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COMMENTARY

Evidence for Vascular Tone Regulation by Resident or Infiltrating Leukocytes

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ABSTRACT. The normal vascular wall contains resident leukocytes, notably tissue macrophages (histiocytes) and mast cells, that confer a rapid, eicosanoid-dependent vasoconstrictor response to agonists typical of leukocytes, such as the complement-derived anaphylatoxin C5a or the formylated peptide f-Met-Leu-Phe (isolated organ methodology). The eicosanoid-dependent vasomotor response is even more intense in pathologies that involve leukocyte infiltration of the blood vessel wall, such as atherosclerosis and serum sickness in the rabbit. The leukocyte compartment of the blood vessel is the likely source of vasoactive mediators (eicosanoids, radicals, cytokines) of physiopathological importance, with possible application in cardiac ischemia, lupus nephritis, vasculitides, and graft rejection. This line of investigation may be compared to the discovery and characterization of endothelium-dependent vasomotor responses. However, the problem is experimentally more demanding: histological correlations, experiments based on leukocyte depletion, reconstitution, and enrichment are useful approaches to document this form of circulatory control. BIOCHEM PHARMACOL 52;10:1481–1488, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. anaphylatoxin C5a; *N*-formyl-methionyl-leucyl-phenylalanine; tissue leukocytes; macrophages; vasoconstriction

Blood flow through various vascular beds is regulated by multiple neurogenic and local mechanisms. The modulation of tone of vascular smooth muscle by the action of other cell types present in the blood vessel structure has been well demonstrated through the discovery of endothelium-dependent vaso-relaxation [1]. We will review, in this paper, evidence for the regulation of blood flow by mediators derived from tissue leukocytes. Much of the current evidence is based on work performed using isolated vascular tissue, maintained in a blood-free medium and exposed to chemical stimuli selective for leukocytes, such as those chemotactic peptides usually studied by immunologists. Vasomotion dependent on vascular wall leukocytes has the potential to become an independent line of investigation different from the much studied effects of circulating PMN* leukocytes on blood flow regulation. Indeed, PMN leukocytes have been shown to release vasoactive mediators [2–6], to contribute to blood viscosity when activated [7], to obliterate microvessels in the "no reflow" phenomenon typical of ischemia–reperfusion models [8], and to impair endothelium-dependent vaso-relaxation [9–11]. These specific topics, and the mechanism of diapedesis [12], are not covered in the present paper.

EXPERIMENTAL APPROACH OF LEUKOCYTES PRESENT IN THE BLOOD VESSEL WALL STRUCTURE

Conventional histologic and immunohistochemical studies show that resident leukocytes are found abundantly in the connective tissue lining blood vessels of all sizes [13]. Mast cells and macrophage-like phagocytic cells (histiocytes) are found most abundantly in the tunica adventitia but also in smaller vessels where the histological distinction between vascular layers is unclear; these leukocytes can reside relatively close to the blood flow. Reticuloendothelial phagocytes and, at least in some species, pulmonary intravascular macrophages are in direct contact with blood [14]. All these cellular elements are ready to be recruited to combat antigens and microorganisms that enter via the bloodstream. In addition, in Western societies, the aorta of most young adults exhibits macrophage-rich pre-atherosclerotic lesions in the subendothelial layer [15]. The possible contribution to vascular tone by leukocytes that have migrated from blood to the vascular wall during a pathologic state has been only superficially explored.

There is no uniformly effective method to stimulate, remove, or inhibit the function of tissue leukocytes when studying the tone of blood vessels. This contrasts with the

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* *Abbreviations:* C5a, complement fragment 5a; f-Met-Leu-Phe, *N*-formyl-methionyl-leucyl-phenylalanine; f-Nle-Leu-Phe-Nle-Tyr-Lys, *N*-formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tyrosyl-lysine; LT, leukotriene; PG, prostaglandin; PMN, polymorphonuclear; and TX, thromboxane.

easy removal of the endothelium, which has been instrumental in proving the vasomotor role of the endothelium [1]. Therefore, several circumstantial lines of evidence must be considered together to support a hypothesis of leukocyte-dependent vasomotricity. We will do so by reviewing and discussing a limited number of studies that may support the hypothesis of a tissue leukocyte-dependent circulatory control.

