Pharmacological Research 108 (2016) 46–56

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

Modulation of endothelial function by Toll like receptors

Beatriz Salvador[a,*], Alicia Arranz[b], Sara Francisco[a,b], Laura Córdoba[a], Carmen Punzón[a], Miguel Ángel Llamas[a], Manuel Fresno[a,b]

[a] Diomune SL, Parque Científico de Madrid, Madrid, Spain
[b] Centro de Biología Molecular "Severo Ochoa", Universidad Autónoma de Madrid, Madrid, Spain

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 30 March 2016
Accepted 30 March 2016
Available online 9 April 2016

Keywords:
Endothelial cell
Immune response
Pattern recognition
Toll-like receptors
Angiogenesis
Endothelial dysfunction

ABSTRACT

Endothelial cells (EC) are able to actively control vascular permeability, coagulation, blood pressure and angiogenesis. Most recently, a role for endothelial cells in the immune response has been described. Therefore, the endothelium has a dual role controlling homeostasis but also being the first line for host defence and tissue damage repair thanks to its ability to mount an inflammatory response. Endothelial cells have been shown to express pattern-recognition receptors (PRR) including Toll-like receptors (TLR) that are activated in response to stimuli within the bloodstream including pathogens and damage signals. TLRs are strategic mediators of the immune response in endothelial cells but they also regulate the angiogenic process critical for tissue repair. Nevertheless, endothelial activation and angiogenesis can contribute to some pathologies. Thus, inappropriate endothelial activation, also known as endothelial dysfunction, through TLRs contributes to tissue damage during autoimmune and inflammatory diseases such as atherosclerosis, hypertension, ischemia and diabetes associated cardiovascular diseases. Also TLR induced angiogenesis is required for the growth of some tumors, atherosclerosis and rheumatoid arthritis, among others. In this review we discuss the importance of various TLRs in modulating the activation of endothelial cells and their importance in immunity to infection and vascular disease as well as their potential as therapeutic targets.

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* Corresponding author at: Diomune SL, Parque Científico de Madrid, C/Faraday 7, 28049 Madrid, Spain.
E-mail addresses: bsalvador@diomune.com (B. Salvador), alicia.arranz@csic.es (A. Arranz), sfrancisco@diomune.com (S. Francisco), lcordoba@diomune.com (L. Córdoba), cpunzon@diomune.com (C. Punzón), mallamas@diomune.com (M.Á. Llamas), mfresno@cbm.csic.es (M. Fresno).

http://dx.doi.org/10.1016/j.phrs.2016.03.038
1043-6618/

1. The endothelium

The endothelium consists of a monolayer of **Endothelial Cells** (ECs) that form the inner lining of the blood vessels (arteries, veins and capillaries) and the lymphatic system. It forms an interface between the lumen and the vessel wall and consequently is in constant interaction with the circulating blood. The endothelium forms a vast network that not only provides a physical barrier to control the vascular permeability, coagulation pathways and vasomotor tone but also serves a multitude of functions that help to maintain organ homeostasis.

The ECs are therefore located at the interface between circulating blood and the surrounding tissue. These unique multifunctional cells are able to react to physical and chemical stimuli within the bloodstream thanks to membrane receptors. These stimuli include growth factors, coagulant, and anticoagulant proteins, cytokines and chemokines, low-density lipoprotein (LDL), nitrous oxide, *etc*. Thanks to this capacity the endothelium plays an active role in the regulation of vascular tone, cellular adhesion and aggregation, vascular smooth muscle migration and resistance to thrombosis. Physiologically, ECs favour a relaxed vascular tone and low levels of oxidative stress by releasing mediators such as nitric oxide (NO), prostacyclin-2 (PGI2) and endothelin (ET)-1 and by regulating angiotensin II activity. The endothelium also regulates vascular permeability, leukocyte adhesion and aggregation and thrombosis and is involved in angiogenesis. Thus, endothelial function is essential for vascular integrity.

Nonetheless, ECs unique location, widespread distribution and constant contact with circulating fluids also justify their key roles during infection and disease. Although ECs are not regarded as classical immune cells, they are critically involved in the inflammatory response and are actively participating in innate immunity. This is mediated through pattern recognition receptors (PRR) contributing to host defence against pathogens and tissue repair [1]. However, their inappropriate activation also contributes to tissue damage during autoimmune and inflammatory diseases.

Endothelial activation refers to any change in the endothelium homeostatic control mechanisms. In response to a variety of stimuli, the endothelium undergoes changes that allow its participation in the inflammatory response [2,3]. This condition is also known as endothelial dysfunction and results in expression of pro-inflammatory chemokines and cytokines (such as interleukin IL-1, IL-6, Tumor Necrosis Factor alpha (TNF-α) and interferon gamma (IFN-γ)), and adhesion molecules (such as ICAM-1, VCAM-1 and E-selectin) designed to interact with leukocytes and platelets and target inflammation to specific tissues and pro-thrombotic factors. It is also accompanied by oxidative stress and abnormal vascular tone modulation. Endothelial dysfunction can be triggered by a wide range of agents including pathogens and cell/tissue damage signals. Endothelial dysfunction has been linked to a variety of disease conditions, including atherosclerosis, systemic autoimmune diseases, diabetes mellitus, coronary artery disease, hypertension and hypercholesterolemia [2–4].

