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CONTENTS

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Rismawati Yaswir, Purwanto AP, Sidarti Soehita, July Kumalawati, Aryati,
Rahayuningsih Dharma, Adi Koesoema Aman, Yolanda Probohoesodo, Puspa Wardhani

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LITERATURE REVIEW

MACROPHAGE AUTOPHAGY IN IMMUNE RESPONSE

(Otofagi Makrofag dalam Respons Imun)

Jusak Nugraha

ABSTRAK

Otofagi adalah mekanisme yang digunakan oleh sel untuk menyerap, membuang dan mendaur ulang sampah. Makrofag dapat berfungsi untuk menangkap, mengonsumsi dan mencerna antigen eksogen, keseluruhan mikroorganisme, partikel yang tidak larut dan bahan endogen misalnya: sel inang yang sekarat atau rusak yang dipajankan oleh limfosit. Saat ini makrofag dapat dibagi menjadi dua jenis aktivasi: Aktivasi klasik (M1); Aktivasi alternatif (M2) yang memiliki efek berbeda. Aktivitas M1 meningkatkan respons Th1 misal menyebabkan peradangan, pembunuhan patogen intraselular, DTH (tipe hipersensitivitas tertunda) dan kerusakan jaringan. Aktivitas M2 menyebabkan peningkatan respons Th2 sebagai imunomodulator, deposisi matriks dan remodeling jaringan. Peran makrofag pada infeksi M.tuberculosis akan menentukan kondisi inang. Jika makrofag dapat melakukan fungsi fagositosis M.tuberculosis akan dimusnahkan dan inang tidak terinfeksi. Mycobacterium TB yang patogen dapat dengan mudah menghindari fagositosis dan berhasil menghambat otofagi makrofag. Peningkatan otofagi akan meningkatkan efikasi BCG maupun vaksin lainnya dan dengan menggunakan pendekatan merangsang otofagi untuk membasmi TB sangat berguna sehingga pengobatan berbasis otofagi untuk TB dapat segera diwujudkan.

Kata kunci: *Makrofag, otofagi, respons imun*

ABSTRACT

Autophagy is a mechanisms used by cells to sequester, remove and recycle waste, Macrophage can function to catch, eat and digest exogenous antigen, whole microorganism, insoluble particle, and endogenous material for example : host cell which is dying or damaged that presented by lymphocyte. Nowadays macrophage could be divided into two kinds of activation: Classical activation (M1); Alternative activation (M2) which has different effect. M1 activity increase Th1 response e.g. causing inflammation, intracellular pathogen killing, DTH (delayed type hypersensitivity) and tissue damages. M2 activity cause increasing Th2 response as immunomodulator, matrix deposition and tissue remodeling. The role of macrophage in M.tuberculosis infection will determine host condition. If macrophage could completely doing the phagocytosis function M.tuberculosis will be eliminated and host is not infected. Pathogenic Mycobacterium tuberculosis could easily avoid phagocytosis and successfully inhibit macrophage autophagy. Enhancing autophagy will increases the efficacy of BCG or another incoming new vaccine and by using autophagy-inducing approaches to combat TB are very challenging and an autophagy-based therapy for TB may be realized.

Key words: Macrophage, autophagy, immune response

INTRODUCTION

Autophagy comes from the Greek language, which is auto-meaning alone and phagein which means eating. So, autophagy is defined as eating itself. The concept of autophagy first appeared in 1960, when researchers discovered that cells could destroy their own content by enveloping using membranes to form bags such as vesicles to be delivered to recycled compartments, called lysosomes for degradation (Fig. 1).[1]

In the early 1990s, Yoshinori Ohsumi used baker's yeast to identify important genes in the autophagy. The discovery of Ohsumi raises a new understanding of how cells recycle their contents. These findings pave the way for understanding the importance of autoophagy in various physiological processes, such as adaptation to hunger or response to infection.

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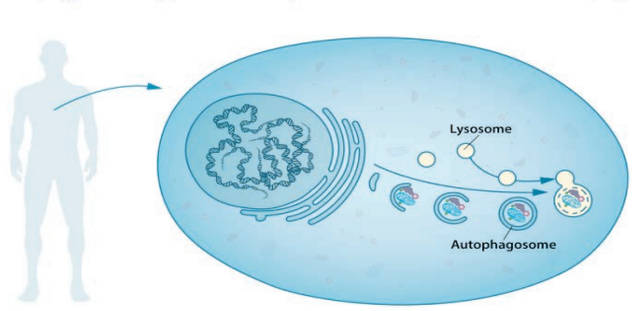

