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Mini Review

The Role of Endothelial Cells in the Resolution of Acute Inflammation

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ABSTRACT

Endothelial cells are key regulators of the inflammatory response. Lining blood vessels, they provide in the steady state an antiinflammatory, anticoagulatory surface. However, in the case of injury or infection, endothelial cells control the adhesion and migration of inflammatory cells, as well as the exchange of fluid from the bloodstream into the damaged tissue. Thus, expression of endothelial adhesion molecules, cytokines, and changes in permeability need to be tightly regulated to allow for a controlled inflammatory response. Acute inflammation is characterized by tissue infiltration of neutrophils, followed by monocytes/macrophages. For successful tissue regeneration and healing, the acute inflammatory response needs to be actively shut down, a process called resolution of inflammation. Unsuccessful resolution may lead to excessive tissue damage and ultimately results in chronic, self-promoting inflammation. This review will summarize recent advances in the field of endothelial biology, which point to an active participation of the endothelial barrier in the resolving process. *Antioxid. Redox Signal.* 7, 1744–1754.

INTRODUCTION

A SELF-LIMITING ACUTE INFLAMMATION caused by injury or infection is characterized by early vascular leakage, with extravasation of plasma components and fluid, and massive recruitment of neutrophils that ingest invading pathogens and release proinflammatory mediators and reactive oxygen species (ROS) leading to tissue damage, and oxidation of membrane lipids; subsequently, neutrophils undergo apoptosis, an important step in the resolution of inflammation (for reviews, see 82, 83). As a consequence, specific monocyte infiltration and maturation into macrophages are initiated to remove apoptotic cells, fibrin and protein clots, and bacterial and cellular debris; additionally, proinflammatory mediators are dissipated and antiinflammatory, proresolving mechanisms are induced; finally, normal vascular permeability has to be restored, ultimately leading to a cessation of leukocyte emigration and restoration of normal tissue function.

A continuous imbalance between initiation of proinflammatory mechanisms and those that promote resolution leads to prolongation of an inflammatory response. Thus, chronic inflammation may arise from failure and inefficiency of mechanisms normally responsible for resolution of inflammation and restitution of tissue homeostasis. Although much has been learned about the proinflammatory pathways over the last years, mechanisms that lead to the equally important resolution of acute inflammation are less well understood. Recent evidence demonstrates that resolution is an active process involving apoptosis of neutrophils, selective attraction and infiltration of monocytes and their differentiation into macrophages and dendritic cells, and up-regulation of proresolving genes such as heme-oxygenase-1 (HO-1) and cyclooxygenase-2 (COX-2) (109). Hence, the mechanisms involved in the endogenous inflammation-resolving process are currently extensively investigated, as they could offer possible targets in the treatment of chronic inflammation (89, 91, 109).

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ENDOTHELIAL CONTROL OF LEUKOCYTE ADHESION AND BARRIER FUNCTION

The endothelial cell layer forms a barrier between blood and tissue. At sites of acute inflammation, leukocytes have to pass this barrier in order to exert their function toward invading microbes. During resolution of acute inflammation, the physiological barrier has to be restored to limit cell and fluid extravasation. Therefore, the endothelium plays a key role not only in promoting, but also ending inflammatory responses after injury or infection (Fig. 1).

Endothelial cells can actively regulate the migration of leukocytes through the vessel wall by expressing adhesion molecules and chemokines (67) on their luminal surface, allowing leukocytes to adhere and finally migrate into the tissue. For successful resolution, endothelial cells must first selectively let pass mononuclear cells for the clearance of

