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Review Article

Role of vascular permeability and its signaling cascade in inflammation

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Abstract

Endothelial hyperpermeability is associated with pathogenesis of a several diseases including diabetes, atherosclerosis, and sepsis. The mechanism underlying this process is the existence and excessive leakage of plasma rich protein fluids across the endothelial barrier. The maintenance of barrier function is critical to maintain the role of vascular endothelium as a barrier. However, a number of inflammatory mediators are found to contribute towards endothelial dysfunction. The key mechanisms that lead to defective endothelial barrier function are disintegration of endothelial cell-cell junctions as well as remodeling of action cytoskeleton, resulting in the formation of intracellular gaps that mediate transendothelial migration. Great progress has been made in understanding the underlying processes that include activation of intracellular signaling molecules involved in endothelial barrier disturbance. Thus, by seeking an agent which targets the signaling molecules that disrupt the endothelial-junction-cytoskeleton, may help to establish a therapeutic potential to enhance endothelial barrier function during inflammation.

Keywords: inflammation, endothelial hyperpermeability, endothelial barrier

1. Introduction

Inflammation is a nonspecific response of a tissue evoked by injury or infection. It is an essential immune response as well as first line of host defence system against the entry of foreign invaders. Pathologically, inflammation is defined as the local infiltration and activation of leukocytes and it is a complex process which involves the interaction between cell surface receptors, extracellular matrix, and inflammatory mediators (Eming, Krieg, & Davidson, 2007). Numbers of inflammatory mediators have been identified and they can be categorized into few groups based on their biochemical properties. They can act either locally or systemically by binding of inflammatory mediators to the cell surface receptors resulting in activation of endothelial cell or perturbed state, which then disrupt the endothelial barrier function that is characterized by increased endothelial permeability (Puerta-Guardo, Glasner, & Harris, 2016). In addition, mediators released from endothelial cells, such as nitric oxide and reactive oxygen species, further contribute to the nature and progression of inflammatory responses. Expression of adhesion molecules and disruption of adherens junctions can be characterized as one of the hallmarks of endothelial activation. However, uncontrolled inflammation could lead to detrimental effects on the vascular endothelium. Endothelial barrier function will be disrupted and thus promotes vascular inflammation, which is one of the hallmarks of numerous cardiovascular diseases including stroke, hypertension (Brades, 2014) and atherosclerosis (Steyers & Miller, 2014). Numbers of endogenous vascular protecting agents have been identified, for instance activation of protein kinase A (PKA) and cyclic adenosine monophosphate (cAMP) (Bogatcheva, Zemskova, Kovalenkov, Poirier, & Verin, 2009). Thus, targeting the signalling molecules that disrupt the endothelial-junction-cytoskeleton establishes a therapeutic potential to enhance endothelial barrier function during inflammation.

2. Mediators of Inflammation

The inducers of inflammation (e.g. physical trauma, chemicals and aggressive agents), which also known as stimuli, initiate the inflammatory response in the body which can be distinguished by dilatation of blood vessels, increased

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permeability, excessive accumulation of fluid into interstitial space, migration of immune cells to the target tissue and swelling formation (edema) (dos Santos Temponi *et al*., 2012). Binding of inducer to their respective receptor triggers the release of numerous inflammatory mediators. Inflammatory mediators are the molecules that act locally or can be systemically (Rankin, 2004). These mediators activate a series of signaling cascade, which in turn alters the functionality of the tissues or organs to be adapted to the conditions indicated by the particular inflammatory mediators (Medzhitov, 2008).

These inflammatory mediators can be either derived from plasma proteins or released by immune cells. For instance, histamine and serotonin are secreted by mast cells and platelets. Others are released as inactive molecules and circulate in the plasma. Apart from that, some mediators are produced directly by specialized leukocytes, mainly macrophages and mast cells, during the acute-phase response (Kreuger & Phillipson, 2016).

Inflammatory mediators can be categorized into seven groups according to their biochemical properties: chemokines, cytokines, fragments of complement components, lipid mediators, proteolytic enzymes, vasoactive amines and vasoactive peptides (Medzhitov, 2008).