The fact that certain pharmacologic stimuli that are relatively selective for leukocytes may influence the circulation is, in itself, a line of evidence for leukocyte-dependent vascular response. Understanding the effects of the chemotactic peptides f-Met-Leu-Phe and C5a is particularly helpful when discussing mechanisms of the leukocyte–smooth muscle cooperation, as the smooth muscle and vascular endothelial cells probably do not respond directly to these agents in most species and organs. The formylated tripeptide f-Met-Leu-Phe results from a purely empirical structure–activity program and is based on the assumption that bacterial and mitochondrial proteins, which possess a formylated Met residue at the NH_2 terminus, would be recognized by phagocytic cells [16, 17]. The receptor for f-Met-Leu-Phe belongs to the family of G-protein-coupled receptors [18].

Another well-characterized chemotactic peptide for phagocytic leukocytes is the anaphylatoxin C5a derived from serum complement. The chemical properties and formation of this 74 residue peptide are reviewed elsewhere [19]. Within the multifunctional complement system, anaphylatoxin C5a plays a prominent role as a pharmacologically active fragment of C5 that activates various leukocyte functions. Mast cells and basophils release histamine and other secondary mediators in response to C5a. In phagocytic leukocytes, namely neutrophils and monocytes/macrophages, several distinct activation pathways are stimulated by C5a; there is a chemotactic and chemokinetic response, an increased eicosanoid formation, a release of reactive radicals and of granule content [19], an increased adherence to cultured endothelium [20], and a potentiation of the synthesis of cytokines in the monocyte [21]. The receptor for C5a also belongs to the G-protein-coupled family of receptors [22].

Receptors for f-Met-Leu-Phe or C5a generally can be inferred to be absent from non-leukocyte lineages [23, 24], but important exceptions may exist; notably, hepatocytes may express receptors for both peptides [25]. Thus, vascular response to these peptides could be attributed generally to a leukocyte target cell, but this should be interpreted with caution in each case. This concern about specificity will be addressed below when considering certain experimental systems.

DO RESIDENT VASCULAR LEUKOCYTES INFLUENCE THE CIRCULATION?

Consistent with a direct vascular response to chemotactic peptides, several animal vascular smooth muscle preparations, maintained in leukocyte-free physiologic solutions, respond mechanically to C5a and also to the unrelated chemotactic peptide f-Met-Leu-Phe. This type of experimental system is particularly helpful in studying the contribution of secondary mediators to biologic effects.

Some isolated guinea pig blood vessels respond to C5a by a contraction that is mediated by a mixture of histamine and prostanoids, based on pharmacologic evidence (suppressive effect of H_1 receptor antagonists and of cyclooxygenase blockers) [26, 27]. Mediator measurements in the physiologic fluids bathing these tissues proved that immunoreactive TXB_2 and histamine are released rapidly when C5a is added [23, 28]. The partial dependence of response upon histamine, as well as the presence of preformed histamine in guinea pig vascular tissues [28], suggests the involvement of at least one type of tissue leukocyte, the mast cell, in this system.