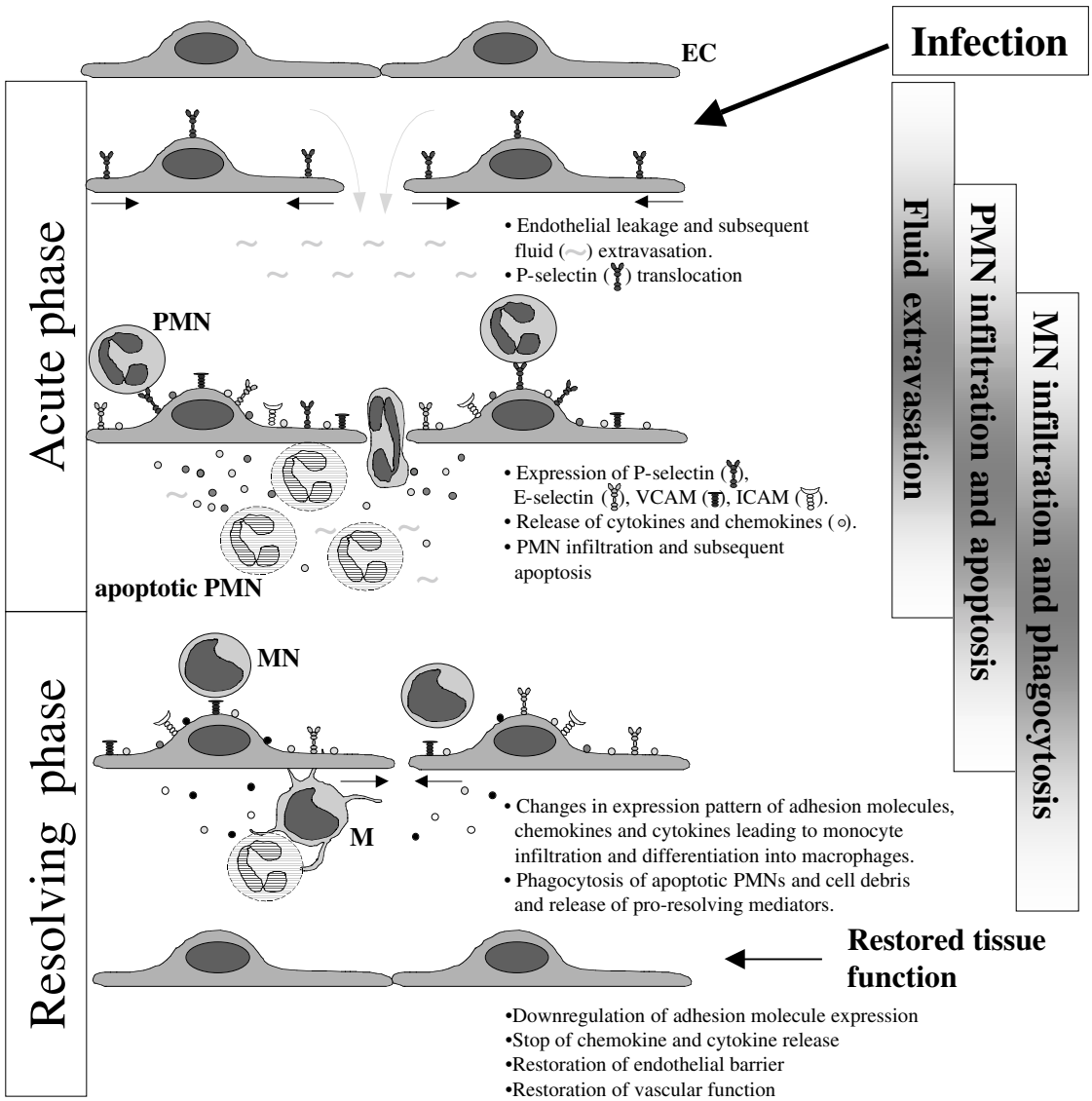

FIG. 1. The role of the endothelial cell in the resolution of acute inflammation. Upon inflammatory stimulation, the endothelial barrier function is rapidly lost, leading to extravasation of fluid. Preformed P-selectin is translocated to the luminal surface of endothelial cells, adhesion molecules such as E-selectin, ICAM-1, and VCAM-1 are expressed, and chemokines are expressed and released. These changes lead to transmigration of neutrophils through the endothelium into the tissue. Eventually, neutrophils undergo apoptosis. During this phase, more and more monocytes are selectively recruited to the site of inflammation, presumably by changes in the expression pattern of adhesion molecules and chemokines on the endothelial cell. The accumulating monocytes differentiate into macrophages to phagocytose apoptotic neutrophils and cell debris. Finally, release of proinflammatory chemokines is abrogated and endothelial barrier and vascular function is restored. During the whole process of acute, resolving inflammation, the endothelial cell serves as a gatekeeper, controlling which cell type enters the site of inflammation. EC, endothelial cell; PMN, polymorphonuclear leukocyte; Mϕ, macrophage.

