The role of Endothelial cells in innate and adaptive immune responses, and as Potential Anti-Inflammatory therapeutic and Preventive Target in Cancer and Autoimmunity

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Abstract: An effective immune response depends not only on the proper activation, regulation, and function of immune cells, but also on their distribution and retention in diverse tissue microenvironments where they encounter a number of stimuli and other cell types. Endothelial cells represent a highly heterogeneous population of cells with the ability to interact with and modulate the function of immune cells.

The integrity of the endothelial lining of the vasculature is essential for vascular homeostasis and normal organ function. Endothelial injury or dysfunction has been implicated in the pathogenesis of diverse vascular diseases. Studies in vitro have demonstrated that a wide variety of stimuli can induce programmed cell death (apoptosis) of endothelial cells, and have suggested that apoptosis could be an important mechanism of vascular injury, resulting in vascular leak, inflammation, and coagulation.

Allergic disorders commonly involve both chronic tissue inflammation and remodeling caused by immunological reactions to various antigens on tissue surfaces. Recent studies have shed light on the important roles of endothelial cells in the development and exacerbation of allergic disorders.

Previous reports have demonstrated that pro-inflammatory/immune effector cytokines significantly promote endothelial dysfunction while numerous novel anti-inflammatory/immunosuppressive cytokines have recently been identified such as interleukin (IL)-35.

In this article, I describe the role of endothelial cells, in innate and adaptive immune responses, effects of endothelial as anti-inflammatory therapy and mechanisms of endothelial dysfunction as potential therapeutic target in cancer and autoimmune diseases

Key Words: Endothelial cells, Anti-Inflammatory, Immunoregulatory, Cancer and Autoimmunity

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1. Introduction

The endothelium is a highly specialized cellular system that is composed of 1–6 -10^{13} endothelial cells (ECs)[3] lining a total surface area of 4000–7000 m2(1),(2), and plays a key role in physiological processes such as bloodsupply, nutrient delivery, metabolic homeostasis, and immunecell trafficking, as well as pathological processes such as inflammation(3),(4),(5).Inflammation can be seen as a vascular response(6),where ECs become activated, display increased leakiness,enhanced leukocyte adhesiveness, and procoagulant activity,and form new vessels(7).Thus, an immune response resulting in inflammation depends strictly on a permissive microvasculature, which normally exerts the opposite function of preventing the indiscriminate influx of immune cells into a tissue. Compared with large blood vessels, the microvascular bed constitutes the bulk of the overall endothelial surface, covering an area 50 times greater than that of all large vessels combined(8).Major qualitative differences exist between macro- and microvascular ECs, the latter being able to generate a range of mediators, to display distinct adhesion molecule patterns, to activate unique sets of genes, and to form capillaries (9),(10),(11).Much of the information on the contribution of ECs to immunity and inflammation derives from HUVECs(12),butthese cells do not reflect the highly specialized nature of microvascular ECs, and the study of ECs from distinct body compartments has confirmed their heterogeneity(9),(10),(13),(14),(15).This is best exemplified by the differential expression of homing ligands involved in immune cell trafficking. Mad-CAM-1 is expressed by Peyer's patches high endothelial venules to recruit-4-7 homing receptor-positive naive lymphocytes(16).Likewise, ECs from brain, liver, and other organs express distinct surface markers, protein transporters, and intracellular enzymes(15),(17),(18).The mechanisms responsible for EC heterogeneity are unclear, but tissue-specific and transcription factors likely contribute to the induction or maintenance of specialized EC features(13),(17),In addition to its function in leukocyte trafficking, distribution, and homing, recent evidence indicates that microvascular ECs play a far more direct role in immunity. This review will show that the multifaceted properties of the organ-specific microvasculature convert the perceived passive role of ECs to an active one that controls innate and adaptive immunity, coagulation, and inflammation.

