The acute inflammatory reaction

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Introduction

Acute inflammation involves a myriad of cellular and humoral mechanisms which produce rapidly developing increases in vascular permeability, changes in blood flow, and the mobilization, accumulation and activation of leukocytes, mainly neutrophils and monocytes. Recent work has illuminated several important aspects of the acute inflammatory response which will be discussed here: (1) adhesive interactions between leukocytes and vascular endothelial cells; (2) leukocyte penetration of the blood vessel wall; (3) leukocyte chemotaxis; (4) mechanisms of tissue injury, and (5) the overall biological role of cytokines in inducing some of these responses.

Adhesive interactions between neutrophils and endothelial cells

Neutrophil adhesion to the blood vessel wall is an early event in the inflammatory response and is necessary for leukocyte accumulation within the inflammatory site. On the basis of *in vitro* studies, several mechanisms that could be responsible for mediating this critical interaction between neutrophils and blood vessel endothelial cells have been described (Fig. 1).

Critical to understanding neutrophil–endothelial cell adhesive mechanisms in inflammation is the idea that the specificity of the reaction *in vivo* seems to reside in an altered endothelium. That is, neutrophils appear to adhere only to modified endothelium, rather than at random, as would be expected if neutrophil activation were the initiator of adhesion. Recognition of this can be found in some of the earliest studies in experimental pathology (Cohnheim, Lectures on General Pathology, New Sydenham Society, 1889). Studies elucidating some of these changes in the endothelium have resulted in new insights into adhesive interactions.

ELAM-1

The best understood mechanism of endothelial-based neutrophil–endothelial cell adhesion results from the synthesis of endothelial leukocyte adhesion molecule (ELAM)-1 by endothelial cells and its expression at their apical surface after activation of the endothelium by cytokines. ELAM-1 was identified using a panel of monoclonal antibodies raised against cytokine-treated endothelial cells. These were screened for their ability to bind selectively to activated endothelial cells and to inhibit neutrophil adhesion to cytokine-activated endothelial cells (Bevilacqua *et al.*, *Proc Natl Acad Sci USA* 1987, 84:9238–9242). Use of the antibodies led to the definition of a molecule that could be immunoprecipitated from activated endothelial cells and the cloning of ELAM-1 from an endothelial complementary DNA (cDNA) library expressed in COS cells [1]. A single transcript of 3.9 kb appears to result in the translation of two peptides of similar molecular weight, probably differing only in post-translational modifications. The ELAM-1 molecule known to induce neutrophil adhesion on its own, as COS cells transfected with ELAM-1 are capable of binding neutrophils.

The kinetics of transcription of ELAM-1 messenger RNA (mRNA), followed using the cloned cDNA, the appearance of protein in the endothelial cytoplasmic membrane detected with an antibody to ELAM-1, and the induction of neutrophil adhesiveness following cytokine stimulation, are all consistent and show a significant induction at 0.5 h, with activity peaking at 4–6 h and waning over 24 h. This also closely follows the kinetics of neutrophil accumulation *in vivo* after cytokine administration and in inflammatory responses generally. ELAM-1 is induced on endothelial cells after treatment with the cytokines tumor necrosis factor (TNF)α, lymphotoxin (TNFβ), and interleukin (IL)-1β, and also after endothelial cells have contacted bacterial endotoxin (Bevilacqua *et al.*, *Proc Natl Acad Sci USA* 1987, 84:9238–9242).

Abbreviations

cDNA—complementary DNA; **ELAM**—endothelial leukocyte adhesion molecule; **ICAM**—intercellular adhesion molecule; **IL**—interleukin; **LFA**—lymphocyte function-associated antigen; **mRNA**—messenger RNA; **NADPH**—reduced nicotinamide adenine dinucleotide phosphate; **NCF**—neutrophil chemotactic factor; **PAF**—platelet-activating factor; **TNF**—tumor necrosis factor.

