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ROLE OF COMPLEMENT IN ACTIVATION OF MACROPHAGES

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INTRODUCTION

When microbes penetrate the defensive barriers of the host, it faces the most essential element of the innate immune system called complement, which is a group of proteins that are found in the blood circulation and other soluble substances in the body, and it is produced in large quantity by the liver (Janeway et al., 2012, Bohlson et al., 2014). It was discovered by Jules Bordet in the 1890 "as a heat-labile component of normal plasma" (Janeway et al., 2012). It also plays an important role in protecting the body from intracellular and extracellular pathogens as well as its participation in the adaptive immune response (Haskard et al., 2008).

Complement activation

Complement system is composed of many plasma proteins that in normally inactive form in the blood (Abbas et al., 2012). Such proteins interact with each other to generate other components which their function is eradication pathogen (Janeway et al., 2012). Microbes and antibodies that are adhered to antigens can activate the complement system (Abbas et al., 2012). Complement activation includes the degradation of proteins in order to create enzyme complexes with proteolytic effectiveness (Abbas et al., 2012). The products that are produced by complement activation either binds covalently to microbial membrane or to antibody coated with antigen (Abbas et al., 2012). However, regulatory proteins, that present normal in the human body and does not exist in microbes, can inhibit the complement activation (Abbas et al., 2012). In addition, complement system can be activated by different pathways (Abbas et al., 2012). Firstly, classiscal pathway is considered the most important mechanism in the adaptive immune system because the immunoglobulin M can bind efficiently with C1q (Abbas et al., 2012). Also, It can be activated by the presence of the C1q protein that is able to recognize microbes by either direct binding to their surface or indirect by attaching to the

Fc region of IgG or IgM coating antigen and resulting in stimulation of other serine proteases (C1r and C1s). When these proteins become active, it cleaves other proteins by protease enzymes (Abbas et al., 2012). Secondly, lectin pathway is stimulated by mannose binding lectin (MBL) or ficoline which recognizes the presence of carbohydrate structures on microbial surface (Janeway et al., 2012). When MBL binds to the target cell, the two zymogens MASP1 and MASP2 that are conjugated with MBL initiate the proteolytic steps (Janeway et al., 2012).

In addition, alternative pathway is activated by binding complement C3 directly to the specific compounds such as LPS that present on the surface of microbes (Abbas et al., 2012). Interestingly, C3 is characterized by its activity in solution, because It can bind to pathogen membrane, even if at low concentration (Abbas et al., 2012). Moreover, these three pathways have similar target which is generating the C3 convertase (Janeway et al., 2012). This enzyme has the ability to cleave the C3 to C3a and C3b, and then creating the C5 convertase which is considered the initiating point of the terminal pathway (Janeway et al., 2012). C5 convertase breaks down C5 protein to a small part C5a and a large part C5b, and this process persists in activating other complement proteins until creating a membrane attack complex (MAC) that degrades the cell wall of microbes (Janeway et al., 2012).

Macrophage and Complement receptors

Macrophage is a monocyte cell characterized by its rapid recruitment at the site of injury (Abbas et al., 2012). It has different names according to their location in the body (Abbas et al., 2012). For example, in the bone, they are called osteoclasts, Alveolar cells in the lung, Kupffer cells in the liver and microglial cells in the brain (Abbas et al, 2012). In addition, Activated macrophages perform different important functions. Firstly, macrophages are able to ingest the microbes in vesicles by the process of phagocytosis and demolish them (Abbas et al., 2012). Secondly, macrophages are responsible for producing pro-inflammatory mediators such as IL-6 and TNF-α that attach to the receptors of other cell which possess a defensive roles (Abbas et al., 2012). For instance, some cytokines promote vascular endothelial cells to recruit a large number of monocytes from the blood to site of inflammation (Abbas et al., 2012).