In response to intrinsic and extrinsic stimuli, firstly, the chemokines, protein molecules or signaling proteins, will be produced by cells that influence the immune system. They are responsible for the cell trafficking, such as recruitment of leukocytes and chemotaxis towards the affected tissues (Medzhitov, 2008).

Second, inflammatory cytokines (TNF-α, IL-1 and IL-6) are small glycoproteins and are released by a number of cell types, predominantly macrophages and mast cells. Cytokines play a pivotal role in both acute and chronic inflammation. This latter group can be subdivided into two: cytokines mediating humoral response (IL-4, IL-5, IL-6, IL-7, and IL-13) and cytokines mediating cellular response (IL-1, IL-2, IL-3, IL-9, IL-10, IL-12, interferons and TNF-α) (Feghali & Wright, 1997).

Third, complement peptides or more commonly termed as anaphylatoxins, are fragments that generated during the activation of complement system. Complement components such as C3a, C4a and C5a, exert a number of effects in inflammatory response. During an acute inflammatory response, they act as potent chemo-attractants for the recruitment of immune cells such as phagocytes (neutrophils, monocytes) to sites of inflamed tissues (Iqbal *et al*., 2013). They are also able to initiate smooth muscle contraction. Complement components trigger the release of histamine from mast cells and involves in the production of cytokines (Sarma & Ward, 2011).

Fourth, lipid mediators are bioactive molecules that are produced via specific biosynthetic pathways in response to extracellular stimuli. They are derived from metabolism of phospholipids and arachidonic acid released from plasma membrane (MurakaMi, 2011). The generation of arachidonic acid by activated cells involves one of two pathways: 1) from the cell membrane phospholipids (in particular, phosphatidylcholine) through activation of phospholipase A_2 (PLA_2), 2) cleavage from diacylglycerol (DAG) by diacylglycerol lipase (Medzhitov, 2008). Once generated, arachidonic acid is further metabolized through two processes: 1) cyclooxygenation (COX1 and COX2), which form the end products of pros-taglandins and thromboxanes, 2) lipoxygenation (lipoxygenases), which produce leukotrienes and lipoxins (Khanapure, Garvey, Janero, & Gordon Letts, 2007). Lipid mediators play essential roles in distinct phases of inflammatory responses with prostaglandins PGE_2 and PGI_2 promoting vasodilation and vascular permeability. Lipoxins may amplify or reduce inflammation by coordinating the production of cytokines, formation of antibody, cell proliferation and migration (Romano, Cianci, Simiele, & Recchiuti, 2015). In addition, they also control the tissue repair process. Another potent inflammatory mediator biosynthesized from cell membrane phospholipids is platelet-activating factors (PAF). During inflammatory response, PAF are generated by acetylation of lysophosphatidic acid and exhibit a wide range of activities. PAF stimulate recruitment of leukocytes, platelet aggregation and degranulation at the sites of inflamed tissues, and as increase the vascular permeability by releasing high concentration of serotonin lipase (Medzhitov, 2008).

Fifth, proteolytic enzymes (including chymotrypsin, cathepsin and matrix metalloproteinases) were reported to have anti-inflammatory activity and vital for the control of inflammation (Viswanatha Swamy & Patil, 2008). These mediators exert many biological activities in controlling inflammation, including cell apoptosis, tissue remodelling and host defence (Sharony *et al*., 2010).

Sixth, vasoactive amines, histamine and serotonin, are generated in granules and released by mast cells and basophils (Theoharides *et al*., 2012). Histamine and serotonin have been established to produce a number of the effects of inflammation, including vasodilation, edema and increased vascular permeability. The immediate release of histamine and serotonin by mast cells can be highly detrimental, resulting in life-threatening anaphylactic shock, uticaria (hives), wheal and flare reactions (Jutel, Blaser, & Akdis, 2006).