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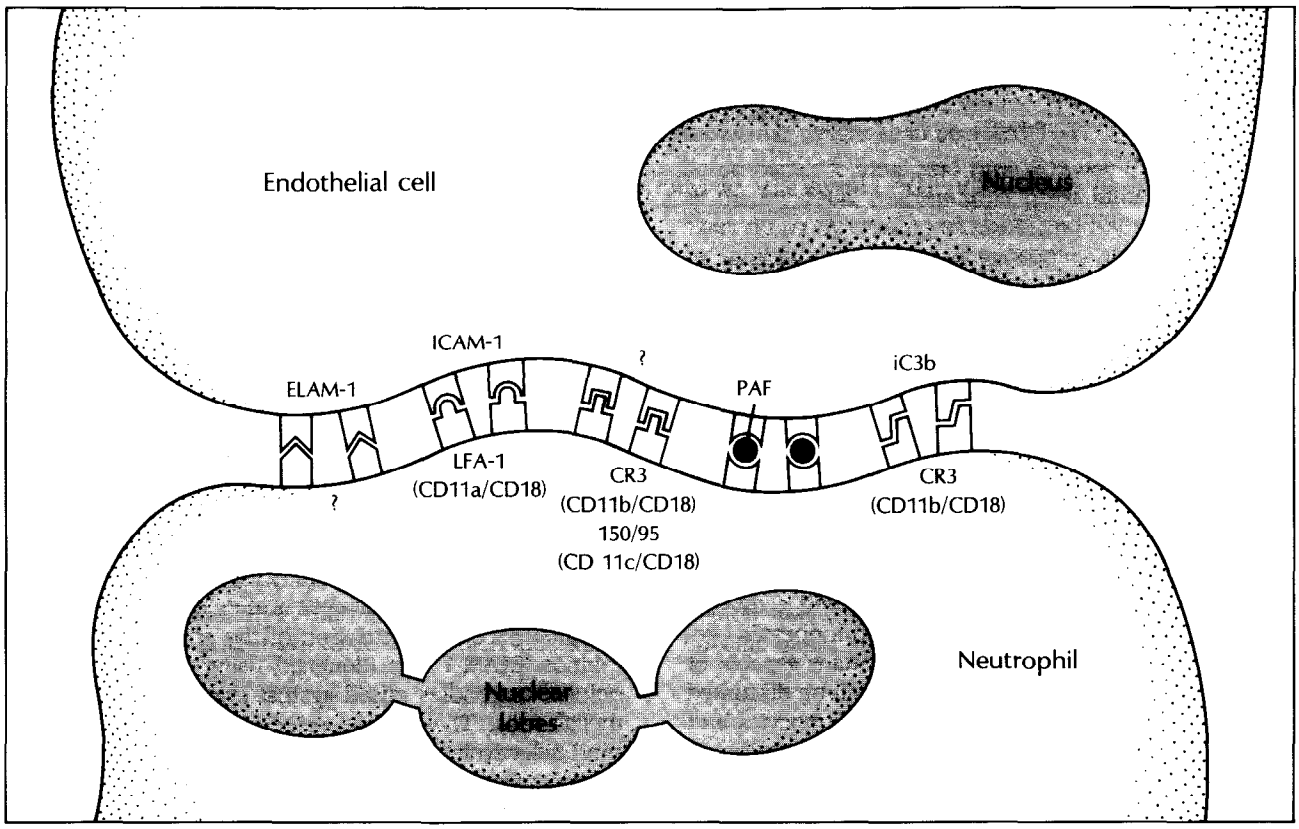

Fig. 1. Schematic diagram of adhesion mechanisms documented *in vitro* that may be relevant to neutrophil–endothelial adhesion occurring in acute inflammation. There are likely to be inter-relationships between these different mechanisms but their nature is unknown. ELAM, endothelial leukocyte adhesion molecule; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; PAF, platelet-activating factor.

The nucleotide sequence of ELAM-1 has revealed intriguing relationships with other classes of proteins, including the lymphocyte homing receptor recognized by the MEL-14 antibody, the platelet and endothelial cell activation molecule, granule membrane protein 140 (GMP 140), and complement pathway regulatory molecules. The significance of these relationships is currently unknown.

Important questions about ELAM-1 include the nature of the neutrophil ligand for this molecule, whether ELAM-1 is expressed on cell types besides the endothelium and whether stimuli other than cytokines and lipopolysaccharide will induce ELAM-1 expression. Although there is compelling evidence that ELAM-1 has an important role in the acute inflammatory response *in vivo*, this remains to be proven.

