Cytokines and vascular reactivity in resistance arteries

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Vila, Elisabet, and Mercedes Salaices. Cytokines and vascular reactivity in resistance arteries. Am J Physiol Heart Circ Physiol 288: H1016–H1021, 2005; doi:10.1152/ajpheart.00779.2004.—Cytokine levels are elevated in many cardiovascular diseases and seem to be implicated in the associated disturbances in vascular reactivity reported in these diseases. Arterial blood pressure is maintained within a normal range by changes in peripheral resistance and cardiac output. Peripheral resistance is mainly determined by small resistance arterioles. This review focuses on the effects of cytokines, mainly TNF-α, IL-1β, and IL-6, on the reactivity of resistance arteries. The vascular effects of cytokines depend on the balance between the vasoactive mediators released under their influence in the different vascular beds. Cytokines may induce a vasodilatation and hyporesponsiveness to vasoconstrictors that may be relevant to the pathogenesis of septic shock. Cytokines may also induce vasoconstriction or increase the response to vasoconstrictor agents and impair endothelium-dependent vasodilatation. These effects may help predispose to vessel spasm, thrombosis, and atherogenesis and reinforce the link between inflammation and vascular disease.

inflammation; cardiovascular diseases

ARTERIAL BLOOD PRESSURE is maintained within normal ranges by changes in cardiac output and peripheral resistance. Peripheral resistance and cardiac output are regulated by the peripheral and central nervous systems, as well as by many humoral factors. Peripheral resistance is primarily determined by the distal part of the arterial vasculature, which consists of the small resistance arteries and the arterioles (8). Disturbances in peripheral resistance are basic contributors to different cardiovascular pathologies. Therefore, alterations in the neurohumoral and mechanical systems that regulate the tone of resistance arteries may play an important role in the pathogenesis and progression of cardiovascular disease. The tone of resistance arteries depends on a complex interplay between endothelial and smooth muscle cells. Endothelial cells contribute to the regulation of vascular tone by releasing several vasoactive factors, some of which, such as nitric oxide (NO), endothelium-derived hyperpolarizing factor, and prostacyclin, are potent vasodilators; others, such as the peptide endothelin (ET)-1, prostanoids, such as thromboxane A2 (TxA2), prostaglandin H2, radical superoxide anion (O2•−) and components of the renin-angiotensin system, are vasoconstrictors. The most studied aspect of endothelial function is the regulation of vascular tone, and the association between endothelial dysfunction and vascular disease is well established. The term endothelial dysfunction refers to an imbalance in the production or bioavailability of endothelial vasodilator mediators, and such an imbalance may promote vasospasm and thrombosis and has been implicated in many cardiovascular disorders.

Inflammation can be the result of infection, trauma, ischemia, or immunologic processes. There is increasing evidence suggesting a link between infection or inflammation and the risk of cardiovascular disease (14, 37, 49). Because the inflammatory response is associated with cytokine release, cytokines may have an important role in the vascular injury induced by inflammation. Cytokines are soluble proteins or large peptides produced by leukocytes and other cell types, and they act as chemical communicators between cells. Some cytokines are involved in the effector phase of the inflammatory response and include proinflammatory cytokines, the most important being TNF-α, IL-1β, and IL-6, as well as anti-inflammatory cytokines, such as TNF-β, IL-4, IL-10, and IL-13. TNF-α is a multifunctional circulating cytokine derived from endothelial and smooth muscle cells as well as macrophages. IL-1β and IL-6 are cytokines with a broad range of humoral and cellular immune effects related to inflammation, host defense, and tissue injury.

Cytokines usually act synergistically on the initiation of the inflammatory cascade, leading to the expression of further factors (37). Thus cytokines can induce the expression of cytokine receptors and other cytokines, thereby constituting an amplification cascade. In addition, cytokines also induce the expression of several enzymes, such as inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2), which will, in turn, produce mediators with actions at the vascular level that contribute to the inflammatory response. During the onset of sepsis, an early appearance of cytokines in the serum is well established in rodents, whereas such patterns are less evident in humans (40). The change in cytokine levels that occurs during sepsis has been characterized in studies in which the endotoxin from gram-negative bacteria, lipopolysaccharide (LPS), is administered. TNF-α, IL-1β, and IL-6 are often generated in response to LPS by a mechanism that is regulated by nuclear
factor-κB (NF-κB) (37), which also regulates the transcription of many other mediators implicated in the inflammatory response (37). Nevertheless, as well as triggering a generalized response that involves increase of the above-mentioned cytokines, LPS increases a large number of other mediators and, in later stages of sepsis, anti-inflammatory cytokines (37, 40). A balance between the effects of the pro- and anti-inflammatory cytokines is thought to determine the outcome of this and other cardiovascular diseases, in the short or the long term.