Figure 1. The body cells have special compartments including lysosomes containing enzymes to digest cell contents. A new vesicle called an autophagosome is present in the cell[1]

Mutations in the autoophagy genes can cause disease and the autophagic process plays a role in several conditions including cancer and neurologic disease.[1]

Autophagosomes that form will cover the contents of cells, such as damaged proteins and organelles. Then the Autophagosome will coalesce with lysosomes, where the contents autophagosomes will be broken down. This process will provide nutrients for the cells and is important in the process of cell renewal. To date there is only one mechanism possessed by eukaryotic cells to clean intracellular organelles and pile up proteins that are too large to be degraded by proteasome. This mechanism which uses a lysosome degradation path called autophagy is also used to clean microorganisms (such as viruses, bacteria and protozoa) that invade intracellularly.[2] Macro-autophagy (commonly referred to as autophagy) is a process of catabolism that regulates cellular balance with using lysosomes. The auto-process involves placing proteins and cytoplasmic contents into a double membrane vesicle compartment which will then be delivered to lysosomes to be degraded.[3]

Autophagy is thought to have evolved as a stress response allowing eukaryotic unicellular organisms to survive under difficult conditions, possibly by regulating the energy balance and/or through the quality control of proteins and organelles.[2] The metabolic outcome of lysosomal activity will then be reused as the energy needed for cells. The autophagy process serves as an intracellular recycling mechanism.[3]

Autophagy will be activated in response to various cellular stresses and often have cytoprotective function. Depend on the triggers of the autophagy process, autophagy can develop into a non-selective degradation process or may be a selective degradation process for a particular substrate. Lack of nutrients, damage or excess of cell organelles, protein buildup, endoplasmic reticulum stress, oxidative stress, toxins, radiation and hypoxia can be autophagic triggers.

In this literature review will be discussed about the relationship between autophagy and immunity and inflammation, the factors that can trigger the emergence of autophagy as well as laboratory tests to assess the autobiographic process.

AUTOPHAGY MECHANISM

Characteristic of the autobiographic process is the formation of a double membrane vesicle called the autophagosome. The autophagy process consists of four main steps: initiation, elongation, closure, fusion (Figure 2). The source of the autophagosomal membrane and the factors underlying the dynamics of the autophagosomal membrane are complex, can be

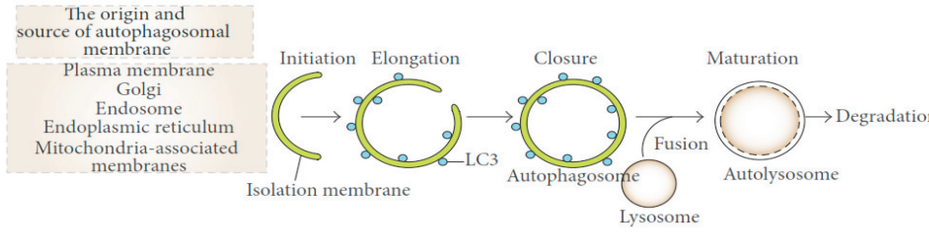

Figure 2. The general scheme of the autophagy process, beginning with the formation of an insulating membrane (phagophore) derived from various intracellular membrane sources. The initiation process will be followed by elongation and closure that will form a complete autophagosome. The process of unification between the autosomal and the lysosomes will form autolysosomes, which will degrade the contents of the autophagosome[3]

from plasma membrane, golgi apparatus, mitochondria, endoplasmic reticulum.[3]

Autophagosomes appear in the cytoplasm as an insulating membrane (phagophore) in the form of a cup-shaped bulge called omegasome. Omegasome often arises from endoplasmic reticulum rich in phosphatidylinositol-3-phosphate kinase (PI3K) and double-FYVE-containing protein 1 (DFCP1). The Golgi apparatus, the mitochondria and the plasma membrane also play a role in the formation of phagophore. Many types of proteins are related in the autobiographic biogenesis. These proteins are first found in yeasts and are referred to as autophagy-related genes (ATGs) proteins.[3]

Several important molecular events have been recognized in studies of autophagy induced by nutritional deficiency[3], where autophagy will be induced by nutritional deficiency, through inhibition of mammalian target of Rapamycin (mTOR) which causes the displacement of mTOR complexes from the cytosol to a particular area in RE.[4,5] After the autophagy is induced, the ULK1 complex will be activated and transferred to the RE and transiently associated with Vacuole Membrane Protein I (VMP1), resulting in activation of the Class III PI (3) K {Endoplasmic Reticulum (ER) localized autophagy specific class III phosphatidylinositol-3-OH kinase (PI (3) K)} which further forms phosphatidylinositol-3phosphate (PtdIns (3) P) on the RE membrane and then binds to double FYVE-containing protein 1 (DFCP1) and WD-repeat domain phosphoinositide interacting (WIPI). WIPIs and complex autophagy related genes (ATG12-ATG5-ATG16L1) are present on the outer membrane and LC3-phosphatidylethanolamine (LC3-PE) is present in the outer and inner membranes of the insulating membrane and ATG2 and ATG9 form omegasom (Figure 3).[2,6]