Seventh, vasoactive peptides (e.g. substance P) can be stored in vesicles or formed by proteolytic processing of a protease in the extracellular fluid (e.g. kinins and fibrin degradation products). Substance P is secreted by sensory neurons and inflammatory cells such as macrophages, eosinophils and lymphocytes and is known to elicit vasodilation and alter vascular permeability (O'Connor *et al*., 2004). Other vasoactive peptides are generated through activation of Hageman factor. Thrombin and plasmin have profound, direct effects on inflammatory and endothelial cells by inducing vasodilation and increased vascular permeability (Medzhitov, 2008). As mentioned earlier, activation of Hageman factor is dependent on both high molecular weight (HMW) kininogen and plasma prekallikrein (Meier, Pierce, Colman, & Kaplan, 1977). It has dual functions as both a sensor of vascular damage and an inducer of inflammation. Activation of Hageman factor results in kallikrein–kinin system activation, which catalyses the formation of bradykinin. Release of bradykinin is believed to contribute to increased vascular permeability and act as a potent mediator in pain perception (Renné, Schmaier, Nickel, Blombäck, & Mass, 2012; Greenbaum, 1997).

3. The Endothelial Barrier and Inflammation

The barrier function is the central nature of the endothelium. The endothelium forms the interface between blood and interstitial space and functions as a physical barrier to control the passage of blood constituents to the surrounding tissues and forms a transendothelial protein gradient that is required for tissue fluid homeostasis (Fischetti & Tedesco, 2006). The endothelium maintains the transport of solutes and

controls the flux of tissue fluid across the vessel wall via two different routes. Apart from that, the endothelium restricts passage of macromolecules such as proteins and lipids to the tissues as well as regulates the transmigration of cells into the tissues (Bubik, 2009).

Generally, endothelial transport can be divided into two common pathways; paracellular and transcellular pathways (Komarova & Malik, 2010). The transcellular pathway is responsible for the active transport of high-molecular-weight molecules such as proteins and lipids across the barrier in the resting state. However, in response to intrinsic and extrinsic stimuli (in context of inflammation), unrestricted passage of plasma proteins, solutes and fluid is allowed across the endothelial barrier via the paracellular route (Mehta & Malik, 2006). The paracellular pathway is maintained by the interendothelial junctions, which connects adjacent endothelial cells into a monolayer restricting the leakage of macromolecules from the blood vessel to the interstitial space. Two major types of interendothelial junctions present in the endothelium, tight junctions (TJs) and adherens junctions (AJs). Both of the protein junctions play an important role in the maintenance of the endothelial barrier. TJs are composed of occludes and claudins, which directly limit paracellular permeability (Schlegel & Waschke, 2014). On the other hand, the major components of AJs composed the vascular endothelial (VE)-cadherin complexes with a family of catenins, which are present dominantly in most blood vessels (Bazzoni & Dejana, 2001; Komarova & Malik, 2010). They provide mechanical strength to cohesion between endothelial cells thus enhancing the barrier stabilization (Dejana & Orsenigo, 2013).

Disruption of the interendothelial junctions upon the binding of inflammatory mediators to their receptors has been shown to cause the loss of barrier function, leading to excessive leakage of macromolecules and accumulation of fluid in the interstitial space (Figure 1). For instance, interferon-gamma (IFN-γ), an inflammatory mediator and a cytokine, increases endothelial permeability by activating p38 MAP kinase and altered the actin cytoskeleton (Ng *et al*., 2015). Other than that, a recent study documented that interleukin 4 (IL-4) disrupted the endothelial barrier functions via the Wnt5A signaling cascade (Skaria, Burgener, Bachli, & Schoedon, 2016). As a consequences of the above-mentioned pathologic condition, the endothelial permeability increased and is a hallmark of inflammation-induced diseases such as atherogenesis, sepsis and myocardiac infarction (Bubik, 2009).

4. Inflammation-activated Endothelial Dysfunction

Endothelial dysfunction is characterized by the changes in the actions of the intact endothelial barrier toward a pro-inflammatory state, vasodilation. It is associated with most inflammation-induced diseases such as atherosclerosis, diabetes, myocardial infarction and sepsis (Su, 2015). Free radicals can cause a change in the balance of nitric oxide (NO) by promoting excessive production of NO, leading to the collapse and overly permeability of the endothelium, allowing leakage of macromolecules and fluids into body tissues. Once the endothelium is damaged and the action of NO is inhibited, the endothelial signalling becomes impaired, resulting in the development of widespread diseases (Rajendran *et al*., 2013).