The purpose of this review is to provide an insight into the effect of cytokines, mainly TNF-α, IL-1β, and IL-6, on the vascular reactivity of resistance arteries. As pointed out by Christensen and Mulvany (8), relatively large “feed arteries” offer substantial resistance. Thus, in agreement with these authors, we will use the term resistance arteries for arteries with an internal diameter of <400 μm. First, we will describe the effects of cytokines on vascular reactivity. Next, we will focus mainly on the effects of cytokines on endothelium-dependent vasodilator responses to assess the putative role of cytokines on endothelial dysfunction. Finally, we will summarize the most important changes in resistance arterial function in some vascular pathology with elevated cytokine levels.

**CYTOKINES AND VASCULAR REACTIVITY**

Cytokines induce genes that synthesize other peptides in the cytokine family and several mediators, such as prostanoids, leukotrienes, NO, bradykinin (BK), reactive oxygen species, and platelet-activating factor, all of which can affect vascular function. The vascular response appears to be related to the balance between all the vasoactive factors released under the influence of cytokines, and regional differences in release and responsiveness to these factors appear to contribute to the dilator or constrictor response observed within a specific vascular bed. The effects of cytokines on vascular reactivity also seem to rely on the exposure time, which, in turn, will influence mediator release. The possible mediators involved in the changed vascular reactivity in resistance arteries induced by cytokines are summarized in Fig. 1.

**Short-term exposure to cytokines.** Cytokines could exert rapid vasoactive effects in blood vessels, likely by acting on the endothelial and smooth muscle receptors of the vessels. Receptors for TNF-α and IL-1β have been found on endothelial and smooth muscle cells (23, 47). Iversen et al. (23), using distal ends of human internal mammary arteries, reported that elevated concentrations of TNF-α, IL-6, and IL-10 contracted the arteries but failed to relax norepinephrine-precontracted vessels. The observed vasoconstrictor effects were mediated by ETα receptors and were endothelium dependent. In contrast, in isolated human resistance arteries, IL-1β and TNF-α (alone or in combination), at a concentration similar to that found physiologically, did not exert any direct vascular effects on precontracted vessels (38).

In rat skeletal muscle arterioles, 2 min of incubation with TNF-α had no direct vasomotor effect. However, pretreatment with endotoxin allowed the TNF-α to cause arteriolar dilation, possibly through a mechanism involving COX and NOS (17). In these arterioles, 1 h of incubation with IL-1β and IL-6 produced a potent vasodilator effect in vivo, but not in vitro, suggesting that cytokine interaction with parenchymal or intravascular factors elicits arteriolar relaxation (32). On the other hand, IL-10, an anti-inflammatory cytokine, did not affect vascular responses to phenylephrine or ACh, although it did prevent the loss of vascular tone in skeletal muscle arterioles exposed to the endotoxin for 1 h (43).

Bronchial vascular resistance in sheep decreased after 20 min of infusion with TNF-α but increased and remained elevated 2 h after the start of infusion. The observed increase in bronchial vascular resistance was due to a secondary release of ET-1 (50). In agreement, exposing cultured endothelial cells to TNF-α enhanced ET-1 secretion (10). On the other hand,
ET-1 could induce cytokine release via NF-κB activation (49). Vasooconstriction and increase in ET-1 circulating levels have also been observed in rat coronary vessels after 15 min of TNF-α infusion (28).

In human forearm resistance artery, 1 h of exposure to TNF-α raised basal vascular resistance by increasing basal bioavailability of the vasoconstrictor prostanoids and reducing the basal bioavailability of NO (33). In rat aorta, 1 h of exposure to IL-1β enhanced the vasoconstrictor responses to angiotensin II by a mechanism that involves prostaglandin H2/ TxA2 (48). In agreement, serotonin-mediated vasoconstriction was enhanced by an increase in TxA2 production from COX-2 in rat middle cerebral arteries after 1 h of incubation with LPS (20).