Furthermore, macrophages are considered APCs that are similar to other antigen presenting cells (APCs) (Abbas et al., 2012). This is because they perform an important function, which is presenting the antigen to and activating T lymphocytes, and they also carry out a

significant role in repairing the damaged tissue by catalyzing the growth of new blood vessel (Abbas et al., 2012). Furthermore, there are several numbers of complement receptors that are located on the surface of macrophage receptors namely, CR1(CD35), CR3 (CD11b/CD18), CR4 (CD11c/CD18), Scavenger receptor, C5aR1, C5aR2, C3aR, C3a-desArg, gC1qR and cC1qR (Bohlson et al., 2014). This essay will present the how complement activates macrophage in terms of opsonization and phagocytosis and regulate the macrophage production of cytokines.

Complement opsonine regulate macrophage activation

Oposonization and phagocytosis

C3b and iC3b, which generated from cleaving of C3 component, have an ability to capture and opsonize the microbes(Bohlson et al., 2014). When the microbes enter the body, it activates the complement pathways to generate C3b, C3b and C4b (Abbas et al., 2012). C3b exists highly in the serum more than any other components (Abbas et al., 2012). C3b and iC3b bind covalently to the surface of microbes and perform as opsonine by binding to their receptors on macrophage (Abbas et al., 2012). For example, CR1 receptor, which a single chain transmembrane protein, attaches to C3b and C4b, whereas CR3 and CR4, are heterodimer of the 95KDa-β chain and 165-KDa-α chain, binds to iC3b (Sutterwala et al., 1996). CR1 alone is unable to catalyze "the phagocytosis of C3b coated microbes", yet it is considered very efficient in promoting this process when it binds with antibody coated microbes (Abbas et al., 2012).

When the macrophage is activated by complement it releases IFN-γ in order to activate itself and promotes CR1-mediated phagocytosis (Abbas et al., 2012). It also releases enzymes known as oxidative enzymes that alter molecular oxygen into reactive species (ROS) such as superoxide radical that can kill pathogens (Abbas et al., 2012; Hartung & Hadding, 1983). Human monocytes can easily ingest the complement coated particles that possess fibronectin (FN), because this protein stimulates the C3b and iC3b receptors (Wright et al., 1983). Furthermore, iC3b and C3b-dependent phagocytosis are the main components in the innate and adaptive immunity to protect the body from the bacteria, such as *pneumococci* and *meningococci* (Abbas et al., 2012).

C1q promotes phagocytosis of apoptotic cell by macrophages

Phagocytosis of dead cell in short time is tremendously important to maintain the balance of the healthy tissue (Lillis et al., 2008). It plays an important role in resolving "the

inflammatory state" and contributes in immune response (Lillis et al., 2008). Remarkably, it has been found that C1q contributes to enhance this process, however the mechanism how it recognizes and enhance the ingestion of apoptotic cells is still unclear (Lillis et al., 2008). Further, C1q, which falls under classical complement cascade, is one of the defense collagen family, namely "C1q, ficolin, the collectins MBL, adiponectin and pulmonary SP-A and SP-D" (Galvan et al., 2012b). These collagen group are discriminated by their capability to promote the phagocytic action of macrophage, monocyte and dendritic cells (Lillis et al., 2008). They also have a similar characteristic to C1q by possessing a carboxyglobular head region and elongated N.terminal collagen like sequences. C1q complement is hexamer of trimers composed of monomers that consist of amino terminal globular domains and carboxy terminal domain in per monomer, and Gathering six trimers together creates C1q molecule (Janeway et al., 2012). Structurally, MBL is similar to C1q, they serve as "bridge molecule" which their spherical heads bind to apoptotic cell, whereas their collagen like tail connects dead cell to macrophage by binding to their receptors on phagocyte and send signal to promote ingestion. (Lillis et al., 2008).