2. Structure and fundamental functions of endothelial cells

Blood vessels are composed of a sheet of inner endothelium, which is a monolayer of endothelial cells that surrounds functional cells, including pericytes and vascular smooth

muscle cells, and extracellular matrix (19). The endothelial and supportive mural cells are tightly bound to each other, partly through integrins, thereby maintaining vascular integrity. The endothelium is composed of 1-6 $x10^{13}$ endothelial cells that cover more than 1000 m^2 of surface area throughout the body (20),(2).In the absence of inflammation, vascular endothelial cells serve as an essential barrier between the bloodstream and vesselwalls. In addition to being a physical barrier, endothelial cells have various indispensable functions, which can be classified into three major groups: 1) modulation of metabolic homeostasis (trophic action), 2) control of vascular hemodynamics (tonic action) and 3) regulation of vascular permeability, coagulation and cell extravasation (trafficking) (21).These functions of endothelial cells change during the transition from quiescent to inflammatory conditions. The initial change of the vessels in acute inflammation is characterized by increased blood flow secondary to arteriolar and capillary bed dilation (erythema and warmth)(7),(22).Increased vascular permeability, as a consequence of either widening of interendothelial cell junctions of the venules or direct endothelial cell injury, results in an exudate ofprotein-rich extravascular fluid (tissue edema)(23),(24). Endothelial cells normally maintain a balance between the pro- and anticoagulant activities, but they express numerous pro-coagulant factors in response to injury or inflammatory cytokines. Endothelial injury exposes the underlying subendothelial von Willebrand factor and basement membrane collagen, stimulating platelet adhesion, platelet activation and aggregation. These interactions of platelets and endothelium have a profound impact on clot formation as a defense mechanism. Concurrently, leukocyte recruitment from the bloodstream into the extravascular tissue at sites of pathogen invasion or tissue damage is a multi-step process: 1) loose attachment to and roll-over on the endothelium (mediated by selectins and carbohydrates on endothelial cells), 2) firm and selective attachment to the endothelium (mediated byintegrins and chemokines), 3) migration through interendothelial spaces and 4) detachment from the blood vessel (5),(25),(26). Thus, these structural and functional changes of endothelial cells facilitate leukocyte recruitmentand alter gene expression profiles in inflammatory conditions and are collectively called “endothelial activation” (27).

3. Pathophysiology of endothelial dysfunction

The endothelium maintains normal vascular tone and blood fluidity, with no or little expression of proinflammatory factors under normal homeostatic conditions.However, both traditional and novel cardiovascular risk factors including smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerotic disease are all associated with alteration in endothelial function(28),(29),(30).This results in a chronic inflammatory process accompanied by a loss ofantithrombotic factors and an increase in vasoconstrictor and prothrombotic products, in addition to abnormalvasoreactivity, therefore elevating risk of cardiovascular events(31).More recently,endothelial dysfunction has also been associated with obesity, elevated C-reactive protein, and chronic systemicinfection(32),(33),(34),(35),(36),(37).

4. Oxidative stress and endothelial cell dysfunction

Reactive oxygen species (ROS) are generated at sites of inflammation and injury and at low concentrations can function as signaling molecules participating in the regulation of fundamental cell activities such as cell growth and cell adaptation responses; whereas at higher concentrations, ROS can cause cellular injury and death. The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissues, is a major target of oxidative stress, playing a critical role in the pathophysiology of several vascular diseases and disorders. Specifically, oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesion, which is coupled with alterations in endothelial signal transduction and redox-regulated transcription factors(38).

5. Endothelial Dysfunction

Endothelial dysfunction is a systemic pathological condition which can be broadly defined as an imbalance between vasodilating and vasoconstricting substances produced bythe endothelium or overall functions of the endothelium. Normal functions of endothelial cells include production of nitric oxide (NO), regulation of platelet adhesion, coagulation, immune function, control of volume, and electrolyte content of the intravascular and extravascular spaces. Endothelial dysfunction is primarily due to reduction in NO bioavailabilty, and a marker for vascular health. Endothelial dysfunction can result from and/or contribute to several disease processes, as occurs in diabetes mellitus, hypercholesterolemia and hypertension, and also due to environmental factors, such as smoking tobacco products and exposure to air pollution(39).Specifically, endothelial dysfunction is associated with reduced nitric oxide production, anticoagulant properties, increased platelet aggregation, increased expression of adhesion molecules, increased expression of chemokines and cytokines, and increased reactive oxygen species production from the endothelium(40).These all play important roles in the development of diabetic vascular complications including atherosclerosis and other vascular pathologies. Importantly, endothelial dysfunction has been shown to be of prognostic significance in predicting vascular events(41),(42),so endothelial function testing may potentiate the detection of cardiovascular diseases such as myocardial infarction, peripheral vascular disease, ischemic stroke, and others(43),(44).An important feature of endothelial dysfunction is the inability of arteries and arterioles to optimally dilate inresponse to an appropriate stimulus by vasodilators acting on the endothelium. This endothelial dysfunction is notoriously associated with decreased NO bioavailability, which is due to impaired NO production by the endothelium and/or increased inactivation of NO by reactive oxygen species(45),(46).Reduced NO bioavailability decreases the ability of endothelial cells to execute their functions in regulating vascular tone andgrowth, thrombosis, immune cell responses, and vascular barrier functions.