cellular corpses, whereas the drainage of extravasated fluids is facilitated by the lymphatic system at the site of inflammation. Furthermore, proinflammatory chemokines have to be dissipated. In this context, the importance of the chemokine receptor D6 in resolution was recently illustrated. It was shown that D6, which is mainly expressed on lymphatic endothelial cells, binds, internalizes, and subsequently degrades a variety of chemokines and thus promotes resolution of cutaneous inflammation (44). Finally, endothelial cells have to actively shut down the expression of adhesion molecules (Fig. 1). Recent data demonstrate that neutrophils by themselves down-regulate adhesion molecule expression in endothelial cells and reduce binding of inflammatory cells (96). On the other hand, *in vitro* experiments showed that angiopoietin-1 (Ang-1) down-regulates expression of E-selectin (26) and of thrombin-induced interleukin-8 (IL-8), and thus inhibits neutrophil binding to endothelial cells (78). Furthermore, overexpression of Ang-1 in mice abrogated lipopolysaccharide (LPS)-induced up-regulation of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) and preserved normal endothelial nitric oxide synthase (eNOS) expression (110), significantly improving survival of mice challenged with lethal doses of LPS. Moreover, vessels of mice overexpressing Ang-1 exhibited resistance to vascular leakage (100, 101), implicating Ang-1 as an interesting target in promoting resolution.

Superoxide dismutase (SOD) and ROS scavenging: Influence on redox signaling

Many proinflammatory intracellular signaling pathways in endothelial cells critically depend on free radicals. Whereas in granulocytes ROS generation by NADPH oxidase is necessary for successful antimicrobial defense during acute inflammation, activation of this enzyme in endothelial cells has been implicated in the progression of endothelial dysfunction and atherosclerosis (81). Therefore, inhibiting ongoing inflammatory responses by reducing oxidative stress is another potential mechanism to promote resolution. Copper, zinc superoxide dismutase (CuZn-SOD) scavenges superoxide radicals by production of hydrogen peroxide and oxygen. A recent study shows that mice overexpressing CuZn-SOD were protected against LPS-induced endothelial dysfunction (20). Furthermore, endothelial cells overexpressing SOD showed upon tumor necrosis factor-α (TNFα) stimulation decreased superoxide production, decreased surface expression of VCAM-1 and ICAM-1, and thus decreased neutrophil adhesion. Moreover, in these cells, TNFα-induced activation of activator protein-1 (AP-1) and nuclear factor-κB (NFκB) were significantly reduced (63). Besides SOD, selenoproteins such as glutathione peroxidases and thioredoxin reductases were shown to prevent oxidative events in endothelial cells (for review, see 8). Whether these enzymes are up-regulated during a self-resolving inflammation and whether their induction would support resolution remain to be investigated.

Leukocyte specific protein 1 (LSP-1)

LSP-1 was described to be a substrate for protein kinase C and for mitogen-activated protein kinase-activating protein (MAPKAP) kinase, and to play an important role in the regulation of cytoskeletal architecture and motility. Initially, LSP-1 was thought to be restricted to leukocytes; however, recently LSP-1 was shown to be present also in endothelial cells. Surprisingly, using chimeric mice that lack LSP-1 either in endothelial cells or in leukocytes, it was clearly shown that endothelial, but not leukocyte LSP-1 controlled the process of leukocyte transmigration toward TNFα (64). By using a zymosan-induced peritonitis model (45) or a zymosan-induced knee inflammation model (106), it was shown that mice that lack LSP-1 exhibited an increase in neutrophil accumulation. Whereas rolling and adhesion of leukocytes remained unaltered, extravasation was clearly increased in LSP-1-deficient mice. Therefore, LSP-1 displays a new target in endothelial cells that could contribute to the resolution of inflammation.