In addition, apoptotic cell (AC) can be opsonized directly by C1q for ingestion (Lu et al., 2008). It is distinguished by having Calreticulin (CRT) in a small blisters that present on its surface (Lu et al., 2008). Calreticulin is a protein that presents inside the cell particularly in the endoplasmic reticulum and on the outer layer of macrophages, and also it is named collectin receptor (cC1qR) (Vandivier et al., 2002). In order to opsonize AC and promote phagocytosis, C1q has to adhere to CRT and then interact with CD91, which is called 2-macroglobulin receptor or Lipoprotein receptor- related protein 1 (LRP-), on the phagocytes (Lue et al., 2008). Vandivier et al (2002) demonstrated that "C1q and MBL drive apoptotic cell uptake through engagement of cell surface Calreticulin and CD91". They said that C1q or MBL opsonizes the dead cells and then adheres to cC1qR by their collagenous tail, and when it becomes conjugated to CD91 it transmit an intercellular signal to begin phagocytosis.

On the other hand, some studies reported that deficiency in LRP receptor on the macrophage has no effects on the phagocytosis process (Lillis et al., 2008., & Bohlson et al., 2014). This because it participates only in ingestion of particles that possess LRP ligand on their outer layer, and it is not related to apoptotic cell phagocytosis (Lillis et al., 2008). As a result of this, C1q does not need LRP to enhance phagocytosis, but it has capability to control the production of Mer tyrosine kinase from macrophage in order to enhance the

phagocytosis (Galvan et al., 2012a). Galvan et al (2012a) demonstrated that C1q possesses proteins such as Gas6 (growth arrest- specific 6) which can bind to both phosphatidlserine. This component exists on the surface of apoptotic cells and tyrosine kinases, including Tyor3, Axl and Mert that are produced by macrophages in order to ingest the apoptotic cells (Galvan et al., 2012a). They Further demonstrate in their experiment that protein expression that are stimulated by C1q are requested for apoptotic cell ingestion. They performed experiment in vitro by taking macrophage from a mouse that has tyrosine kinase deficiency (Mertk-/-) and wild type. These two types of macrophages are exposed to C1q to see what extent their ability in response to C1q and express Mer protein. They noticed that macrophage that possesses tyrosine kinase deficiency lost its capability in both response to C1q and secretion Mer.

Additionally, Benoit et al (2012) state that C1q attaches to of apoptotic lymphocyte (ALs) and promotes their ingestion by HMDMs (human monocyte-drived macrophages). They performed an experiment by taking lymphocyte and monocyte from human and incubated with C1q coated ALS. They found that "C1q binds with the same efficiency to EALs (early apoptotic lymphocytes) and LALs (late apoptotic lymphocytes) and enhances their uptake by human HMDMs". Moveover, It serves as immunoregulator and immunosuppressive to control the cytokins, chemkines, gene expression and action of inflammsome of HMDMs. They further demonstrated that when C1q binds to EALs and LALs it elevates the production of CX3CL1, IL-13 and IL-37 and reduces the production of M1- associated cytokines CXCL9 in HMDM. Also, It can assist to produce IL-10, IL-27 and interferons by activating TLR (Toll like receptor) on macrophage (Benoit et al., 2012).In addition, scavenger receptor which presents on the macrophage surface has an important role in clearing the apoptotic cell by binding to C1q complement (Ramirez- Ortiz et al., 2013). Deficiency of C1q probably causes autoimmune disease such as systemic lupus erytheromatosus and atherosclerosis (Ramirez- Ortiz et al., 2013). Therefore, it seems that there is agreement that C1q contributes and enhance the phagocytosis of apoptotic cell but the precise mechanism is still unclear.

Complement anaphylatoxins regulate macrophage production of cytokines

C3a and C5a are considered an important pro-inflammatory cells (Grailer et al., 2013). C3a is produced from the division of C3 into C3a and C3b by means of C3 convertase, while C5a manufactured from binding C3b with other components to create an enzyme namely C5