6. Mechanisms of Endothelial Dysfunction

While it is clear that multiple chronic inflammatory diseases are associated with endothelial dysfunction and cardiovascular morbidity, the mechanistic links between inflammatory diseases and CVD have not been fully elucidated. The role of traditional cardiovascular risk factors in patients with RA and SLE has received considerable attention, though traditional factors alone are insufficient to explain the excess burden of CVD in these populations. It seems likely that chronic inflammation, a shared feature of these diseases, is involved in the pathogenesis of accelerated endothelial dysfunction. Several potential mechanisms are explored

below. The vascular endothelium is known to be a target of TNF-α. On a cellular level, TNF-α induces the expression of genes associated with inflammation, coagulation and proliferation. Decreased nitric oxide (NO) bioavailability appears to be a common and critical step linking TNF-α to endothelial dysfunction. Multiple groups have shown that eNOS protein expression is reduced via TNF-α- inducedinhibition of eNOS promoter activity and mRNA destabilization(47),(48).NO availability is also compromised in the presence of TNF-α secondary to impaired degradation of ADMA, an endogenous inhibitor of NOS. Furthermore, TNF-α induces CAM expression on the surface of vascular endothelial cells. This effect is mediated via isoform one of the TNF-receptor (TNFR1). Activation of TNFR1 leads to increased CAM expression via induction of NF-Kb(48),(49).NO is also known to be an inhibitor of CAM expression(50).TNF-α may therefore lead to increased CAM expression by multiple pathways.The effect of TNF-α on NO availability and subsequent endothelial dysfunction has also beendemonstrated in vivo in both animal and human models. Intravenous delivery of TNF-α in rats leads to impaired endothelium-dependent vasodilation(51).Intra-arterial infusion of TNF-α in humans also impairs local endothelium-dependent vasodilation measured by FBF(52).Non-specific induction of an acute systemic inflammatory response by Salmonella typhi vaccination also causes reduced FBF(53).This effect is mediated by impaired NO bioavailability as demonstrated by rescue of vascular reactivity with the NOS inhibitor L-NNMA (L-NG-monomethyl Arginine)(52).The downstream effects of TNF-α-mediated inflammation are illustrated in an apoE, TNF-α mouse model. Mice deficient in TNF-α develop less athero-sclerosis than those with intact TNF-α expression (i.e., apoE single knockout)(54).This is associated with decreased expression of ICAM-1, VCAM-1 and monocyte chemotactic protein-1 (MCP-1). It is well known that TNF-α plays a critical role in the inflammation associated with RA, SLE, IBD, psoriasis and spondyloarthritis. This common feature is illustrated by the efficacy of anti-TNF-α agents in many of these diseases. Given the central role of TNF-α in the pathogenesis of many chronic inflammatory diseases and its well-characterized effects on the endothelium as described above, it is reasonable to conclude that increased circulating TNF-α is implicated in the induction of endothelial dysfunction and initiation of atherosclerosis in these diseases. This hypothesis is supported by the beneficial effects of anti-TNF-α agents on endothelial function in patients with chronic inflammatory diseases, as discussed later.