**Long-term exposure to cytokines.** Proinflammatory cytokines play an important role in the systemic inflammatory response and secondary tissue damage in patients with sepsis (37). Endotoxins, in addition to other bacterial molecules, trigger a generalized response that involves the generation of the cytokines. Furthermore, long-term exposure to TNF-α and IL-1β induces a hyporeactivity to vasoressor agents that seems to be related to the large decrease in systemic resistance usually found in sepsis (1). In cerebral arterioles, application of TNF-α induced a progressive dilation, with a maximum increase in diameter at 4 h that was inhibited by aminoxygguanidine and dexamethasone, suggesting an important role for NO from iNOS in this vasodilation (5).

Cytokines can also enhance the vasoconstrictor responses mediated by different agonists. Thus segments from human temporal artery incubated in organ culture for 48 h with TNF-α or IL-1β enhanced the vasoconstrictor response mediated by ETβ receptors (51). Similarly, subchronic treatment for 3 days with IL-1β and IL-6 potentiated the ET-1- and norepinephrine-induced perfusion pressure without modifying perivascular nerve stimulation-evoked contraction in isolated rat mesenteric vascular bed. This increased contraction could be related to the impairment of endothelium-induced relaxation observed in the same study (12). In rat middle cerebral arteries, LPS increased vasooconstriction to serotonin from the 1st to the 4th h of incubation, whereas after 5 h the contraction to serotonin returned to the control value (20). An increased production of TxA2 from COX-2, O2, and H2O2 seems to enhance vasoconstriction to serotonin during the first hours of LPS exposure, and this would be counteracted by the increased iNOS and superoxide dismutase expression at 5 h (20). These results are interesting, because the use of pharmacological agents to inhibit the synthesis of NO by iNOS has been proposed in patients with septic shock. The presence of these inhibitors could unmask cytokine enhancement of the vasooconstriction induced by such important vasoactive agents as angiotensin II, ET-1, and serotonin.

All these results seem to suggest that changes in vascular reactivity as a result of short-term contact of vessels with cytokines are mainly due to the effects of the cytokines; however, the participation of mediators such as ET-1 or those derived from COX-2 expression, which is rapidly upregulated when exposed to cytokines (21), should not be excluded. However, the long-term effects of cytokines on vascular reactivity do involve the release of other mediators, such as NO from iNOS and prostanoids from COX-2.

**Cytokines and Endothelial Dysfunction**

Endothelial dysfunction and elevated levels of proinflammatory cytokines are observed in several cardiovascular diseases, such as congestive heart failure (CHF), atherosclerosis, septic shock, diabetes, and hypertension, and in aging (9, 14, 30, 37, 42, 49). Under the influence of cytokines, the endothelium-dependent dilatation can be impaired and the endothelium may lose its ability to respond to circulating hormones or autacoids. This effect may favor a predisposition to vessel spasm, thrombosis, or atherogenesis.

The influence of cytokines on endothelium-dependent relaxation has been analyzed in conductance and resistance arteries from humans and animals. In healthy volunteers, a mild systemic inflammatory response impairs endothelium-dependent dilatation to ACh and BK but does not influence endothelium-independent relaxation to nitroglycerin in resistance and conduit vessels (22). Similarly, brief exposure of human forearm resistance artery to TNF-α impairs vasodilatation to ACh (33, 39), probably through an increased basal bioavailability of vasoconstrictor prostanoids and reduced NO bioavailability (33). In isolated rat mesenteric resistance arteries, the effects of cytokines on endothelium-dependent relaxation have been studied at different exposure times. Thus, De Salvatore et al. (12) found that subchronic (3 days) in vivo treatment with IL-1β and IL-6 impaired the reduction of perfusion pressure induced by ACh. The incubation of these arteries in organ culture for 14 h with IL-1β almost abolished ACh-mediated relaxation, at least partly through increased O2 production in endothelial and smooth muscle cells (24). In contrast, when the incubation time was only 30 min, IL-1β had no effect on ACh-mediated relaxation, whereas TNF-α impaired the NO-dependent component of endothelium-dependent relaxation in response to ACh and BK without modifying responses to sodium nitroprusside (52). This effect may be attributable to the ability of TNF-α to increase the levels of O2, thereby inactivating NO (52). In fact, TNF-α has been seen to stimulate NADPH oxidase to generate sustained amounts of O2 in vascular smooth muscle cells (34). In agreement, in vitro LPS treatment (1–5 h) of rat middle cerebral arteries reduced the BK-induced endothelium-dependent relaxation by mechanisms that include production of NO from iNOS and release of O2, generated in part from COX-2 (21).