Macroscopic rabbit blood vessels contain a relatively dense population of resident leukocytes, mainly subendothelial macrophages, with the notable exception of the thoracic aorta where this population is much less dense (reportedly, 1 cell/mm^2) [29, 30]. Accordingly, the vasomotor effect of both families of chemotactic peptides (anaphylatoxins and formylated peptides) has been described in isolated vascular strips from the rabbit [31–35]. The rabbit aorta is not responsive to either type of peptide, whereas the portal vein and main pulmonary artery are sensitive to both [31, 33]. A biphasic response, composed of a short contraction followed by a longer relaxation, is recorded when these tissues are exposed to nanomolar concentrations of the chemotactic peptides. This relaxation is best observed in tissues that have been precontracted with another agent because these isolated tissues exhibit no significant tone [32]. There was no significant tachyphylaxis observed in these systems when 90 min was allowed between challenges [34]. Pharmacologic analysis revealed that the structure–activity relationships for the peptides that had been derived from the study of human PMN receptors are well conserved in the rabbit vessels. Notably, the deformylated peptide Met-Leu-Phe is a low potency, but full agonist. The f-Met-Leu-Phe antagonist Boc-Phe-D-Leu-Phe-D-Leu-Phe has no direct effect on vascular tone, but acts as a competitive antagonist of the formylated peptide-induced effects [32]. This also holds true with C5a receptors in rabbit vessels as shown by a set of small peptides recently developed as C5a agonists on human PMN leukocytes that retained their relative activity and the biphasic myotropic profile of the parent anaphylatoxin [35]. Release of a secondary mediator was studied in rabbit vascular strips challenged with f-Met-Leu-Phe or C5a. This revealed a major, but not exclusive role for cyclooxygenase products of the arachidonate cascade as mediators of smooth muscle contraction and relaxation (based on the effect of inhibitory drugs and on the measurement of the major eicosanoid produced in these tissues, 6-keto-$PGF_{1\alpha}$) [31, 32, 34]. Very little TXA_2 is produced by these large rabbit vessels, either under basal or C5a-stimulated

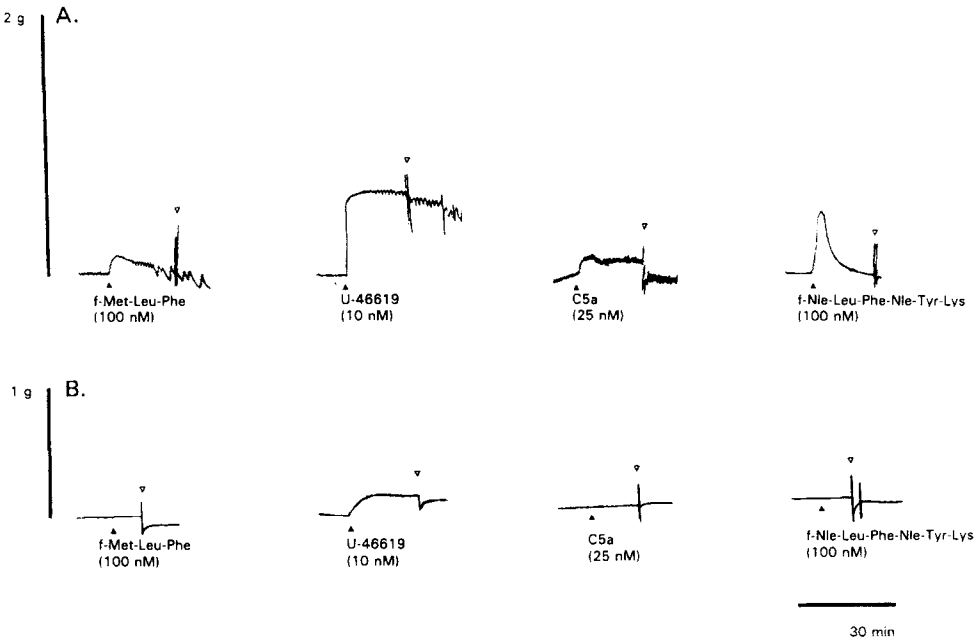

FIG. 1. Effect of chemotactic peptides and other agents on the human isolated umbilical artery. (A) Responses of a fresh artery obtained post-partum, prepared as a strip [28]. (B) Responses in vascular equivalents made of pure smooth muscle cell cultures [40]. Abscissa scale: time; ordinate scale: isometric contraction. Closed triangles refer to the application of agents; open triangles refer to the first washout of stimulants.

conditions [31]. This may not be representative of the general circulatory effect of C5a because the evidence reviewed below points to the major role of vasoconstrictor prostanoids in the *in vivo* effect of C5a in this animal [36]. In addition, the relaxant phase that follows the application of either type of peptide on the rabbit portal vein is partly dependent on nitric oxide of non-endothelial origin [34].