The ATG8 protein along with other factors will result in the elongation and closure of the phagoophages, which further form the multiple membrane autophagosomes. The autophagosome can coalesce with lysosomes, where the ability to digest the contents of autophagosome comes from the lysosomal hydrolytic enzyme. The fusion process is mediated by the translocation of SNARE protein syntaxin 17 to the outer membrane of the autoto-mosomes.[6]

AUTOPHAGY FUNCTIONS

Macrophages are an important cell type in the acquired immune response. Macrophages have a PRR that aids in the introduction of pathogen-associated molecular patterns (PAMPs) from microbes and danger-associated molecular patterns (DAMPs). Lipids, nucleic acids, proteins, lipoproteins, glycans derived from various bacteria, viruses, parasites and fungi are included in PAMPs. Depending on the specific receptor PAMPs/DAMPs and the number of activated PRRs, various pathways can be activated, which will prepare the cells to fight the invasive agent by activating the degradation pathway and forwarding signals such as cytokines to provide warnings against other cells in the acquired and adaptive immune system in the surrounding network.[3]

The function of the protein autophagy in the immune system is also included in the development and balance of the immune system and in antigen presentation (Figure 4).[2] The discovery of different autophagy genes in certain lymphocyte populations in mice has demonstrated the important role of protein autophagy in maintaining normal numbers

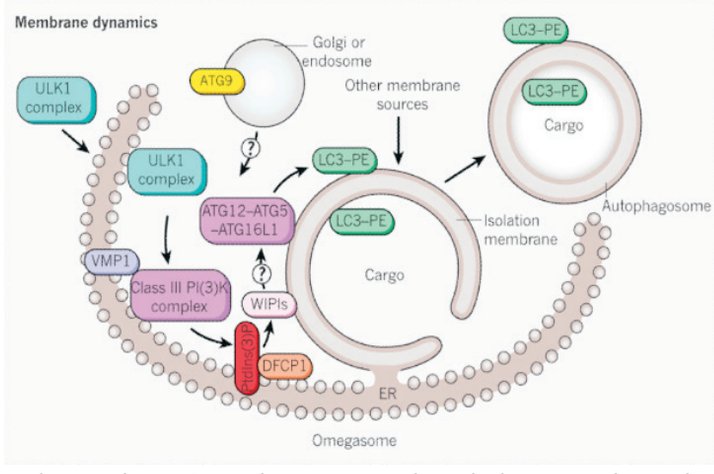

Figure 3. After autophagy induction, the ULK1 complex migrates to the endoplasma reticulum and is associated with VMP1, which activates the Class III PI (3) K and PtdIns (3) P complexes that will bind DFCP1 and WIPIs. Complex WIPIs and ATG12-ATG5-ATG16L1 along with LC3-PE will form omegasom and proceed with elongation and closure process[2]

of B cells, CD4 + T cells, CD8 + T cells and fetal hematopoietic stem cells.[7] In T cells, where the number of mitochondria is set during the transition from thymocyte to mature T cells, the presence of auto-cell deficiency disorders is associated with mitochondrial clearance disorder (Fig. 5).[8] Another function of autotophagy cells is the clearance of autoreactive T cells in the thymus gland.[8]

Autophagy also has another function in the process of lymphocyte differentiation, possibly indirectly, through the effects of cytokine expression. It is not known for certain whether autotophagy also plays a role in the development and/or balance of immune cell populations other than lymphocytes and hematopoietic stem cells.[2] Autophagy protein is needed for antigen presentation during infection in vivo.[9] MHC class II molecules, which were originally thought to just display lysosomal products of endocytosed proteins to $CD4^+$ helper T cells, can also present intracellular substrates of autophagic pathways.[10]

Autophagy and Resistance to Infection

The discovery of the association between microbial infection and auto-activation has resulted in the identification of additional auto-adapter and mechanisms that specifically govern the target, defense, and degradation of various bacteria.[3] Autophagy and autophagy proteins have an important role in resistance to bacterial, viral infections and protozoa. The function of autophagy protein in controlling infection by intracellular pathogens is cell-autonomous. The mechanisms by which the autophagic genes mediate resistance to infection in vivo remain uncertain, but may involve combinations of xenophagy, the influence of autophagy protein dependent on microbial replication or microbial resistance, activation of the innate immune response and immune response and changes in cell death induced by pathogens (as in Figure 4).[2]