Cytokines may affect endothelial function in resistance arteries through a number of signaling mechanisms. Thus TNF-α is able to impair the stability of endothelial NO synthase mRNA (14). In addition, cytokines and LPS may also induce iNOS expression in vascular smooth muscle and endothelial cells (6, 20), and this would account for an excessive basal NO increase that might participate in the impairment of endothelium-dependent relaxation observed in the presence of LPS and cytokines. The nitration of protein tyrosine residues by peroxynitrite, due to the simultaneous generation of NO from iNOS and O2, could inhibit the enzymes involved in endothelium-dependent relaxation. It has been reported that a high concentration of NO, such as would be produced after iNOS, could downregulate endothelial NO synthase and soluble guanylate cyclase activity (7, 35).
CYTOKINES AND PATHOLOGY OF BLOOD VESSELS

As pointed out above, cytokine levels are elevated in several vascular pathologies. Current data indicate that plasma levels of inflammatory biomarkers, such as TNF-α or IL-1β, can be clinically useful in identification of individuals at high risk for future cardiovascular events (3). The cellular events triggered by these cytokines could participate in the initiation, progression, and tolerance of the inflammatory process associated with many vascular diseases in a way similar to that reported for TNF-α in stroke (18). We will describe alterations in the vascular function of resistance arteries that occur in some cardiovascular diseases and in aging, where plasma levels of cytokines are reported to be elevated.

Diabetes. The increased expression and action of various cytokines and growth factors in diabetes appear to be a consequence of hyperglycemia-induced events and may play a role in vascular complications, including atherosclerosis and alterations in the vascular reactivity associated with diabetes (9). Nevertheless, the changes observed in the reactivity of resistance arteries from diabetic patients and from animal models of experimental diabetes remain controversial. Thus increases in response to norepinephrine-mediated vasoconstriction and no changes in response to serotonin-, vasopressin-, and norepinephrine-mediated vasoconstriction have been reported in mesenteric resistance arteries from diabetic rats (9, 46). Furthermore, subcutaneous resistance vessels from patients with type 1 diabetes showed an increase in vasoconstrictor response to ET-1 but not to norepinephrine (31). In contrast, contraction to ET-1 was diminished in resistance arteries from patients with type 2 diabetes (41). Experimental and clinical evidence for the presence of impaired endothelium-dependent vasodilation in types 1 and 2 diabetes are widely reported (13). However, normal endothelial function has also been reported in patients with type 1 diabetes (31). Although TNF-α has been implicated in the endothelial dysfunction that appears in diabetes, lowering the levels of this cytokine with pentoxifylline in type 2 diabetic patients had no effect on endothelial dysfunction in conductance and resistance vessels (2).

Hypertension. Increasing evidence indicates that hypertension could be considered a chronic inflammatory disease with elevated proinflammatory cytokine blood levels (49). In animals and humans, the proinflammatory role of angiotensin II has been clearly established by upregulation of adhesion molecules and cytokine expression through NF-κB activation. In addition, angiotensin II increases oxidative stress, which will contribute to inflammation and decrease NO bioavailability. The role of angiotensin II in triggering the inflammatory response has been clearly established in large conduit vessels, but its participation in small resistance arteries has been less studied (49). ET-1 is also an important mediator of inflammation in the arterial wall that can upregulate cytokines and adhesion molecules. A correlation between elevated plasma levels of inflammatory mediators and ET-1 has also been reported in hypertensive patients (36). Hypertension can predispose to and accelerate atherosclerosis through an activation of the inflammatory mechanism, in which angiotensin II and ET-1 also play an important role. Arterial reactivity to ET-1 is altered in hypertension, but some discrepancies exist between the vasoconstrictor effect of ET-1 in vitro, in small arteries from human subcutaneous biopsies, and in vivo, for forearm blood flow from hypertensive patients (45). These discrepancies could be related to differences in the vascular bed studied, the degree of endothelial dysfunction, and/or the presence or absence of atherosclerosis (45).