The mechanism of action of C5a and f-Met-Leu-Phe also has been analyzed in detail in the isolated human umbilical artery preparation, where these peptides elicit consistent contractile effects in nanomolar concentrations [28] (Fig. 1A). Apparently, this particular tissue contains neither histamine nor mast cells [28] and is not innervated by the sympathetic nervous system [37]. The effect of C5a or f-Met-Leu-Phe was not abrogated by endothelium removal or by the drug AA-861 (a 5-lipoxygenase antagonist), but was reduced dramatically by treatment with indomethacin, dazmegrel (a TXA_2 synthase inhibitor) or SQ-29548 (a TXA_2 receptor antagonist) [28]. Autoradiography of ^{125}I-labelled C5a or of a formylated peptide analogue showed specific binding of the peptide to cells dispersed in the vessel wall, most frequently at the periphery. Cells staining positively for α-naphthyl acetate esterase, a macrophage marker, showed a distribution similar to that of cells binding ^{125}I-labelled chemotactic peptides. Endothelial or smooth muscle cells apparently did not bind to the labelled peptides. Pure cultures of smooth muscle cells derived from the umbilical artery failed to release prostanoids when exposed to C5a or f-Met-Leu-Phe, whereas fresh strips of this artery released more TXB_2, compared with the baseline level, in response to the peptide. It was concluded that macrophage-like cells, present in the vessel wall, were the likely target cells for the chemotactic peptide. These cells trigger a contractile effect on the smooth muscle by generating cyclooxygenase products such as TXA_2 and perhaps PG endoperoxides [28]. It is well known that human and animal macrophages produce PGs and TXA_2 in response to various stimuli [38]. Additional circumstantial evidence of the involvement of tissue macrophages may be derived from the fact that LTB_4 and interleukin-8, relatively selective neutrophil stimuli, produce virtually no response in the umbilical artery ([28] and unpublished results). Rings of human coronary artery also respond to f-Met-Leu-Phe by an eicosanoid-dependent contraction that appears to be very similar to that documented in the umbilical artery [39]. This model may explain many of the circulatory effects of C5a in various animal models and vascular beds.

Tissue-engineered vascular equivalents made from homogeneous cultured smooth muscle cells and endothelial cells derived from human umbilical vessels have been reported [40]. The purity is strictly controlled in these experiments, as histiocytes cannot maintain themselves during subculture of the vascular cells. We have used equivalents made only from smooth muscle, as they retain part of their contractile effect.[†] While these were consistently responsive to several agonists, including the TXA_2 mimetic U-46619, none of the equivalents tested responded to f-Met-Leu-Phe, f-Nle-Leu-Phe-Nle-Tyr-Lys, or C5a (Fig.

[†] L'Heureux N, Germain L and Auger FA, unpublished results. Cited with permission.

1B). These experiments support the existence of a cell compartment different from the smooth muscle cell which is sensitive to chemotactic stimuli.

Significant evidence supports the vasoconstrictive role of the chemotactic peptides C5a and f-Met-Leu-Phe in the coronary circulation in many species with the notable exception of the dog (Table 1). There is controversy as to whether circulating PMN leukocytes participate in increasing coronary resistance during challenge with C5a and f-Met-Leu-Phe; the conflicting evidence, sometimes from the same system, is summarized in Table 1 (see also Gardinali *et al.* [41]). It can be seen that the chemotactic peptides are generally vasoconstrictor agents, even in hearts perfused with cell-free medium. The presence of PMN leukocytes in whole blood, however, may potentiate the vasoconstrictor effect under certain circumstances. The PG-dependent contractile effect of nanomolar concentrations of f-Met-Leu-Phe in strips of human coronary arteries incubated in a blood-free medium strongly suggests that the vessel wall cells are sufficient to account for a vasoconstrictor effect of chemotactic peptides [39]. An interesting line of investigation using the porcine heart suggests that TXA_2, derived from mast cells, accounts for an important component of the coronaroconstrictor effect of C5a due to a parallel release of histamine and the preventative effect of lodoxamide, an agent that inhibits mast cell degranulation [42].