5. Signalling of Endothelial Activation

5.1 Nitric oxide

Nitric oxide is a short-lived, soluble free radical that is continuously synthesized in the endothelium in response to the activation of nitric oxide synthase (NOS). The major isoforms of NOS involved in inflammatory responses comprise: endothelial (eNOS), inducible (iNOS), and neuronal (nNOS). Once NOS is activated, catalytic activity of amino acid L-arginine (Arg) occurs, which is then converts and generates copious amounts of citrulline and NO (Yuan & Rigor, 2010).

NO plays a pivotal but complicated role in regulating the endothelial barrier function. The influence of NO has complex effects on endothelial permeability depending upon the organs or tissues examined in an endothelial model system and the type of stimulus (Hatakeyama *et al*., 2006). NO functions to regulate vascular tone and vasorelaxation in response to increased blood flow when the concentration is at basal level. However, hyper-production of NO in response to proinflammatory stimuli causes destabolization of adherens junctions and

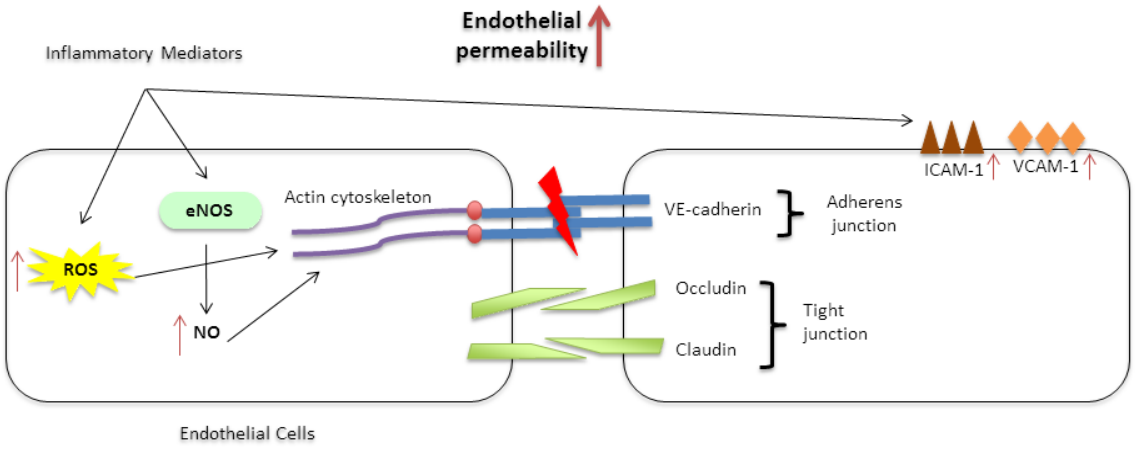

Figure 1. Schematic of inflammatory mediators-induced increased endothelial permeability. Inflammatory mediators could increase endothelial permeability via production of ROS and NO, which in turn results in disorganization of the endothelial barrier. In addition, it also leads to adherens molecules such as ICAM-1 and VCAM-1 being highly expressed.

leads to increased endothelial permeability (Guequén *et al*., 2016; Marin *et al*., 2012). NO may act as a potent inducer in stimulating endothelial hyperpermeability in microvascular beds (Aramoto, Breslin, Pappas, Hobson, & Durán, 2004; Breslin, Pappas, Cerveira, Hobson, & Durán, 2003). In contrast, NO can also elicits barrier-protective effects in endothelial cells and in some organs such as skin, kidney and intestine by inhibiting the activation of NOS (Kurose, Wolf, Grisham, & Granger, 1994).