convertase which cleaves C5 into C5a and C5b (Grailer et al, 2013). Moreover, C5a has ability to control the production of IL-17and IL-23 that produced from T cells and macrophage (Grailer et al., 2013). IL-23 has an efficient role in promoting the production of IL-17 (Grailer et al., 2013). This enzyme (IL-17) partakes progression of several diseases such as autoimmune, sepsis, ischemia- reperfusion injury and acute lung injury (Grailer et al., 2013). This because it possesses a crucial role in promoting the production of cytokines and chemokines, namely IL-6, TNF, IL-1β, IL-8 and CCL2 by conjugating to its receptor that presents on the surface of leukocytes and endothelial cells(Grailer et al., 2013). However, it has been shown that C5a can reduce the secretion of IL-17 and IL-23 indirectly by binding to its receptors, including C5aR & C5L2 and activate macrophage to release IL-10 that "reduces the IL-17 and IL-23 in an autocrine /paracrine manner" (Grailer et al., 2013). For example, C5a serves as a negative regulator to control the secretion of IL-17 in asthma and leads to decrease airway hyper-responsiveness, while in autoimmune arthritis, it induces "tissue macrophage" to secret IL-6 and "drive TH17 differentiation" (Grailer et al, 2013).

In addition, Yan et al (2011) demonstrated that C5a plays a critical role in enhancing the production of cytokines and chemokines from macrophage by activating IgG IC (immune complex). Activated IgG IC stimulates the expression of C/EBPβ (CCAAT/enhancer-binding protein β) and C/EBPδ (CCAAT/enhancer-binding protein δ) to elevate secretion of TNF-α, MIP-2 and MIP-1 α in alveolar macrophage, and it has been noticed that lack in C5aR had no effect on NF-κB DNA- binding activity (Yan et al, 2011). In addition, C5a is able to regulate the production of inflammatory mediator in cells (Yan & Gao., 2012). For instance, in peripheral blood mononuclear cells C5a has a crucial role in synthesizing and secreting TNF-α, IL-1β and IL-6, and also it may participate indirectly in producing IL-17F in mouse peritoneal macrophages (Yan & Gao., 2012). This is because it probably activates the LPS through promoting "Akt phosphorlation in MyD88-dependent manner"(Yan & Gao., 2012). Moreover, C5a has been described as an effective regulator because it acts important functions in vivo (Yan & Gao., 2012). It is considered a positive regulator in sepsis due to its ability to regulate the level of IL-17F (Yan & Gao., 2012).

Furthermore, Soruri et al (2003) report that C5a can stimulate the recruitment of monocytes and differentiation into dendritic cell, because it is able to recruit and stimulate the leukocytes and monocytes to the site of injury through interacting with its receptor (C5aR) that is presented on the surface of mononuclear phagocytes (Soruri et al., 2003). They further

demonstrate that monocytes release IL-1β, IL-6 and TNF-α a enzymes**.** Additionally, macrophages, that are activated by binding C3a with C3aR, can release a significant amount of thromboxane A2 (Hartung et al, 1983). C3a with lipopolysacchraid modulate the production of IL-1β in human monocytes, macrophages and dendritic cells (Asgari et al., 2013). It regulates the influx of ATP from intracellular to extracellular space by mediating extracellular signal regulated kinase 1/2 (ERK1/2) (Asgari et al, 2013). IL-1β that produced from monocytes through binding with C3a, can induce Th17 response (Asgari et al, 2013).

CONCLUSION

Complement system is one of the major component of the innate immune system which is needed for defending against foreign bodies that enter the body. It can be activated by three different ways, including classical, lectin and alternative pathway. Moreover, complement components activate macrophage either directly by binding to their receptors or indirectly by stimulating the macrophage to produce cytokines to activate itself. Further, C3b is a major complement protein that present in considerable amount in the blood. It opsonizes the pathogens and enhance phagocytosis process by binding to CR1 receptor on the surface of macrophages. C1q promotes phagocytosis of apoptotic cells by macrophage. It binds apoptotic cell to macrophage through binding its globular head to dead cell, whereas collagen tail attaches to C1q receptor, and if there is deficiency in this component, the dying cell will not be eliminated, result in autoimmune disease. In addition, C3a and C5a catalyze macrophage to produce cytokines such as IL-6, TNF-α and IL-1β and IL-10. Moreover, the studies which carried out recently showed that there is a strong relationship between complement and macrophage. The mechanism how complement activates macrophage is still unclear. Therefore, This issue requires more studies with greater accuracy in the future.

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