Chemotactic peptides produce vascular spasm in other vascular beds, even in the absence of circulating leukocytes. Zymosan-activated plasma, a crude source of C5a, is a potent and TXA_2-dependent vasoconstrictor in the perfused lung of the dog, in the absence of leukocytes in the perfusion fluid [43]. Another study suggested that a material, apparently identical to C5a, explained the vasoconstrictor effect of heterologous plasma in isolated perfused pig kidneys [44]. This is a possible mechanism for complement-dependent hyperacute graft rejection.

Both the formylated and the anaphylatoxin families of chemotactic peptides elicit important hemodynamic effects when injected *in vivo* in normal animals. For instance, f-Met-Leu-Phe is hypotensive in rabbits [45] and C5a is generally hypotensive and elicits pulmonary hypertension in several species [23, 46]. Since the circulating leukocytes are an obvious target for these peptides, such systems may not illustrate the role of resident vascular leukocytes, unless specific additional measures are taken. For instance, C5a-induced hemodynamic effects in the rabbit are not prevented by nitrogen mustard-induced neutropenia [36]. These responses to human purified or recombinant C5a consist of dose-dependent hypotensive effects that are suppressed by either indomethacin or dazoxiben, a TXA_2 synthase inhibitor [35, 36]. The effector role of vasoconstrictor prostanoids can be reconciled with hypotension, as the primary cause for this circulatory change was a drop in cardiac output; the peripheral resistance was not changed in most

TABLE 1. Do chemotactic peptides influence coronary artery resistance via circulating cells or by an action on the vessel wall?

Species	Experimental system	Main observations	Secondary mediators	Ref.
Pig	Heart perfused *in situ* with C5a	Non-tachyphylactic vasoconstriction	TXA_2	[63]
		Vasoconstriction, ischemia, no role for circulating PMN leukocytes	TXA_2, LTs	[64–66]
		The inhibition of mast cell degranulation prevents the coronaroconstrictor effect of C5a	No role for histamine	[42]
		Massive PMN sequestration; little effect of C5a in cell-free medium	Vascular obliteration with PMN leukocytes proposed	[67]
		Prevention by anti-CD18 antibodies of C5a-induced vasoconstriction and PMN extraction from blood	TXA_2 formed in blood by a PMN–platelet interaction	[68, 69]
		Platelet and PMN depletion does not prevent the effect of C5a	TXA_2 or tissue origin	[47]
Rabbit	Heart perfused *in situ* with f-Met-Leu-Phe	Intense vasoconstrictor effect if perfused with blood, not with cell-free medium	PMN leukocytes	[70]
		Vasoconstrictor effect abolished by leukocyte depletion or a platelet-activating factor antagonist	Platelet-activating factor	[71]
	Heart perfused *in vitro* with f-Met-Leu-Phe	Significant vasoconstrictor responses	Sulfidopeptide LTs, TXA_2	[72, 73]
		Vasoconstriction much increased in infarcted hearts infiltrated with leukocytes	Sulfidopeptide LTs	[52]
Human	Isolated coronary arteries challenged with f-Met-Leu-Phe	Concentration-dependent contraction	Vasoconstrictor prostanoids	[39]
Dog	Heart perfused *in situ* with C3a or C5a	Increased coronary blood flow with no adverse consequences		[74]

organs as demonstrated by the microsphere technique [36]. A specific target vascular bed in the rabbit appeared to be the lung circulation, as the central venous pressure increased during C5a-induced hypotension.

TXB_2 release and vasoconstriction in porcine heart infused *in situ* with C5a also occur in animals depleted of platelets and PMN leukocytes [47]. This experiment also tends to indicate a C5a-activable tissue source for TXA_2 release. Also, the infusion of zymosan-activated plasma (a crude source of C5a) into the femoral artery sharply increases the downstream vascular resistance in the pig; cyclophosphamide-induced neutropenia or α-adrenergic blockade failed to prevent this vasoconstrictor effect [48].

CIRCULATORY CONTROL BY LEUKOCYTES THAT HAVE MIGRATED INTO INJURED TISSUES

The possible contribution to vascular tone by leukocytes that have migrated from blood to the vascular wall during a pathologic state is still largely speculative but is supported by some experimental evidence. In pathologic states such as atherosclerosis or various forms of vasculitis, specific types of leukocytes migrate from blood to the injured vascular structures, and there is evidence that macrophages can even proliferate in atherosclerotic lesions in humans and rabbits [49].