7. Innate immunity

The primary function of innate immunity is to recognize pathogen- associated molecular patterns (PAMPs) through “pattern recognition receptors.” Among these, TLRs are surface molecules that trigger signals resulting in proinflammatory gene expression, leukocyte chemotaxis, phagocytosis, cytotoxicity, and activation of adaptive immune responses(55).Several reports have demonstrated TLRs on ECs(56).EC expression of TLR1 is still in question. One report showed TLR1 immunoreactivity in atherosclerotic endothelium(57),while another failed to demonstrate TLR1 expression by human microvascular EC lines in vitro, even though TLR1 transfection inhibited TLR4-dependent signaling(58).TLR2 has been identified on atherosclerotic endothelium, expressed by von Willebrand factor-positive ECs and markedly up-regulated in vascular inflammation(57).Microvascular EClines express low levels of TLR2 mRNA and protein, which are up-regulated upon stimulation with LPS, TNF-_, and IFN-_ in a NF-_B- and MyD88-dependent manner(59),(60),(61).Neutrophil NADPH

oxidase is involved in EC TLR2 up-regulation, as neutropenic mice show decreased endothelial TLR2 expression(61).This indicates a "cross-talk" between polymorphonuclear neutrophils and ECs that would enhance vascular defenses by up-regulating TLR2. Dunzendorfer et al.(60).reported that human coronary ECs are hyporesponsive to TLR2-specific ligands. Given the current belief that TLRs are proatherogenic, flow suppression of TLR2 expression may be atheroprotective. Functional expression of TLR2 may not be universal because human dermal microvascular ECs fail to respond to TLR2 agonists such as *Mycobacterium tuberculosis* 19-kDa lipoprotein or phenol-soluble modulin unless transfected with TLR2(62),(63).TLR3 is spontaneously present on HUVECs, and ligation by poly(I:C) up-regulates its expression together with that of IFN-_, IL-28, IL-29, and STAT1(64).TLR4 expression has been demonstrated on various ECs and significantly increases under inflammatory conditions. TLR4 is expressed in coronary ECs(65),and is overexpressed and colocalizes with the p65 subunit of NF-_B in coronary atherosclerotic plaques, suggesting activation of TLR4 at this site(57).This possibility is supported by the demonstration that LPS activates NF-_B in dermal microvascular ECs and that LPS, IFN-_, and TNF-_ up-regulate TLR4 mRNA and protein(59).LPS stimulation of coronary ECs induces production of IL-6, IL-8, and MCP-1, transcription of IL-1_ and TNF-α mRNA, as well as expression of ICAM-1, VCAM-1, and endothelial leukocyte adhesion molecule-1(66).Neutrophil accumulation appears to depend on TLR4 expression by ECs rather than leukocytes as sequestration of neutrophils in the lung is deeply impaired in endothelial TLR4_/_ mice (67).The latter observation contrasts with the significant decrease of leukocyte binding caused by LPS in human intestinal microvascular ECs, perhaps reflecting a tolerance of ECs to high levels of endotoxin to which they constantly exposed in the gut microenvironment(68).Finally, LPS can directly initiate angiogenesis through TNFR-associated factor 6-dependent signaling pathways(69).Because epithelial bacterial translocation exposes sub-epithelial micro-vessels to bacterial products, Maaser et al.(70),studied the effect of the TLR5 ligand flagellin on HUVECs, human intestinal microvascular ECs, and dermal ECs. They found that all three ECs constitutively expressed high levels of TLR5mRNA and protein, and Salmonella-infected intestinal epithelial cells induced ICAM-1 expression in cocultured ECs. The functional role of EC TLR5 was demonstrated by induction of leukocyte adhesion and transmigration by flagellin, pointing to a previously unrecognized role of endothelial TLR5 in innate Immunity(70).In the only report investigating the expression of TLR7 or TLR8 by ECs, neither TLR was found to be expressed in HUVECs(64).In contrast, TLR9 is spontaneously expressed by mouse and rat lung ECs, and exposure to CpG DNA induces an inflammatory response manifested by IL-8 and ICAM-1 induction through p38 MAPK- and NF-Bmediated pathways(71).Other receptors mediating innate immunity include the nucleotide- binding oligomerization domains (NODs) 1 and 2, two cytosolic proteins that function as sensors for microbial peptides and regulators of inflammation(72).Both NODs have been detected in ECs and are up-regulated in response to LPS and proinflammatory cytokines.HUVECinvasion by Listeria monocytogenes induces IL-8 production, NF- activation, and p38MAPKsignaling in a NOD1-dependent fashion(73).Muramyl dipeptide enhances IL-6 release by ocular ECs spontaneously expressing NOD2 and induces NF-B transcriptional activity in transfected HUVECs overexpressing wild-type NOD2(74),(75).