A recent human genetic study explains that immunity-related GTPase human (IRGM), which regulates the *Mycobacterium* clearance based on autophagy-dependent[11], is identified as one of the genetic risks of tuberculosis in West African populations.[11] *Mycobacterium tuberculosis* inhibits maturation of phagosomes by impairing the phagosome maturation pathway. In contrast, the survival rate of *M.tuberculosis* will decrease after autophagy induction of infected macrophages, so it is concluded that the degradation of phagosome containing *M.tuberculosis* is stronger than the inhibitory effect of *M.tuberculosis*.[3]

Studies have also demonstrated the important role of autophagy as a defense against *Mycobacterium* infection in human cells and genetic analysis of host genes regulating replication of *Mycobacterium tuberculosis* is dominated by autophagy regulators.[12]

Autophagy may plays an important role in resistance to one of the most important global infectious diseases, tuberculosis.[2]

Mutations in Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), which encodes recognition receptors for intracellular pathogens that function on bacterial autofibs[2], are also associated with sensitivity to infection by *Mycobacterium leprae*, the leprosy organism.[13] Metabolic inflammasome a complex molecular marker such as Protein Kinase RNA-activated (PKR), eukaryotic translation Initiation Factor 2-alpha kinase 2 (eIF2α), Jun N-terminal Kinase (JNK), Insulin Receptor Substrate (IRS), Inhibitor κB Kinase (IKK) acts as a liaison between stress RE and other general stress, including inflammation and metabolic disorders (such as insulin resistence and obesity).[14]

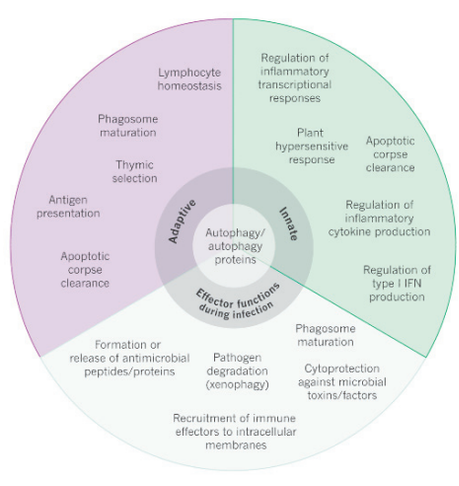

Figure 4. Autophagy function in acquired and adaptive immunity and as an effector at the time of infection[2]

In line with the protective effect of this autophagy, hepatic suppression of the Atg7 otofagi gene in mice will result in increased RE stress and insulin resistance and mice lacking the proteins of the p62 autophagy adapter will be obese and insulin resistant.[15] Protein misfolding and ER stress are evident in various renal diseases, including primary glomerulonephritides, glomerulopathies associated with genetic mutations, diabetic nephropathy, acute kidney injury, chronic kidney disease and renal fibrosis.[16] Relationships between genes such as IRGM, NOD2 and other autophagy genes are associated with resistance to *Mycobacterium* infection and other infections and whether the resistance is mediated by autotophagy remains to be investigated.[2]

LABORATORY EXAMINATION FOR MACROPHAGE AUTOPHAGY

Detection of autophagy with an Electron Microscope Laboratory

Examination for macrophage detection of autophagy was first about 50 years ago using Transmission Electron Microscopy (TEM) and TEM is still the most common and sensitive examination in detecting the presence of auto- autophagy vesicles. Currently the TEM examination is combined with a tomography approach to identify areas of the RE. Transmission electron microscopy examination is a qualitative examination, by detecting the presence of morphologically intact organisms in the early autophagosome process or the degraded organelle in the late autolysosome.[17]

Detect LC3B-II by Western Blot

LC3 is a light chain of proteins found in the autophagic process. After translation, proLC3 will be broken down with the enzyme of Atg4 protease into LC3-I. At the time of auto-induction, LC3-1 will be conjugated by Atg7, Atg3, Atg12-Atg5-Atg16L and highly lipophilic phosphatidylethanolamine (PE) to form LC3-II. The presence of PE will result in LC3-II integrating with the lipid layer on the phagophore and autophagosome. To date, LC3-II is the only protein known to be specifically present in the autophagy structure during the process from the phagophore to the lysosomal degradation.[17]