Endothelial CD39 and adenosine release

CD39, an ecto-nucleoside triphosphate diphosphohydrolase (NTPDase), which is expressed on quiescent vascular endothelial cells and can be induced by hypoxia (23), efficiently hydrolyzes extracellular ATP and ADP to AMP and adenosine. Mice lacking CD39 showed exacerbated inflammatory responses to chemical skin irritants, due to their inability to hydrolyze extracellular accumulating ATP (69). Furthermore, it was shown that ATP released from activated neutrophils during transcytosis is metabolized by endothelial CD39, especially under hypoxic conditions. The liberated adenosine leads via activation of its receptor ($AdoRA_{2B}$) to an increase in endothelial cylic AMP, which finally leads to enhanced barrier function and, in *in vivo* models, to decreased vascular leakage (23; 35). *In vitro*, adenosine attenuates polymorphonuclear leukocyte (PMN) transendothelial migration (105). Moreover, it was shown that endothelial cells themselves release ATP upon LPS stimulation that precedes IL-1α secretion. When those endothelial cells overexpressed CD39, secretion of IL-1α was abrogated (41). Additionally, endothelial CD39 expression inhibited ATP-induced apoptosis and NFκB activation and abrogated E-selectin expression (33). Therefore, induction of CD39 and thus increased adenosine liberation followed by improved barrier function would result in a stop of leukocyte migration during the resolving phase of inflammation.

AOC3 (VAP1): oxidative modification of adhesion molecules

Recently, it was suggested that leukocyte transmigration is dependent on the enzymatic activity of endothelial amine oxidase, copper-containing-3 (AOC3), also known as vascular adhesion molecule 1 (VAP1) (53). Moreover, *in vivo* inflammation studies clearly showed that when AOC3 activity is lost either by treating animals with antibodies against AOC3 or by using mice lacking the enzyme, leukocyte transmigration through the endothelium is significantly decreased, whereas the rolling velocity of neutrophils is greatly increased (95, 102). Whether endothelial AOC3 is actively shut down, as well as the mechanism behind it, still remains unclear; however, regulating the function of this enzyme may be important in promoting resolution of inflammation.

ENDOTHELIUM-DERIVED GASEOUS MEDIATORS IN THE RESOLUTION OF ACUTE INFLAMMATION

HO-1

Heme exerts several proinflammatory actions, such as up-regulation of cytokines and chemokines, and inhibition of neutrophil apoptosis (3). Therefore, degradation of heme is important for tissue homeostasis. HO-1 is the rate-limiting enzyme of heme catabolism, catalyzing the breakdown of heme into carbon monoxide (CO), iron, and bilverdin, which is further metabolized to bilirubin. It is well established that expression of HO-1 is cytoprotective in a variety of cell types, including endothelial cells. Moreover, HO-1 is highly up-regulated during the resolution of inflammation (107, 108), and inhibition of HO-1 leads to increased inflammatory cell extravasation and prolongation and potentiation of inflammation. Therefore, a central role of HO-1 and its products in the resolution of inflammation, but also in wound healing and angiogenesis, was suggested.

In vivo experiments clearly demonstrated that induction of HO-1 inhibited oxidant-induced (37) and shock-induced (70) leukocyte rolling and adhesion. HO-1 affected expression of P-selectin on the endothelium, whereas expression of adhesion molecules on leukocytes remained unchanged (37), again pointing to an important role of the endothelium in control of leukocyte migration during resolution. Indeed, overexpression of HO-1 in endothelial cells attenuated TNFα-induced NFκB activation and protected against proinflammatory responses, including increased VCAM-1, E-selectin, and monocyte chemotactic protein-1 expression and decreased eNOS expression (52). Interestingly, these antiinflammatory and potentially proresolving effects were mediated by HO-1-derived bilirubin, but not by CO (94).

HO-1-derived CO has been described to be beneficial in a variety of inflammatory models (for review, see 75). For instance, CO suppresses LPS-induced TNFα production in leukocytes *in vivo* and *in vitro* (71, 76). However, on endothelial cells, CO seemed to mediate mainly antiapoptotic effects (93). For example, it was shown that administration of CO abolished anoxia- (*in vitro*) or ischemia- (*in vivo*) induced apoptosis of lung endothelial cells in a p38 mitogen-activated protein kinase-dependent way (112). On the other hand, protection of TNFα-induced apoptosis in endothelial cells (56) by CO required basal transcription of NFκB-dependent antiapototic genes (9).