8. Endothelial-leukocyte interactions

The distribution of leukocytes is tightly regulated by numerous homing and adhesion molecules (receptor and counterreceptor pairs) on the surface of microvascular and immune

cells(76).EC-mediated leukocyte distribution displays specialized features depending on the tissue where lymphoid cells are destined to reside(77).In inflammation, ECs still control the type and number of immune cells that extravasate into the interstitium but in a dysregulated fashion(25).Multiple reviews are available on this subject, and the contribution of ECs to adaptive immunity through leukocyte distribution will not be discussed here. A different type of leukocyte-endothelial interaction relevant to adaptive immunity occurs in the induction of transplantationtolerance. Alloantigen-specific CD8_CD28_ T suppressor cells induce expression of inhibitory receptors anddown-regulate adhesion molecules on ECs, rendering them tolerogenic(78).In addition, alloantigen presentation by EC to CD4_ T cells induces CD4_CD25_Foxp3_ regulatory T cells capable of suppressing proliferation of alloreactive T cells in vitro and in vivo(79).

9. Endothelial-platelet interactions

Platelets normally circulate without attaching to the endothelium,but do so when ECs become activated, and platelet adherence triggers inflammation(80).The molecular pairs allowing adhesion of platelets to endothelium include Pselectin glycoprotein ligand 1/P-selectin, GPIb/von Willebrand factor, GPIb/P-selectin, and GPIIb/IIIa/fibrinogen/ ICAM-1, respectively(81).Recently, EC-derived fractalkinehas also been show to contribute to platelet activation and adhesion(82).Activated platelets produce massive amounts of proinflammatory mediators and cross-talk with and activate different cells; in turn, platelets are activated by EC-derived proinflammatory substances binding to cognate receptors on the platelets' surface(83),(84).Platelets' mediators are kept in the _-granules and dense body systems(85),and are promptlyreleased upon activation, including histamine, serotonin, thromboxane A2, platelet-activating factor, PGE2 and PGD2, TGF-, platelet-derived growth factor, multiple chemokines (RANTES, epithelia-derived neutrophil-activating 78, MCP-3, growth-related oncogene, and MIP-1), IL-1, and thrombocidins, all of which target immune cells(80),(86).Some of these products control vascular tone and permeability, but platelets also release trophic factors for ECs like vascular endothelial growth factor (VEGF), which promotes angiogenesis(87).In addition, platelets release heparanase, causing degradation of extracellular matrix and facilitating leukocyte extravasation(88).In addition to molecules that alter EC function, platelets produce molecules that directly impact on adaptive immunity, like membrane-bound and soluble CD40L, which engages CD40 on the surface of ECs, leading to adhesion molecule up-regulation, chemokine secretion, and leukocyte recruitment(89).In this regard, activated platelets mimic the action of activated T cells, which express and release CD40L(90).In doing so, platelet modulate the immune response by establishing a link between innate and adaptive immunity(91).Finally,CD40 ligation by platelet CD40L not only promotes immune activation and inflammation but also tissue factorinduction and blood coagulation(92).

10. Endothelial cell apoptosis in vivo Detection

Endothelial cell apoptosis in vivo can be assessed in situ by one or more of several techniques, including immuno-staining for endothelial specific markers, detection of DNA strand breaks by terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL), assay for caspase activation, and measurement of phosphatidyl serine exposure by annexin V binding (e.g.Refs(93),(94).As the TUNEL technique can detect DNA breaks associated with proliferation, it should ideally be supported by other assays for apoptosis. Apoptotic cell death, in general, and endothelial cell apoptosis, in particular, may be difficult to observe in vivo as apoptotic