As mentioned above, the rabbit aorta has a low density of resident macrophages [29, 30] and, when tested *in vitro*, very little response to either f-Met-Leu-Phe or C5a [31, 32]. However, experimental pathologies that favor aortic wall infiltration by leukocytes, dietary atherosclerosis or serum sickness, sensitize the rabbit aorta to these peptides [50]. Aortic rings isolated from hypercholesterolemic animals exhibited a rapid and relatively sustained (10–20 min) contractile response when challenged by f-Met-Leu-Phe or C5a [50]. Aortic rings derived from rabbits with serum sickness (13 days post BSA injection) exhibited brief contractions in response to either peptide. In both models, tissues from normal weight-matched animals were not consistently responsive to these peptides. The cyclooxygenase inhibitor indomethacin extensively reduced the contractile effect of either peptide on precontracted aortic rings in both models. Chemotactic peptide-induced increased prostanoid secretion was evident only in the fluid bathing atherosclerotic aortic rings. Morphological correlations included the demonstration of cells positive for a rabbit macrophage marker (RAM-11, a monoclonal antibody) and the C5a receptors (CD 88 antigen) in tissues from rabbits with hypercholesterolemia (numerous clusters of cells) or serum sickness (modest infiltration). Control aortic rings responded to f-Met-Leu-Phe by a significant contraction if cultured for 2 hr in the presence of resident peritoneal cells (84% macrophages), but not of a high density of peripheral blood leukocytes (<0.5% monocytes). It was concluded that infiltrating or adherent macrophages in the blood vessel wall confer to some phagocyte activating peptides the role of eicosanoid-dependent vasoconstrictor agents [50]. Related to these findings, aortic rings from cholesterol-fed rabbits release more superoxide anions than controls, either under basal conditions or following stimulation of the respiratory burst of infiltrating leukocytes with phorbol 12-myristate 13-acetate [51]. This observation suggests additional types of vasomotor control by infiltrating leukocytes, as superoxide anions functionally antagonize nitric oxide.

Myocardial infarction is followed by massive leukocyte infiltration composed initially of PMN leukocytes and, after a few days, monocytes/macrophages. In a model of myocardial infarction in the rabbit, the capacity of cardiac tissue to release cyclooxygenase products (TXB_2, PGI_2, PGE_2) in response to f-Met-Leu-Phe or bradykinin increased sharply and was correlated with the presence of leukocyte infiltration [65]. The perfused coronary preparation (Langendorff's technique) was used to show that the vasoconstrictor effect of f-Met-Leu-Phe is comparatively weak in normal rabbit hearts, but very intense in 4-day infarcted hearts; pharmacologic evidence points out the major role of sulfidopeptide LTs (LTC_4, LTD_4) as secondary mediators of this coronaroconstrictor response [52]. Thus, the cell element responsive to f-Met-Leu-Phe, most likely the infiltrating leukocyte, triggers a series of events that may lead to further ischemia and inflammation. Similarly, the formylated peptide-induced increase in TXA_2 and PGE_2 in the venous effluent was detected only in the inflamed perfused rabbit colon [53].

A model of dietary atherosclerosis in the cynomolgus monkey has been used repeatedly to illustrate the role of activated leukocytes in vascular control [54–56]. f-Met-Leu-Phe infusion *in vivo* increased the resistance of large arteries of the affected monkeys, but not that of normal animals; the effect of C5a was inhibited by the TXA_2 antagonist SQ 29548. In this model, there was a dense accumulation of monocytes-macrophages in the intima and media of atherosclerotic arteries [56]. Thus, there is a correlation between the vasomotor effect of the chemotactic peptides and the presence of infiltrating leukocytes in vessels of a size affected by atherosclerosis. However, the role of circulating leukocytes and other blood elements (such as platelets) is difficult to exclude in this system.