CD11/CD18

There is evidence for the involvement of molecules other than ELAM-1 in adhesive interactions between neutrophils and endothelial cells, most notably the CD11/CD18 group of leukocyte molecules. This topic was extensively reviewed last year [2]. Briefly, these three related heterodimers all share a common β-chain (CD18), and have unique α-chains (CD11a,b,c) linked non-covalently to the β-chain. CD11a is also known as lymphocyte function-associated antigen (LFA)-1, CD11b is the type 3 complement receptor, and CD11c is 150/95 or the type 4 complement receptor (Sanchez-Madrid *et al.*, *J Exp Med* 1983, 158:1785–1803). There is considerable *in vitro* and *in vivo* evidence linking these molecules to important adhesive interactions involving neutrophils, monocytes and lymphocytes, as well as evidence from an 'experiment of nature' [3,4]. The basis of the congenital leukocyte adhesion deficiency syndrome is failure of expression of the CD11/CD18 complex; an important characteristic of this syndrome is neutrophil leukocytosis combined with inadequate neutrophil mobilization into sites of bacterial infection, strongly suggesting that neutrophils require adequate expression of CD11/CD18 to cross the blood vessel wall.

Experiments using monoclonal antibodies leave little doubt that the CD11/CD18 complex of the neutrophil has a significant role in adhesion of neutrophils to cytokine-stimulated endothelium, including a recent study of the relative significance of the ELAM-1 and CD11/CD18 molecules [5]. Partial inhibition of *in vitro* adhesion was caused by antibodies to ELAM-1 or by antibodies to CD11a, CD11b and CD18 after a 4-h exposure of the endothelium to IL-1, with an additive effect from combinations of ELAM-1 and CD11/CD18 antibod-

ies. The precise interactions, if any, between the neutrophil CD11/CD18 molecules and the endothelial cell-associated ELAM-1 molecule, which lead to intercellular adherence, remain to be identified.

ICAM-1

Intercellular adhesion molecule (ICAM)-1 is a widely distributed cell surface molecule that mediates adhesive reactions and is a ligand for CD11a (LFA-1; Marlin *et al.*, *Cell* 1987, 51:813–819). It is expressed at relatively low levels on untreated endothelial cells and is markedly upregulated by cytokines (Pober *et al.*, *J Immunol* 1986, 137:1893–1896). On the basis of the use of antibodies to ICAM-1, it seems that this molecule is involved in the adhesion of neutrophils to cytokine-treated endothelial cells [6]. Another ligand for LFA-1, ICAM-2, has recently been characterized and its role, if any, in these reactions is unknown [7].

Mechanisms of rapidly induced adhesion

It is unlikely that neutrophil adhesion to endothelial cells previously exposed to cytokines represents the sole adhesion mechanism in acute inflammation. Neutrophil adhesion to endothelial cells can occur very rapidly (within minutes) after injury (Allison *et al.*, *J Exp Med* 1955, 102:655–668; Atherton *et al.*, *J Physiol* 1972, 222:447–474). Cytokine-mediated adhesion involving transcription, translation, post-translational events, and membrane insertion of the newly synthesized protein(s)] requires a minimum of 0.5 h to become detectable and only becomes prominent after 1–2 h. Two adhesion mechanisms with a rapid onset may be relevant to very early adhesive interactions between neutrophils and endothelial cells.

Appropriately stimulated endothelial cells produce the phospholipid platelet-activating factor (PAF). Endothelial cell-generated PAF or exogenous PAF will induce neutrophil–endothelial adhesion (Zimmerman *et al.*, *J Clin Invest* 1985, 76:2235–2246). The adhesion is detectable after 1 min, peaks at 5 min, and rapidly wanes thereafter. Thrombin is able to stimulate PAF production in endothelial cells, suggesting that there is a nexus between platelets, the coagulation cascade and the inflammatory response. Interestingly, thrombin-stimulated adhesion does not seem to depend on CD18 molecules to any marked extent [8].

An adhesion mechanism that depends on complement fixation on the endothelium has recently been defined [9]. The complement system is a humoral cascade capable of rapid amplification and is well known to be involved in many inflammatory processes. Complement fixation on endothelial cells leads to neutrophil adhesion within 1 min of complement activation, with adhesion peaking at 20 min. The adhesion is maintained as long as a source of complement is available. This adhesion requires no components distal to C3 (i.e C5–C9) and is mediated by the complement activation product iC3b, which is fixed to the endothelial cell and functions as a bridge to the type 3 complement receptor (CD11b/CD18) on the neutrophil. This adhesive mechanism provides a clear role for the involvement of neutrophil CD11b/CD18 binding to a known ligand.

Leukocyte penetration of the vascular wall

Following attachment, the neutrophil must cross the blood vessel wall and then the subendothelial extracellular matrix to gain access to the extra-vascular compartment. Recent studies using tissue culture models of the vessel wall have provided some interesting and novel results.