In addition, a role in angiogenesis was ascribed to HO-1 (for review, see 22). New vessel formation is important for tissue repair after acute inflammation; moreover, a relation between ongoing angiogenesis and chronic inflammatory disorders has been described (43). Recently, it has been shown *in vitro* that induction of HO-1 induces expression of vascular endothelial growth factor and promotes angiogenic activities in endothelial cells; this effect was attributed to a CO-mediated elevation of cyclic GMP (46). Furthermore, using an *in vivo* Matrigel plug assay, it was demonstrated that induction of HO-1 during angiogenesis led to a significant decrease of concomitant leukocyte infiltration (11), and it was suggested that HO-1 induction maximizes angiogenesis associated with the resolution of tissue injury, whereas it inhibits inflammatory angiogenesis.

Taken together, products of HO-1 enzymatic activity beneficially modulate the inflammatory response toward resolution. Bilirubin seems to down-regulate inflammation in endothelial cells, whereas endothelial-derived CO exerts proresolving effects on neighboring cells, such as smooth muscle cells and leukocytes.

Nitric oxide synthase (NOS)

NOSs are oxidoreductases that convert L-arginine, tetrahydrobiopterin (BH4), and oxygen in the presence of electron donors such as FAD, FMN, and NADPH to nitric oxide (NO) and L-citrulline. Three forms of the enzyme exist: neuronal (nNOS, NOS1), inducible (iNOS, NOS2), and endothelial NOS (eNOS, NOS3).

It is the inducible form (iNOS) that is generally controlled by inflammatory mediators. However, endothelial cell-derived NO regulates basal and stimulated vascular tone inducing vasodilation (25), displays potent antiinflammatory properties, and improves endothelial barrier function (36, 111). Mice lacking eNOS were shown to have, besides increased blood pressure (38), attenuated vascular leakage and edema formation during acute inflammation (10). Furthermore, it was shown that these mice are resistant to LPS-induced systemic hypotension (14).

Could endothelial cell-derived NO contribute to resolution of acute inflammation? In endothelial cells, NO inhibits expression of P-selectin (15), VCAM-1, ICAM-1, and E-selectin, but also of IL-6 and IL-8 by scavenging superoxide anions and thereby inhibiting the NFκB pathway (18). Furthermore, the oxidation product of NO and superoxide anion (O_2^-), peroxynitrite ($ONOO^-$), inhibits adhesion of leukocytes *ex vivo* (72), which is, however, dependent on its concentration (73), and attenuates rolling of neutrophils *in vivo* by down-regulating P-selectin (59). However, analysis of leukocyte transmigration in eNOS (−/−) mice revealed controversial results. Whereas Bucci *et al.* showed that in a footpad-swelling model, as well as in the air-pouch model, leukocyte traffic remained unchanged (10), Lefer *et al.* showed that basal leukocyte rolling, as well as thrombin-induced rolling and adherence, was greatly enhanced in eNOS (−/−) and in nNOS (−/−), but not in iNOS (−/−) mice. Furthermore, increased PMN extravasation into the peritoneal cavity after thioglycollate stimulation in eNOS (−/−) and in nNOS (−/−) mice was demonstrated (60).

An imbalance of L-arginine and BH4 leads to an uncoupling of eNOS, using oxygen as a substrate and generating superoxide (104). This would cause, on the one hand, further oxidation of BH4, perpetuating the uncoupled reaction, and, on the other hand, the formation of high concentrations of peroxynitrite by using the remaining NO. Thus, the bioavailability of NO would be reduced, and highly reactive oxygen species formed, resulting in impairment of endothelial function. Such impairment of endothelial function is commonly seen during acute inflammation and normally vanishes during resolution. Indeed, prolonged endothelial dysfunction resulting from diminished bioactivity of NO induced by oxidative stress (99), lipid overload (24), or hyperglycemia (16) has

been implicated in the development of chronic vascular diseases. It is tempting to speculate that in these situations a dysfunctional endothelium has lost the full capacity to induce resolution of acute inflammation.

ENDOTHELIUM-DERIVED LIPID MEDIATORS IN THE RESOLUTION OF ACUTE INFLAMMATION

Phospholipase A_2 (PLA_2)

Polyunsaturated fatty acids, such as arachidonic acid, are hydrolyzed from the *sn-2* position of membrane phospholipids by PLA_2 and can be further metabolized in endothelial cells by several enzymes releasing proinflammatory lipid mediators (74).