Detection of p62 protein content

Protein p62 is used to indicate a defect in the poly-ubiquitinated protein aggregate turnover. The p62 protein will interact with poly-ubiquitinated protein aggregates in the ubiquitin-binding region and with LC3 in the LC3-binding region, which will then be the degradation target of the otolisosome. The p62 accumulated protein aggregate in the liver of mice with Atg7/autophagy deficiency and a targeted deletion of p62 will prevent the accumulation of these proteins, so it is concluded that p62 accumulation is a good measure when there is a selective autobiographic disturbance of poly-ubiquitinated protein aggregates.[18] However, p62 is affected by oxidative stress, oncogene races and activity of NF-κB, so examination of LC3B-II levels is still needed to validate changes in aggregate protein turnover by autophagy.[19]

Autophagy Detection with Fluorescence Microscope

Transfection procedures performed in GFP-LC3 binding can induce the occurrence of autoophagy; GFP-LC3 is sensitive to acidic pH and will stop fluorescence at the time of the autophagosomes united with lysosomes, thus not being able to see the final stages of autophagy.[20]

Currently, detection of LC3B-II endogenous proteins with immunofluorescence is preferred. Just as with the Western Blot LC3B-II examination, it also requires controls that demonstrate that LC3-positive otophagosome levels in cells depend on the auto-regulators, such as Beclin-1 and accumulate when agents that counteract LC3 degradation and autootagic turnover in the lysosome (such as Bafilomycin A1).[17]

Autophagy Detection with Flowcytometry

Quantitative examination by using Fluorescence-Activated Cell Sorting (FACS) has now been used for autobiographic measurements. This examination is based on fluorescence differences between Enhanced Green Fluorescence Protein-Light Chain 3 (EGFP-LC3) bonds in the autophagosome and decrease as they reach the lysosome. This examination eliminates frequent errors in manual calculations of LC3-II microscopically and speeds up the checking time.

The disadvantage of this method is that this examination does not distinguish between EGFP-LC3-I and EGFP-LC3-II (associated with autoto- mosomes),

so this examination is not sufficiently accurate in calculating the auto-drug activity, whereas fluorescence and western blot microscopy can distinguish between EGFP-LC3-I and EGFP-LC3-II.21 Recent studies use saponin detergent to exclude LC3-I (unrelated to autophagosomes), so that more specific fluorescence can be calculated from EGFP-LC3-II.[21]

CONCLUSION

Phagocyte cells from monocytes/macrophages play an important role in the immune response obtained by taking antigen microorganisms, metabolized by proteolysis enzymes into peptide fragments and presented in a form that can activate T cell responses. Other cells included in derivatives such as Langerhans cells in the epidermis, Kupffer cells in the liver, and microglial cells in the central nervous system. The most potent Presenting Cells (APC) antigen is the class of dendritic cells present in most body tissues and is concentrated in secondary lymph tissue.

Autophagy plays an important role in innate immune mechanisms, in resistance to infections such as tuberculosis and leprosy, as well as in inflammatory diseases and autoimmune diseases such as Crohn's, SLE, obesity and diabetes through the mechanism of increasing insulin resistance. Autophagy also has another function in the process of lymphocyte differentiation, possibly indirectly, through the effects of cytokine expression.

Recruitment of LC3 to *M.bovis* Bacilli Calmette Guerin (BCG) containing vacuoles depends on exogenous stimulation of autophagy. On the contrary, targeting of LC3 to *M.tuberculosis* containing vacuoles seems to be triggered without any extrinsic stimulatory signal. One of the major differences between BCG and virulent mycobacteria is the lack of ESX-1 from the BCG strain, which may explain their different effectiveness in replicating within macrophages and in activating innate immune responses by the host.

Laboratory examination for macrophage detection of autophagy the first time around 50 years ago by using Transmission Electron Microscopy (TEM) is still a widely used examination despite many shortcomings. Detection of LC3B-II by Western Blot using anti-LC3B antibodies has been recommended in analyzing otofagi. Interpretation of results in autobiographical examination with Western blot method is still causing controversy. Thus a consensus was made that all LC3-II levels were normalized by loading control, using β-actin or α-tubulin and using controls from inhibited cells such as Bafilomycin A1 or hydroxyquinolone

Other laboratory tests by detecting p62 proteins, mitochondrial proteins and with fluorescence microscopy have been developed to measure autobiographic activity but there are several factors that may affect the results of the examination and still require inhibitor control as in the Western Blot autophagy detection examination method. Flowcytometry and image-based cytometry examinations are a new breakthrough in detecting autophagy, but the advantages and disadvantages of each method should be considered to obtain accurate analysis results.

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