PROGRAMMED CELL DEATH BY PRIMARY PHAGOCYTOSIS: PHAGOPTOSIS

Charu Kharbanda

Associate professor; Swaminarayan Institute of Medical Sciences & Research, Kalol, Gandhinagar. Email: charupiplani1@gmail.com .Mobile: 9426260369.

Abstract : Phagoptosis, also called as primary phagocytosis, is a recent conception of cell death caused by phagocytosis of viable cells. It is instigated by display of 'eat me' signals and or loss of 'don't eat me' signals by viable cells **.** Phagoptosis balances the reproduction and death of erythrocytes, neutrophils and other cells. It also protects against pathogens and regulates inflammation and immunity. Cells are phagocytosed as a result of i) expressing 'eat me' signal or ii) loosing 'don't eat me' signal. 'Eat me' signal is expression of XKr4 protein while 'don't eat me' signal is expression of CD47 protein. There are several advantages of apoptosis both in intrauterine & adult life as disappearance of webs of fingers in humans and disappearance of tail in frog. Abnormalities of phagoptosis include neurodegenerative diseases like attention deficit hyperactivity disorder (ADHD), Alzheimer's disease and cancers.

Key words – Phagoptosis, Macrophages, Cell in cell, Scrambleses.

Author for correspondence: Associate professor; Swaminarayan Institute of Medical Sciences & Research, Kalol, Gandhinagar. Email: charupiplani1@gmail.com .Mobile: 9426260369.

Introduction – Phagoptosis, also called as primary phagocytosis, is a recent conception of cell death caused by phagocytosis of viable cells. It is instigated by display of 'eat me' signals and or loss of 'don't eat me' signals by viable cells [1,2]**.** Phagoptosis balances the reproduction and death of erythrocytes, neutrophils and other cells[2]. It also protects against pathogens and regulates inflammation and immunity. However, recent studies show that activated microglia eat viable brain neurons in models of neurodegeneration and cancer cells can escape phagocytosis by displaying a 'don't eat me' signal, suggesting both extremes of apoptosis can cause Pathology[3]. This review article overviews the molecular signals that regulate phagoptosis and related Physiological and Pathological aspects.

Molecular Physiology of Phagoptosis : Phagoptosis is a subtype of phagocytosis in which a viable cell is internalized by a phagocyte. This is in contrast with efferocytosis, i.e., phagocytosis of cellular debris. In most cases, phagoptosis causes death of the internalized cell[4]. The internalization may either be heterotypic (between cells of different type) or homotypic (between cells of same type). This Phenomenon is also called as cell in cell (CIC)[5].

From the previously published studies, it is evident that CIC formation can either serve to obtain nutrients[6,7] or to escape from unfavorable environmental conditions[8]. It can also be related to cancer progression and worse prognosis[9]. CIC process can be triggered by external factors such as nutrient deprivation[10,11], hypoxaemia[12], infection, non – binding state[13,14] and presence of chemotherapeutic agents in extracellular milieu[15,16].

In simpler words, cells undergo apoptosis when they are recognized as stressed, senescent, damaged, pathogenic, non – self or mis organized (loose contact in homophilic binding). Cells are phagocytosed as a result of i) expressing 'eat me' signal or ii) loosing 'don't eat me' signal and or iii) binding of opsonins. Phagoptosis is probably the most common form of cell death in the body as it is responsible for erythrocyte turnover and there is increasing evidence that it mediates physiological death of neutrophils, T- cells, platelets, and stem cells; therefore, regulates inflammation, immunity, clotting and neurogenesia[17]. Phagoptosis is the major form of host defense against pathogens and cancer cells. However, recent evidences indicate that excessive phagoptosis may kill host cells in inflammatory conditions, contributing to haemorrhagic conditions and neuronal loss in inflamed brain.

Actually, a nuclear protein released in to the cytoplasm initiate the process of display of lipid on the cell surface, signaling other cells an 'eat me' signal.

We already know that dead cells display an 'eat me' signal on the cells surface that is recognized by the phagocytes. What we do not know is that during this process, lipids are flipped between inner and outer parts of the cell membrane via a variety of proteins called as scramblases. Several of these lipid scrambling proteins are now identified.

To identify this, Japanese scientists used an array of screening approaches to study the scrambling protein ,i.e. 'eat me' signal, called XKr4. The broad aim was to single out the genes that are active during cell death and to specifically zoom in on XKr4 and its associated proteins to understand how they interact[18].