The three main classes of PLA_2, *i.e.,* calcium-independent PLA_2 ($iPLA_2$), cytosolic PLA_2 ($cPLA_2$), and secretory PLA_2 ($sPLA_2$), are further subdivided into several isoforms (55). It has been shown that these phospholipases are sequentially induced during acute inflammation; $iPLA_2$ was shown to mediate the onset of acute inflammation by releasing arachidonic acid for the synthesis of proinflammatory eicosanoids, as well as platelet-activating factor, whereas $sPLA_2$ and $cPLA_2$ were shown to be expressed in later phases of inflammation, promoting resolution (31).

COX-2

Inducible COX-2 is expressed at sites of inflammation. Because of the proinflammatory activity of its products, such as prostaglandin E_2 (PGE_2), and because of the beneficial effects of pharmacological inhibitors, COX-2 was generally thought to play a detrimental role. However, also antiinflammatory properties have been ascribed to COX-2 (for review, see 109). Recent data demonstrate that COX-2 is expressed during the resolution phase of inflammation and in areas of wound healing. Furthermore, clinical trials have shown that COX-2-selective inhibitors delayed the healing and tissue restoration, suggesting that COX-2 promotes resolution.

Using the carrageenan pleurisy model, Willoughby *et al*. showed that COX-2 and HO-1 both were essential for the resolving phase because the inhibition of these enzymes delayed the resolution of inflammation (27, 28). Further investigation revealed that the antiinflammatory effects of endothelial COX-2 during the later phase of inflammation are mediated by preferential release of prostaglandin D_2 (PGD_2) and its cyclopentenone breakdown product, 15-deoxy-Δ^{12-14}-prostaglandin J_2 ($15dPGJ_2$), potent-Jially stimulating peroxisome proliferator-activated receptor (PPAR)-dependent transcription and other antiinflammatory mechanisms (for review, see 85). Indeed, COX-2 expression was biphasic, the first peak occurring within the first 2 h and the second, much higher peak occurring after 48 h with a concomitant shift from PGE_2 to $15dPGJ_2$ production (30, 57). This late expression of COX-2 was essential for resolving inflammation, because inhibition of this second peak resulted in a prolonged inflammatory reaction (29). Moreover, COX-2 brings about resolution by inducing both PMN and macrophage apoptosis through the release of $15dPGJ_2$ (30).

Taken together, endothelial-derived PGD_2 and the cyclopentenone prostaglandin $15dPGJ_2$ might contribute to the resolution of inflammation through various mechanisms that include the inhibition of proinflammatory gene expression, the induction of apoptosis, and the activation of PPARs (58).

Ligands for PPARγ

The effects of PPARγ ligands on the inflammatory response have been well studied, but the role of PPARγ therein is still not clear. Whereas in endothelial cells the effects of $15dPGJ_2$ are most likely independent of PPARγ, PPARγ-dependent effects have been shown mainly in macrophages. Moreover, whether $15dPGJ_2$ is produced *in vivo* in sufficient amounts to activate PPARs is still a matter of debate (5). Using a highly sensitive and selective assay, Bell-Parikh *et al.* suggest that the amounts of $15dPGJ_2$ generated *in vivo* are far too low to be compatible with a role for this substance as an endogenous activator of PPARγ (5). Indeed, it was shown in human umbilical vein endothelial cells that 15d-PGJ_2 was a ligand for retinoic acid receptor-related orphan receptor-α, but not PPARγ (68). However, because of its reactivity, $15dPGJ_2$, like cyclopentenone itself, can directly react with proteins and either inhibit or enhance their activity.

Recently, it was found that nitroalkene derivatives of linoleic acid (nitrolinoleic acid) are formed via NO-dependent oxidative inflammatory reactions. Nitrolinoleic acid is a significantly more robust PPARγ ligand than other reported endogenous PPARγ ligands, including $15dPGJ_2$ and synthetic PPARγ agonists such as rosiglitazone and ciglitazone. These findings show that fatty acid nitration products and PPAR-dependent gene expression can transduce NO-mediated cell signaling reactions (86), potentially down-regulating acute inflammation to induce resolution.