2. Toll-Like receptors in ECs

As previously stated, besides circulating chemokines and cytokines, the functions of vascular ECs are modified by exogenous activators such as infectious agents (virus, bacteria entering the bloodstream) or endogenous triggers like Damage-associated molecular pattern molecules (DAMPs) released by injured or stressed tissues and cells. Toll-Like Receptors (TLRs) recognize infectious components (Pathogen-associated molecular pattern molecules (PAMPs) as well as DAMPs and are fundamental in activating innate mechanisms in ECs [5].

TLRs are a family of highly conserved pathogen recognition receptors (PRRs) first discovered in Drosophila [6]. They contribute to the initiation of the innate immune responses by engagement of pathogens or DAMP ligands liberated from host tissues. They belong to the "interleukin-1 receptor/Toll-like receptor superfamily", a class of PRRs and bind diverse microbial products and injury-induced endogenous ligands [7]. PAMPs include various bacterial components such as lipopolysaccharide (LPS, recognized by TLR4), peptidoglycan (TLR 1, 2 and 6), flagellin (TLR5), and viral double-stranded RNAs (TLR3). DAMPs include heat shock proteins (HSPs), high-mobility group Box 1 protein (HMGB1), ATP, uric acid, cholesterol crystals and host DNAs.

TLR1-10 have been identified in both human and mice whereas TLR 11–13 are only expressed in mice. They can be classified as cell membrane TLRs (TLR1, 2, 4, 5, 6, and 11) and intracellular TLRs (TLR3, 7, 8, 9, 10, and 13) localized in intracellular vesicles including the endoplasmic reticulum, endosome, and lysosome [8,9].

TLRs are key mediators of innate immunity and respond to their ligands following two inflammatory cascades (Fig. 1); 1. activation of myeloid differentiating factor 88-dependent pathway (MyD88), leading to nuclear factor kappa-B (NFκB) and Mitogen-activated protein kinases (MAPK) activation and production of pro-inflammatory cytokines [10]; 2. MyD88-independent downstream signalling pathways (TLR4 and TLR3), which triggers the induction of interferon-β (IFN-β) [11].

While TLRs expression is nearly ubiquitous in immune cells, it is less widespread in cells of non-hematopoietic origin, such as ECs [12,13]. However, all TLRs have been detected in one or another type of ECs (see Table 1). In general, TLR2 and TLR4 are ubiquitously present within the vasculature, whereas TLR7 and TLR9 are sparse, and TLR1, TLR3, TLR5, TLR6, TLR8 and TLR10 are expressed in selective patterns. Gene transcripts for TLR1-10 in ECs present different expression levels depending on the specific cell type [14], which may reflect specialized functions of different ECs [15]. Since ECs are heterogeneous, harbouring a specific phenotype and function depending on the tissue/organ in which they are located it stands to reason that TLR expression is dependent on ECs origin/location and therefore on cell type specific. Human vascular beds including aorta and subclavian, carotid, mesenteric, iliac, and temporal arteries express TLR4 and TLR2 (and its TLR1 and TLR6 co-receptors) while TLR3 is mostly expressed in the aorta, and expression of TLR7 and 9 is limited to the iliac artery [16]. Furthermore, it has been shown in humans that ECs from lymph nodes expressed TLR 1–6 and TLR9 but thymus derived ECs lacked TLR5 [15].

The activation of ECs through TLRs can be direct through interaction with ligand molecules, or may require a prior inflammatory stimuli such as IFN-γ, LPS, TNF-α or IL1b. Besides, activation by cytokines and chemokines can also modulate TLRs expression in ECs. Moreover, there is a crosstalk among TLRs. For example, the expression of TLR2 is low in some ECs but its expression can be up-regulated after TLR4 signalling [13,23]. This is particularly relevant when considering the increasing evidence of negative crosstalk between TLR2 and TLR4 where TLR2 ligands signals specifically block TLR4-TRIF signalling pathway [24]. Differential expression of TLRs in the endothelium may also occur in response for example to high glucose conditions that increase the expression of TLR2 and TLR4 in ECs [25,26].

Interestingly, a particular type of ECs, liver sinusoidal ECs (LSECs) may act as unique antigen presenting cells (APC) to T cells being this activity tuned by TLR2, TLR3 or TLR4 ligands [27]. In addition, TLR activated ECs indirectly regulate local inflammation by inducing IL1b release by monocytes and increasing CD14 expression and macrophage survival [28].

As previously stated, in addition to components from infectious agents entering the bloodstream, TLRs can be activated by endogenous ligands released by injured or stressed cells and tis-