They found that a nuclear protein fragment activates XKr4 protein to display an 'eat me' signal to phagocytes. When cell is programmed to die a nuclear protein called XRCC4 , getting cut by an enzyme. A fragment of XRCC4 leaves the nucleus, activating XKr4, which forms a dimer: the linking of identical pieces in to configurations. Both XRCC4 binding and dimer formation are necessary for XKr4 to ultimately transfer lipids on the cell surface to alert phagocytosis.

XKr4 is only one of the scrambling proteins. Others are activated much faster during cell death. The scientists now want to understand when and why the XKr4 pathway is specifically activated. Since it is strongly expressed in brain, it is likely important for brain function.

Lipid that is flipped between inner and outer surface of cell membrane is phoasphatidyl serine. Phosphatidyl serine is an 'eat me' signal that when exposed on the surface of the cell, triggers phagocytosis to eat that cell. That is why it is sometimes called as phagoptosis instead of apoptosis.

Transmembrane protein 16 F (TMEM16F) is a Ca^{+2} gated channel that is required for Ca^{+2} activated phosphatidyl serine shifting on the cell surface. TMEM16F is widely expressed & has roles in platelet activation during blood clotting, bone formation and T cell activation[19].It is recently been found that 2 membrane protein families TMEM16 and XKR, promote phosphatidyl scrambling. The TMEM16 family that has 10 members in humans, contain 8 transmembrane regions. TMEM16A & 16B act as Ca^{+2} dependent Cl^{-} channels, while 16C, 16D, 16F,16G and 16J promote phosphatidyl scrambling at plasma membrane in a Ca^{+2} dependent manner[20]. In particular, TMEM16F is responsible for the phosphatidyl serine exposure on activated

platelets and Scott syndrome, a bleeding disorder, is caused by mutation in the TMEM16F gene.

The XKR family is predicted to contain 6 Transmembrane regions. Among its 9 members, XKR8 carry a recognition sequence for caspases 3 and 7 at their C – terminus and are activated by caspase cleavage to promote phosphatidyl scrambling[21].

Advantages of Phagoptosis: There are several advantages of Phagoptosis both in intrauterine & adult life. Advantages of Phagoptosis are following:

1. Development: During intrauterine life, excess cells are removed by phagoptosis as removal of tail in adult frog while tadpole contains tail and removal of webs in between human fingers. Even during mammalian development multiple cells undergo programmed cell senescence and then phagocytized by macrophages[22]. In the development of brain, microglia eat many otherwise viable neurons to limit neurogenesis[23].
2. Turnover of blood cells: Red blood cells live for roughly 3 months before being phagocytized by macrophages. Old & damaged RBCs display changes in their cell surface as phosphatidylserine exposure, changed conformation of 'don't eat me' signal CD47 & exposure of novel antigens that attract antibodies[24]. Neutrophils having lived their lives show increased expression of XKr4 protein to display an 'eat me' signal to phagocytes. This signal directs them to bone marrow where they are phagocytized by macrophages[25].
3. Host defence agaist pathogen : Dendritic cells phagocytose viable neutrophils and present antigen of bacteria already engulfed by the neutrophils[26]. Thus, phagoptosis contribute to host defense in variety of ways.
4. Host defence against cancer : It has been known for some time now that animals defend themselves against cancer by antibody mediated or antibody independent phagoptosis by macrophages. Most human cancer cells overexpress CD47 or 'don't eat me' signal on their surfaces. If this 'don't eat me' signaling can be prevented then many cancers can be removed from the body[27].

Clinical Pathophysiology:

1. A single nucleotide polymorphism (SNP) of the 'eat me' signal or XKR4 gene has been linked to attention deficit hyperactivity disorder (ADHD). This gene is preferentially expressed in the cerebellum, a brain structure implicated in this disorder.
2. It has also been associated with addiction and substance abuse as well as cognitive deficits including poor self-restraint, memory, executive function and neuropsychiatric symptoms in Mcleod syndrome, a genetically transmitted disorder of XKr4 gene family.
3. Haemophagocytosis is a clinical condition found in many infectious and inflammatory conditions in which macrophages phagocytose viable blood cells to produce reduce count of white and red blood cells (Cytopenia). Interferon – gamma appears to derive haemophagocytosis during infections by activating macrophages for phagoptosis of viable red blood cells thus, causing a consumptive anemia of inflammation[28]. Hemophagocytic lymphohistiocytosis (HLH) is characterized by excessive engulfment of hematopoietic stem cells (HSCs) by bone marrow macrophages, and this has been found to result from down regulation of CD47 expression on HSCs, enabling macrophages to eat them alive[29].
4. Pathological phagoptosis in the brain. Microglial phagocytosis of stressed-but-

viable neurons occurs under inflammatory conditions, and may contribute to neuronal loss in brain pathologies[30].

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