Ligands for PPARα

The role of ligand-induced activation of PPARα in inflammatory control is less controversial. Activated PPARα inhibits the production of inflammatory response markers, such as endothelin-1, VCAM-1, IL-6, and tissue factor, in endothelial cells, smooth muscle cells, and macrophages (4). In endothelial cells, PPARα ligands have been found to inhibit monocyte recruitment and adhesion through down-regulation of chemoattractant and adhesion proteins (for review, see 65). PPARα reduces the activity and DNA-binding capacity of proinflammatory transcription factors such as NFκB and AP-1. This ligand-dependent transcriptional transrepression of proinflammatory genes involves competition for transcription cofactors, as well as direct interaction and interference of PPARα with p65 and c-Jun (17). However, whether the described antiinflammatory mechanisms of PPARα contribute to resolution of acute inflammation remains to be shown. A possible scenario would be the formation of oxidized fatty acids (*e.g.,* hydroxyeicosatetraenoic acids) during neutrophil-dependent ROS production, which then would serve as ligands and activators of PPARα, resulting in inhibition of inflammatory gene expression, up-regulation of proresolving genes, and a switch of the inflammatory reaction. In this context, we have shown that HO-1 is a target gene for PPARα in endothelial cells (Kronke *et al.*, manuscript submitted), further implying a role for PPARs in resolution.

Lipoxins and resolvins

In addition to prostaglandins, endothelial cells generate precursors for lipid mediators that are produced through transcellular metabolism in leukocytes and promote resolution of acute inflammation. During acute inflammation, endothelial cells together with leukocytes produce lipoxins from arachidonic acid via transcellular biosynthesis. These trihydroxytetraene-containing eicosanoids are generated through concerted activation of COX and lipoxygenases. Aspirin acetylates COX and causes the endogenous biosynthesis of so-called aspirin-triggered carbon 15-epimers of lipoxins, which mimic the biologic activities of native lipoxins. These compounds serve as local endogenous antiinflammatory mediators and have been shown to trigger resolution (1, 47, 88). The major cellular targets reported for lipoxins are leukocytes, in particular neutrophils, monocytes, and eosinophils. Lipoxin A_4 (LXA_4) exhibits potent antiinflammatory properties *in vitro* and in animal models of acute inflammation by inhibiting neutrophil and lymphocyte activation. Therefore, LXA_4 is an important endogenous counterregulatory signal that promotes resolution of acute inflammation. Moreover, it has been shown that aspirin-triggered 15-epi-LXA_4 and lipoxin B_4 analogues inhibit neutrophil-mediated changes in vascular permeability (90) and rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages (32).

LXA_4 and 15-epi-LXA_4 are agonists for the LXA_4 receptor, ALX. Recent studies showed that overexpression of ALX in transgenic mice resulted in accelerated resolution of induced inflammation (19, 62). Activation of ALX was shown to regulate leukocyte motility; however, in endothelial cells, where ALX is not expressed, lipoxins may also interact with cysteinyl leukotriene 1 (CysLT1) receptors (34, 98). Therefore, LXA_4 interacts with at least two classes of cell-surface receptors: ALX, specific for LXA_4 on leukocytes, and CysLT1, shared by leukotriene D_4 (LTD_4) on endothelial cells.

On the other hand, endothelial cells produce from docosahexaenoic acid, an omega-3 polyunsaturated fatty acid, metabolites such as 13-hydroxydocosahexaenoic acid (13-HDHA), and if aspirin-triggered, 17*R*-HDHA. Leukocytes can then via lipoxygenase produce so-called resolvins from these precursors (87). Resolvins represent local autacoids that display potent antiinflammatory activity, promoting resolution (91). Recently, a receptor for resolvin E1 has been identified as the orphan G protein-coupled receptor ChemR23 (2).

Together, these lipid-derived "stop signals" may be involved in switching the cellular response from additional PMN recruitment toward monocytes (in a nonphlogistic fashion) that could lead to resolution of the inflammatory response and promotion of repair and healing.