cells are rapidly phagocytosed and their digestion can be completed within 60 min (95).Notably, endothelial cells can engulf apoptotic cells or apoptotic bodies(96),(97).Also, apoptotic endothelial cells may detach from the vessel wall into the circulation. For these reasons it may be difficult to document endothelial cell apoptosis in situ. Consequently, detection of circulating apoptotic endothelial cells or endothelial microparticles has been proposed as an alternative technique to evaluate endothelial cell dysfunction, including apoptosis, in vivo(98),(99).Circulating apoptotic endothelial cells were found in patients suffering from sickle cell anemia(100),and systemic lupus erythematosus (SLE)(101).The determination of whether circulating endothelial cells are apoptotic may be disease specific. For example, at least some of the circulating endothelial cells in sickle cell anemia were show to be viable as they survived for at least 11 days in culture and 66% of the total number of circulating cells were alive when evaluated by fluorescent staining(100).Circulating endothelial cells in acute myocardial infarction and unstable angina were <10% apoptotic as determined by the TUNEL technique(102).Conversely, circulating endothelial cells in SLEwere approximately 89% apoptotic(101).Endothelial cell microparticles have been shown to be elevated in diverse diseases, including thrombotic thrombocytopenic purpura (TTP), lupus, myocardial infarction, and multiple sclerosis(98),(99).However, the circulating microparticles derived from endothelial cells could result from surface blebs on cells as a result of activation rather than apoptosis. Circulating microparticlesfrom patients suffering from congestive heart failure were reported to have elevated phosphatidylserine(103),and others have suggested that these represent release from apoptotic cells(104).

11. The roles of endothelial cells in innate and adaptive immune responses

Allergic disorders commonly involve both chronic tissue inflammation and remodeling caused by immunological reactions to various pathogen-associated molecular patterns (PAMPs) and/orendogenous danger substances released from damaged tissues, i.e., so-called alarmins. Due to their anatomical location, vascular endothelial cells are the final responders to interact with variousalarmins on the epithelial surface. Endothelial cells, as well as epithelial cells, actively participate in both innate and adaptive immune responses, which are crucially involved in the pathogenesisof allergic disorders (105),(106). The primary inflammatory response of tissue cells is initiated byrecognition of various PAMPs and alarmins via pattern-recognition receptors (PRRs); this is called innate immunity. Indeed, vascular endothelial cells spontaneously express class I major histocompatibility complex (MHC) molecules and a wide variety of functional PRRs, including Toll-like receptors (TLRs) and nucleotidebinding oligomerization domain-like receptors (NLRs), as reviewed by Daneseet al.,(107),as well as various cytokine and chemokine receptors. (108),(109).Endothelial cells are also capable of secreting a broad spectrum of cytokines and chemokines in response to stimulation. As a result, endothelial cells participate in the immune response and rapidly produce various inflammatory molecules (e.g., IL-1α, IL-1β, IL-6, IL-8, TNF-α, etc.)(110),(111),that are important for endothelial activation Concurrently, endothelial cells can also produce anti-inflammatory cytokines and chemokines that prevent progression of the inflammatory cascade (112).The inflammatory mediators involved in those inflammatory cascades or released by endothelial cells were reviewed and discussed in depth in a recent review article by Mai et al.(106). In addition, in response to some inflammatory stimuli, endothelial cells express class II MHC molecules that present endothelial antigens to immune cells, leading to long-lasting and highly specific protection, known as adaptive immunity(113),(114),(115).Thus, vascular endothelial cells play

pivotal roles in both innate and adaptive immune responses. Cascade of allergic inflammation regulated by structural cells Allergic inflammation is regulated by complex interactions among several inflammatory and structural cells via inflammatory mediators.A wide variety of mediators, cytokines and chemokinesexerting many different effects on the airways and skin are known to be involved in the pathology of allergic disorders, maintaining chronic allergic inflammation. A well-known cascade of these networks in allergic inflammation is characterized by a type 2 immune response with production of specific cytokines and chemokines initiated by allergen exposure(116).In addition to acute responses, cytokines and chemokines produced by Th2 cells, mast cells and basophils recruit eosinophils from the blood into the airways and skin, leading to allergic asthma and atopic dermatitis. As noted earlier, besides hematopoietic cells, structural cells such as epithelial cells, fibroblasts and smooth muscle cells are known to contribute to allergic inflammation. Nevertheless, the roles of vascular endothelial cells in allergic inflammation had not yet been well studied.