5.2 Reactive oxygen species (ROS)

When endothelial cells are activated, the production of reactive oxygen species (ROS) is accelerated during inflammation. ROS are reactive metabolites of oxygen and are well recognized for serving complex signalling functions in both deleterious and beneficial manners. Under physiological conditions, ROS function as signalling molecules that regulate cell growth, cell adhesion, differentiation and cell apoptosis (Zhou, Shao, & Spitz, 2014). However, an elevated ROS level is implicated as potent mediators of inflammation-induced endothelial barrier dysfunction. Many different mechanisms are responsible for causing barrier failure in endothelial cells including phosphorylation of catenins followed by disassembly of VE-cadherins (Herron, Lowery, Hollister, Reynolds, & Vincent, 2011), reorganization and remodelling of actin-myosin cytoskeleton (Ponce *et al*., 2016), enhanced leukocytes adhesion and transendothelial migration (Yuan, Shen, Rigor, & Wu, 2012).

5.3 Adhesion molecules

The endothelial expression of cell surface adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), is upregulated on the surface of endothelial cells after exposure to various inflammatory cytokines such as TNF-α and IL-6. This process is defined as endothelial cell activation (Liao, 2013). The upregulation of ICAM-1 and VCAM-1 expression mediates firm adhesion of circulating leukocytes to the vessel wall and facilitates transendothelial migration of leukocytes (Albelda, Smith, & Ward, 1994). Activation of ICAM-1 for instance, increases endothelial permeability through involvement of actin cytoskeletal rearrangements (Etienne-Manneville *et al*., 2000) as well as phosphorylation of the adherens junction components (Allingham, van Buul, & Burridge, 2007).

6. Endothelial Barrier Protective Agent: Cyclic Adenosine Monophosphate (cAMP)

Cyclic adenosine 3´,5´-monophosphate (cAMP) is a universal intracellular second messenger which is catalysed from the conversion of ATP by adenylyl cylclase upon activation G-protein coupled receptor (GPCR). Elevation of cAMP levels has been well recognized for its protection of endothelial barrier by enhancing the vascular permeability (Aslam *et al*., 2014). Several reports have been suggested for different mechanisms for the action of cAMP in improving the endothelial barrier structure. First, activation of protein kinase A (PKA) by increased cAMP levels, which induces the contractile force and formation of cortical actin to stabilize the actin cytoskeleton, thereby preventing intracellular gap formation between adjacent cells (Baumer, Drenckhahn, & Waschke, 2008). Second, activated PKA supressed MLCK activity, which results in reduction of MLC phosphorylation and minimises actomyosin contractility (Lampugnani, Resnati, Dejana, & Marchisio, 1991). The increased cAMP levels also result in stabilization of adheren junctions by inhibiting hydrolysis of membrane phospholipids, thus reducing DAG production and PKC activity (Kumar, 2009). However, elevated intracellular calcium, Ca^{2+} may decrease the production of cAMP dramatically (Yuan & Rigor, 2010). Moreover, Fischmeister (2006) and Sayner's team (2006) reported that cAMP could lead to either barrier stabilization or barrier breakdown depending on the site of the production. It was documented cAMP synthesis in the membranous compartment enhances endothelial barrier function, while synthesis outside of the membranous compartment cause destabilization of the barrier function (Sayner, Alexeyev, Dessauer, & Stevens, 2006). Recent data showed that cAMP play an important role in restoration of barrier function after the endothelium has been challenged with thrombin which is associated with Rac1 activation (Aslam et al., 2014).

7. Conclusions

Alteration of endothelial barrier contributes to numerous pathological conditions, such as edema, artherosclerosis, and even cancer. In addition, it is also a significant problem encountered when coming to the aspect of clinical treatments. Extensive studies have been reported according a better understanding of the mechanisms underlying the endothelial hyperpermeability. Moreover, therapeutic strategies have also been developed to control the barrier function. However, many questions and signaling pathways remain to be elucidated. Future studies are needed to fully comprehend the changes that take place in the endothelial barrier structure and function during inflammation. Within this context, the discovery of a potent anti-hyperpermeability drug needs to be developed. A successful strategy should enhance the barrier function while protecting other tissues from undesirable phenomena.

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