Oxidized phospholipids (OxPL) and sphingosine-1-phosphate (S1P)

During inflammation, tissue damage is caused by neutrophil-derived free radicals, originally designated to destroy invading pathogens. As a consequence, oxidative modification of membrane lipids occurs and results in the formation of a variety of biologically active compounds that accumulate in the inflamed tissue. These oxidized lipids induce specific cellular reactions, which profoundly modulate the inflammatory process. The classical view of lipid oxidation products is that they can induce and propagate chronic inflammatory reactions; however, recent data show that cells and tissues respond toward these oxidatively formed stress signals also by activation of antiinflammatory, maybe even proresolving processes.

Data from our laboratory and others indicate that the formation of phospholipid oxidation products potentially contributes to the resolving process at several levels of the inflammatory cascade: OxPL were recently shown to be generated by endothelial cells upon treatment with IL-1 (97) and during apoptosis (12, 13, 39, 49). OxPL (39, 61), but also peroxidation products of arachidonic acid, such as isoprostanes (40), stimulated endothelial cells to bind selectively monocytes, but not neutrophils (39, 61) via expression of connecting segment 1-fibronectin (92). Moreover, OxPL induce maturation of monocytes into macrophages via generation of granulocyte-macrophage colony stimulating factor (80). Furthermore, OxPL induce expression of proresolving genes such as HO-1 (42, 48, 54), COX-2 (79), and IL-10 (103), represent recognition signals for phagocytosis (12, 50), and inhibit acute inflammation induced by LPS (7).

Finally, we have recently shown that OxPL induced a sustained barrier-protective effect, counteracting thrombin-induced endothelial cell barrier disruption. We demonstrated that the effects of OxPL were additive to those of S1P (6). S1P, a biologically active lipid generated by hydrolysis of membrane lipids in activated platelets and various cell types, was demonstrated to be the major barrier-protective product of platelets (21, 84). Additionally, it has been shown *in vitro* that S1P decreases neutrophil chemotaxis and transendothelial migration in response to IL-8 (51). More recently, it was described in *in vivo* models that S1P prevents edema formation and significantly reduces neutrophil accumulation after LPS stimulation (66, 77). Thus, S1P may act in concert with OxPL to restore endothelial barrier function during acute inflammation, an important process for successful resolution.

CONCLUSION AND OUTLOOK

Resolution of acute inflammation involves highly coordinated active mechanisms that need to be switched on at a certain time point in the course of the inflammatory response. Endothelial cells play a major role in resolution, because they are able to secrete a vast array of pro- and antiinflammatory mediators that help to coordinate leukocyte traffic and barrier function. However, prolonged endothelial activation can lead to overwhelming and uncontrolled inflammation that is detrimental and can result in chronic inflammation. Therefore, a tightly balanced inflammatory response followed by timely resolution is essential for successful tissue regeneration. Further research is needed to elucidate the exact endothelial mechanisms that are involved in resolution of acute inflammation, eventually making this process a target for pharmacological intervention.

ABBREVIATIONS

ALX, lipoxin A_4 receptor; Ang-1, angiopoietin-1; AOC3, amine oxidase, copper-containing-3; AP-1, activator protein-

1; BH4, tetrahydrobiopterin; CO, carbon monoxide; COX-2, cyclooxygenase-2; cPLA$_2$, cytosolic PLA$_2$; CuZn-SOD, copper, zinc superoxide dismutase; CysLT1, cysteinyl leukotriene 1; 15dPGJ$_2$, 15-deoxy-Δ^{12-14}-prostaglandin J$_2$; eNOS, NOS3, endothelial NOS; HDHA, hydroxydocosahexaenoic acid; HO-1, heme oxygenase-1; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; iNOS, NOS2, inducible NOS; iPLA$_2$, calcium-independent PLA$_2$; LPS, lipopolysaccharide; LSP-1, leukocyte-specific protein 1; LXA$_4$, lipoxin A$_4$; NFκB, nuclear factor-κB; nNOS, NOS1, neuronal NOS; NO, nitric oxide; NOS, nitric oxide synthase; OxPL, oxidized phospholipids; PGD$_2$, prostaglandin D$_2$; PGE$_2$, prostaglandin E$_2$; PLA$_2$, phospholipase A$_2$; PMN, polymorphonuclear leukocyte; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SOD, superoxide dismutase; S1P, sphingosine-1-phosphate; sPLA$_2$, secretory PLA$_2$; TNFα, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule-1.

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