12. Role of anti-inflammatory cytokines in endothelial dysfunction

The delicate balance between pro- and anti-inflammatory cytokines determines the net effect of an inflammatory response. Perturbations in this equilibrium can drive the host defense immune response either towards chronic inflammation (pro-inflammatory) or towards healing (anti-inflammatory). Exposure of endothelial cells to pro-inflammatory cytokines leads to transient and reversible endothelial dysfunction(117),(118).A number ofanti-inflammatory treatment strategies improve endothelial function by preventing pro-inflammatory cytokinesynthesis. Anti-inflammatory cytokines are a series of immune-regulatory molecules that control the proinflammatory cytokines response, which consequently reduces inflammation and promotes healing. In addition, an elevation in the level of anti-inflammatory cytokines can also be found in the development of vascular disease (119),which reflects an early compensatory mechanism and serves as an indicator of pro-inflammatory reactions. Major anti-inflammatory cytokines include IL-1Ra, IL-4, IL-10, IL-11, IL-13 and TGF-β. Several newly found cytokines, such as IL-33, IL-35, and IL-37 also participate in regulating the function of EC. The following will discuss two of these anti-inflammatory cytokines including IL-10 and TGF-β in detail.

13. Effects of Endothelial as Anti-Inflammatory Therapy

Methotrexate remains the mainstay of therapy for RA and several other rheumatic diseases. An inhibitor of folic acid metabolism, methotrexate sharply reduces systemic inflammation anddramatically improves synovitis in patients with inflammatory arthritis. Methotrexate also appears to improve endothelium-dependent vasodilation in patients with RA, although the data are limited(120).Inhibitors of TNF-α have been employed with increasing frequency for patients with a variety of inflammatory diseases, including RA, spondyloarthritis, IBD and psoriasis. The critical role of TNF-α in the generation of severe systemic inflammation in these conditions likely explains the effectiveness of these agents. TNF-α may also be largely responsible for the endothelial dysfunction and accelerated atherosclerosis in these patients, making anti-TNF-α agents attractive therapeutic options for preventing CVD in this population. Numerous studies have demonstrated improved endothelium-dependent vasodilation in patients with RA after initiation of anti-TNF-α therapy. This has been demonstrated in a vessel-specific manner by measuring FBF immediately after intra-brachial infusion of infliximab. In

this model Cardillo and colleagues demonstrated that the local effect of infliximab on the brachial artery improved brachial artery endothelial function without altering markers of systemic inflammation(121).Multiple other studies have demonstrated that anti-TNF-α agents improve FMD in RA patients who are refractory to conventional disease modifying anti-rheumatic drugs (DMARD) therapy(122),(123),(124),(125),(126),(127).Anti-TNF-αagents also improve endothelium-dependent vasodilation in patients with spondyloarthritis(128),(129),cutaneous psoriasis (130),and IBD(131),although studies are small and few. Improvement in endothelial function with anti-TNF-α therapy may correlate with improvement in disease activity and markers of systemic inflammation(132).The duration of the response has been controversial, however. Several studies in RA have shown that anti-TNF-α agents induce a rapid improvement in FMD that is lost after a period of weeks despite effective control of disease activity and systemic inflammation(124), (126). Other studies have demonstrated sustained improvements in endothelial function(125),(133).Factors contributing to differences in duration of response remain unclear.Anti-TNF-α agents have also been shown to reduce levels of plasma biomarkers of endothelial dysfunction, although results have been inconsistent. Klimiuk et al.(134),demonstrated that etanercept administration reduced levels of soluble ICAM-1, VCAM-1 and E-selectin in patients with RA. Gonzalez-Gay and colleagues found reductions only in soluble ICAM-3 and P-selectin after infliximab infusions for patients with RA (135).Adalimumab therapy in patients with psoriasis has been shown to reduce ICAM-1 levels without affecting other CAMs(130).These findings are similar to results from studies examining levels of CAMs at baseline across various inflammatory diseases: it has been difficult to discern a consistent profile of CAM expression prior to or in response to disease-modifying therapy. Although CAM expression may be a general marker for systemic inflammation and endothelial dysfunction, its utility in clinical and translational research may be limited. Corticosteroids have long been used to manage a variety of inflammatory diseases, but their effects on CVD have been controversial. The association between steroids and insulin resistance and obesity has raised concern for increased cardiovascular risk, while their anti-inflammatory effects might mitigate this risk. Studies addressing the association between long-term steroid use in RA and CVD have yielded variable results. A 2011 systematic review of studies of low-dose steroid use in RA found that corticosteroids are generally associated with mildly increased cardiovascular risk(136).Studies did not reveal an effect of steroids on markers of subclinical atherosclerosis and endothelial function, however. Other observational studies have demonstrated an association between corticosteroid use and lower rates of subclinical atherosclerosis compared to patients not using steroids(137).Veselinovicand colleagues demonstrated that FMD is higher in RA patients treated with corticosteroids versus notherapy(138).This study conflicts with a randomized prospective study, by Hafstrom, showing that addition of steroids to DMARD therapy does not improve endothelial function in RA patients(139).These results highlight the difficulty of studying the effects of single-agent steroid therapy on patients with inflammatory disease in the modern era. Measuring the added benefit of steroids in the context of background immune-suppressing therapy is unlikely to reveal significant improvements, even if corticosteroids may have this effect in isolation.

