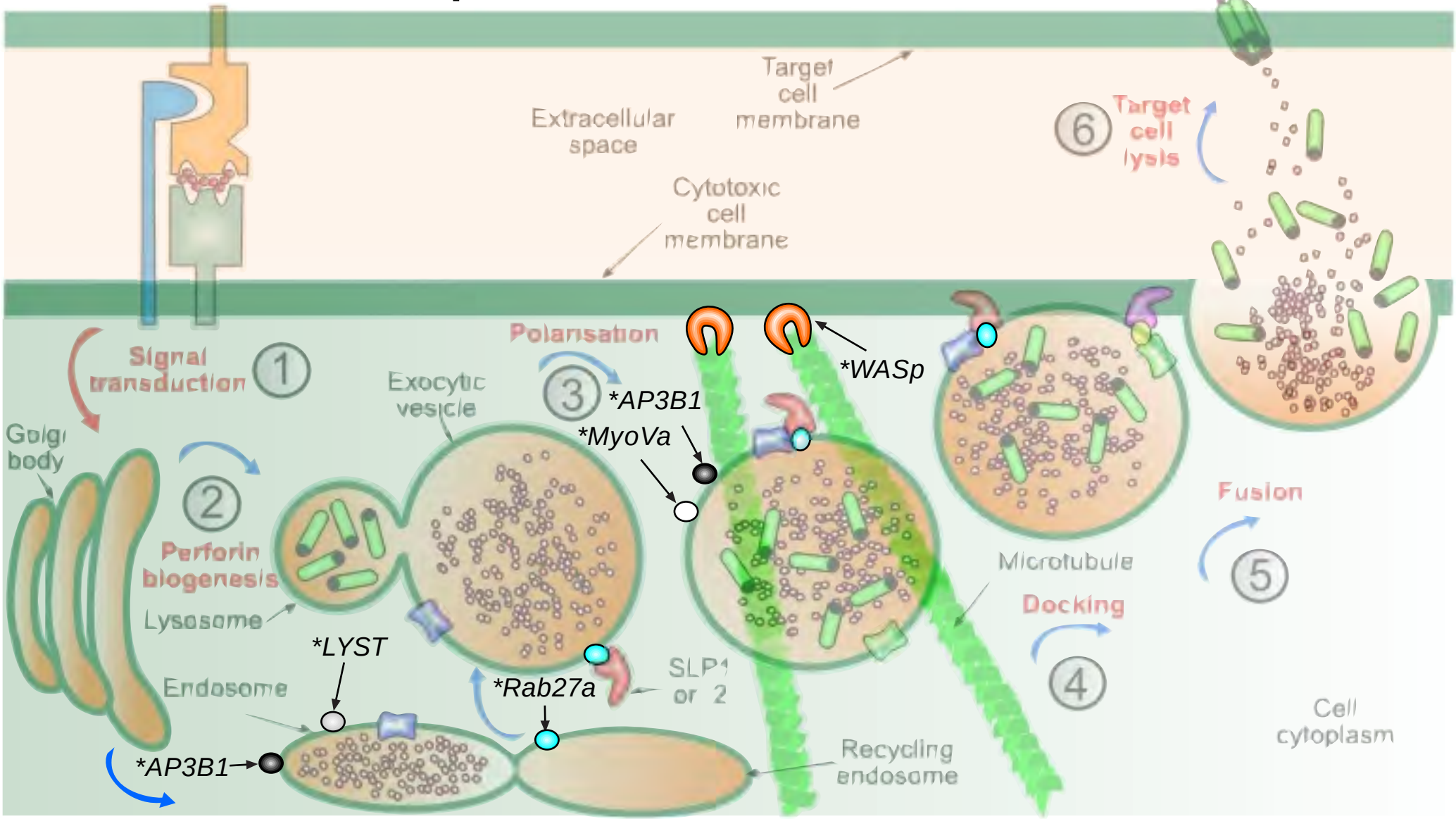
In a calcium-dependent mechanism, perforin monomers complex into a permeable pore in the cell membrane of the target cell. Granzymes penetrate the target cell by passing through the pore and initiate programmed cell death (apoptosis). Loss of water through the pore also contributes to cell death by osmotic shock.

Summary: Defective proteins in familial HLH



In familial HLH a number of defective proteins are known to affect the formation and degranulation of exocytic vesicles containing perforin and granzymes in cytotoxic cells such as CD8+ cytotoxic T cells and natural killer cells. In familial HLH type 1 an unknown gene on chromosome 9 is affected. The most common form of familial HLH is type 2 with defects in the perforin protein. In familial HLH type 3, 4 and 5, defective proteins Munc13-4, Syntaxin II and Munc18-2, respectively, are all involved in pre-fusion docking of the exocytic vesicle at the cell membrane. Failure of cytotoxic cells to control intracellular infection leads to excessive cytokine stimulation of phagocytes.

Defective proteins in SCID-associated HLH



HLH is also associated with certain severe combined-immunodeficiency syndromes where the defective gene encodes a protein important in the formation and degranulation of exocytic vesicles containing perforin and granzymes. In Hermansky-Pudlak syndrome type II, mutations in AP3B1 affects sorting of proteins to endosomes and also polarisation of exocytic vesicles 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 is needed for docking of the exocytic vesicle at the cell membrane. Griscelli syndrome type 1 has defects in MyoVa that affects the polarisation and mobilisation of exocytic vesicles. In Chediak-Higashi syndrome defects in LYST protein affects sorting of proteins to endosomes. Similar to familial HLH, failure of cytotoxic cells to control intracellular infection leads to excessive cytokine stimulation of phagocytes.