CLINICAL IMPORTANCE OF VASOSPASM INDUCED BY VASCULAR WALL LEUKOCYTES

Evidence reviewed above suggests that resident or infiltrating vascular leukocytes may be the source of vasoconstrictor and proaggregant eicosanoids under the effect of suitable stimuli. While the detrimental role of complement on the circulation has been amply documented, available clinical data usually do not allow one to differentiate the relative importance of circulating versus tissue leukocytes in a given syndrome. For instance, in lupus nephritis in humans, a spontaneously occurring renal disease associated with complement activation by immune complexes, a selective TXA_2 receptor antagonist has been shown to improve renal

function due to a favorable effect on the renal circulation [57]. Low doses of aspirin, capable of suppressing TXA_2 release by platelets, did not produce a similar effect. Therefore, it is possible that C5a-induced TXA_2 production takes place in tissue leukocytes in this pathology. Similarly, LTs and TXA_2 are formed during renal rejection episodes and exert a detrimental local circulatory effect on the kidney; these lipid mediators are formed presumably by monocytes-macrophages within the graft [58].

Angina pectoris is commonly caused by a fixed reduction of the coronary artery lumen, although spastic events may contribute to the clinical manifestations in some patients. These individuals are said to suffer from variant (Prinzmetal) angina; histologic evidence obtained at autopsy suggests that mast cells may be more abundant not only in patients with clinical variant angina but also with relatively advanced coronary atherosclerosis [59–61]. In fact, mast cells, macrophages, and T lymphocytes were increased in the coronary vascular wall at the site of erosion or rupture that caused myocardial infarction [61]. Thus, leukocyte stimulation, perhaps via complement peptides [41], may contribute to cardiac ischemia and thrombosis, as some of the typical vasoactive mediators (e.g. TXA_2, PGH_2) released by infiltrating leukocytes are also platelet aggregants.

Vasculitides of various types are associated with blood vessel wall infiltration with various types of leukocytes. Septicemia and pancreatitis are examples of disorders associated with massive systemic complement activation. Iatrogenic systemic complement activation also occurs, as in hemodialysis. In all these situations, both circulating and resident/infiltrating leukocytes may influence the circulation and precipitate ischemic complications. For instance, dietary atherosclerosis in rabbits increases the lethality of bacterial endotoxin and the plasma tumor necrosis factor-α levels in response to endotoxin [62], suggesting that the increased mass of infiltrating vascular macrophages was recruited to produce fatal circulatory complications.

CONCLUSIONS

The lines of evidence for leukocyte-dependent vasomotion can be summarized as follows:

1. Resident leukocytes, particularly mast cells and histiocytes, are commonly found in the blood vessel connective structure. The relative role of these cell types may vary, as such, for the human umbilical artery which contains no mast cells.
2. f-Met-Leu-Phe, a relatively selective stimulus of phagocytic leukocytes, and C5a, a stimulus of phagocytes, mast cells and basophils, often exert direct vasomotor effects on fresh vascular tissue in leukocyte-free medium and also in organs perfused with leukocyte-free medium.
3. Abundant pharmacologic evidence of secondary mediator release is obtained in these systems; some of the mediators are relatively typical of those released by leukocytes (histamine, LTs, nitric oxide). Vasoconstrictor cyclooxygenase products are often prominent secondary mediators of the vascular reactions to chemotactic peptides. The results of several experiments suggest that a cascade of mediator release originates from a leukocyte population.
4. Cultured vascular smooth muscle or endothelial cells generally fail to respond to chemotactic peptides.
5. Pathologies that favor leukocyte infiltration of the vascular wall and surrounding tissues confer a vasomotor effect on chemotactic peptides.

In this paper, emphasis was put on two peptides that stimulate G-protein-coupled receptors in leukocytes. It is expected that some of the recently characterized immunological hormones, the cytokines, may also influence the circulation by more or less selective actions on leukocytes. It is also well known that leukocyte-derived radicals or cytokines (e.g. interleukin-1 and tumor necrosis factor-α) affect vascular functions in several ways. Many other issues remain unresolved, such as the state of activation of infiltrating leukocytes and the relative expression of some genes controlled by stress, such as cyclooxygenase-2, in models where prostanoids are involved. Consequently, there are ample opportunities for more experimentation in this field.

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