Preeclampsia is characterized by a severe increase in vascular resistance and arterial pressure associated with changes in endothelial function and smooth muscle contraction. Placental ischemia during preeclampsia may promote the release of several factors, including cytokines. Thus the plasma levels of TNF-α and IL-6 are elevated in women with preeclampsia (26). These cytokines may decrease the production and/or the bioavailability of endothelium-derived relaxing factors, stimulate the release of endothelium-derived contracting factors and oxidative stress, and activate the renin-angiotensin system, which could contribute to a greater vasoconstriction and the increased peripheral resistance and arterial pressure observed in preeclampsia (26).

CHF. There is increasing evidence that cytokines in general and TNF-α in particular play an important role in CHF (15). A relation between plasma levels of TNF-α and the degree of endothelial dysfunction has been found in patients with CHF (25). TNF-α modulates cardiac contractility and peripheral resistance, the two most important hemodynamic determinants of cardiac function. The deficit in peripheral vasodilator capacity, at least in part, results from attenuated vascular endothelial function and has been attributed to a loss of the ability of the endothelium to release NO. The administration of etanercept, a recombinant TNF receptor that binds to and functionally inactivates TNF-α, has been reported to improve the functional status of patients with CHF (4) and the systemic endothelial vasodilator capacity in patients with advanced heart failure (16). Because an impaired functional capacity of peripheral blood vessels to dilate in response to increased blood flow is a major determinant of the degree of exercise intolerance in patients with CHF (19), the beneficial effects of etanercept on systemic endothelial vasodilator capacity might help improve the functional status in these patients. However, clinical trials to evaluate TNF-α inhibition therapy using etanercept or infliximab in patients with CHF (27) did not show a clear beneficial effect of these drugs over the placebo. The participation of other cytokines in CHF and their complex interactions could help explain the apparent contradictions in studies of the clinical benefits of TNF-α inhibition.

Aging. Diminished endothelial relaxation and increase, decrease, or no change in contractile responses to several agonists are associated with aging (29). Repeated inflammatory episodes are thought to be among the environmental factors that might be involved in the aging process and alter the homeostasis of the affected organs (29). Aging is also associated with a proinflammatory shift in the cytokine expression profile in endothelial and smooth muscle cells in rat coronary arteries (11). The age-related increase in arterial wall cytokine production could be related to age-associated changes in vascular function through altered production of mediators synthesized by iNOS and COX-2. We observed that incubating mesenteric resistance arteries with IL-1β in organ culture for 14 h upregulated iNOS and COX-2 expression. Thus age differences in NO and prostanooid production could be responsible for the diminished contractile response to phenylephrine observed in young rats after IL-1β treatment compared with the response in similarly treated arteries from older rats (6). In small mesen-

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teric arteries from aged rats, a reduced vasodilatation to ACh, probably due to an alteration in the balance between endothelial NO and vasoconstrictor products from COX, has also been reported (30, 42). In addition, the NO-dependent mechanisms that modulate vessel contraction decreased, whereas COX-2 mediators increased, in rat mesenteric resistance arteries, resulting in an increase in contractile response in older rats (42). However, a clear relation between elevated levels of cytokines and changes in vascular reactivity observed in resistance vessels from aged animals has not been reported.

In summary, cytokines may induce vasoconstriction per se and decrease or increase vasoconstrictor responses induced by different agonists. Regional differences in the release and responsiveness of mediators released by cytokines appear to contribute to the vasodilator or vasoconstrictor response observed in a specific vascular bed. In addition, an impairment of endothelium-dependent vasodilator responses has been widely described in conductance and resistance arteries exposed to cytokines. The fact that cytokine levels are elevated in several cardiovascular pathologies where the existence of endothelial dysfunction has also been described supports a link between inflammation and these cardiovascular diseases. Vascular cells may also develop anti-inflammatory counterregulatory mechanisms, including anti-inflammatory cytokines, that maintain vascular wall integrity and homeostasis (44). Although current data clearly indicate elevated plasma levels of inflammatory cytokines in several cardiovascular pathologies, an anticytokine therapy has not been proven clearly effective. An understanding of the anti-inflammatory pathways could identify future targets with promising novel therapeutic possibilities.

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