14. Hydroxymethylglutaryl-CoA Reductase Inhibitors (Statins)

Statins exhibit pleiotropic properties influencing the vasculature that are thought to contribute to their clinical benefit beyond the lipid-lowering effect. Although the mechanisms are incompletely characterized, statins have been shown to improve endothelial dysfunction in

patients with traditional cardiovascular risk factors. Potential mechanisms include upregulation of eNOS, leading to enhanced bioavailability of NO and improved vasoreactivity(136).The use of statins as disease modifying agents and as primary prevention for CVD in patients with chronic inflammatory diseases has also received interest. Statin therapy has been shown to reduce disease severity in patients with RA and has gained attention for use as a disease-modifying agent in other inflammatory diseases(138), (139),(137),(140),(141).Statins have been also been shown to improve endothelium-dependent vasodilation in patients with RA and SLE(142),(140),(143),(144).This effect appears to correlate positively with measures of systemic inflammation and disease severity(144).There is current interest in studying the long-term effects of statin therapy on hard cardiovascular endpoints. The Trial of Atorvastatin in Rheumatoid Arthritis was the first randomized controlled trial designed to study the effects of statin therapy in RA patients(143).At 6 months, statins significantly improved several markers of disease severity and markers of systemic inflammation compared to placebo. Endothelial function was not assessed, however, and the duration of follow-up was not long enough to detect changes in cardiovascular endpoints. Only two studies to date have addressed the effect of statins on cardiovascular events. Sheng and colleagues conducted a population-based cohort study designed to evaluate the effects of statins on lipid levels, cardiovascular events and all-cause mortality in RA and osteoarthritis (OA) patients(145).Statins similarly reduced lipid levels and were protective of cardiovascular events and mortality in RA and OA patients without prior CVD. There was no protective effect in the secondary prevention setting for either cohort, however. Semb*et al.*(146).demonstrated that statins had a similar effect on cardiovascular events in RA and non-RA patients when used for secondary prevention. Unfortunately, there are no randomized controlled trials addressing the effect of statin therapy on patients with RA.

15. **Conclusion**

Many stimuli associated with inflammatory and immune vascular diseases have been reported to induce endothelial cell apoptosis in vitro. Moreover, there are several human diseases in which endothelial cell apoptosis has been observed.Transgenic mouse models that constitutively or conditionally express endothelial-restricted transgenes have been developed (e.g. Refs). With this approach it should be possible to protect endothelial cells specifically from apoptosis by over-expression of anti-apoptotic proteins (e.g. Bcl-2 or IAPs) and thereby to determine experimentally the precise role of endothelial cell apoptosis. Recent studies have shed light on the importance of endothelial cells in the development and exacerbation of allergic disorders.These cells produce several key molecules involved in allergic disorders, such as periostin and TARC, which are not only critical to the pathogenesis of the disorders but also already serve as reliable biomarkers reflecting disease progression.Anti-inflammatory cytokines protect against the impairment of endothelial function by counteracting theeffects of pro-inflammatory cytokines and suppressing oxidative stress.A better understanding of the different functions fulfilled by endothelial cells result not only in a better comprehension of these diseases but also in their prevention and more effective therapeutic treatments. New pharmacological strategies are also being designed to target endothelial cells specifically, and their development is an exciting challenge of and goal for future research.

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