The cytokines TNF and IL-1, apart from causing adhesion, also cause neutrophils to migrate through a confluent endothelial cell monolayer [10], independently of a chemotactic gradient. The endothelial cell response to the cytokine requires protein synthesis and is due to an activity associated with the apical surface of the endothelium. This migration is not a simple sequelae to adhesion, since adhesion induced by other means (Fc interactions) is not associated with migration. The molecular basis of this migration is entirely unknown.

A recent study investigated the next event in neutrophil transport into an inflammatory focus, namely migration through the extracellular matrix (vascular basement membrane) [11]. This endothelial matrix normally forms an impermeable barrier. Once neutrophils have migrated beyond the endothelium, they cross the subendothelial extracellular matrix in a way that is associated with its local physical disruption as defined by increasing permeability of the matrix to colloids, by an as yet unknown mechanism. After the neutrophil has passed through the matrix, the endothelial cell brings about a resealing of the vascular basement membrane by a process requiring protein synthesis that is presumably related to the generation of new basement membrane components.

Leukocyte chemotaxis

Many molecules are capable of causing the migration of phagocytes along a concentration gradient and these have been assumed to be important in attraction to the sites of inflammatory foci. Recently, new families of peptide chemotaxins, which are likely to be of major significance as physiological mediators of chemotaxis, have been defined.

The appearance of phagocytes in inflammatory lesions is characterized by marked differences in the time course of accumulation of the various cell types (Wilhelm. *In* Pathology edited by Anderson and Kissane. Mosby, 1977, pp 25–89). The molecular basis for this heterogeneity was, until recently, unexplained, but there is now

strong evidence for the existence of cell type-specific chemotactic signals. A peptide, neutrophil chemotactic factor (NCF), was purified from activated macrophages (Yoshimura *et al.*, *Proc Natl Acad Sci USA* 1987, 84:9233–9237), cloned and sequenced [12]. It is one member of a family of small peptides and is a potent neutrophil chemotaxin and activator. NCF has target specificity in that it has no effect on macrophages. A macrophage-specific (and neutrophil-inactive) chemotaxin has also recently been identified [13]. Although these factors have been characterized from macrophages and cell lines, it is now known that they are also produced by endothelial cells [14] as well as other cellular constituents of normal tissues. It is likely that endothelial cells as well as other cells represent an important source of chemotaxins for the acute inflammatory response.

Mechanisms of tissue injury

Until recently, thinking about the mechanisms by which phagocytes cause tissue damage has evolved in two separate fields, oxidants derived from activity of the plasma membrane oxidase for reduced nicotinamide adenine dinucleotide phosphate (NADPH), and proteinases released from cytoplasmic granules. There is little doubt that phagocyte cell-derived oxidants including superoxide ions, hydrogen peroxide, hypochlorous acid, and the hydroxyl radical are toxic to cells and tissues, resulting in cell damage or death, increases in vascular permeability, fibrosis and amplification of the inflammatory response by mechanisms such as the generation of chemotactic lipids (Warren *et al.*, *Pathol Immunopathol Res* 1987, 6:301–315). Proteinases derived from phagocytes, while not directly toxic to cells, can bring about hydrolysis of proteins, resulting in tissue disruption.

Recent results have led to an ecumenical view that both oxidants and granule enzymes function in a synergistic manner to produce the complete toxic capacity of the neutrophil [15]. For example, oxidants are capable of blocking the anti-proteinase activity of blood, allowing proteinase activity to become manifest. Chlorinated oxidants (e.g. HOCl) are also capable of converting the latent forms of collagenase (Wiess *et al.*, *Science* 1985, 227:747–749) and gelatinase (Peppin *et al.*, *Proc Natl Acad Sci USA* 1986, 83:4322–4326) into active enzymes. Exposure of the basement membrane to oxidants renders it more susceptible to attack by leukocyte proteases (Vissers *et al.*, *Biochem Biophys Acta* 1986, 889:277–286). Thus, the way in which products of activated phagocytic cells cause tissue injury is considerably more complex than previously realized.

Cytokines

It is apparent from the above discussion that cytokines such as TNF and IL-1 have important effects on tissue constituents, such as endothelial cells, and produce multiple reactions responsible for the accumulation of phagocytes in inflammatory lesions. Evidence is accumulating that cytokines have other potent pro-inflammatory effects. Cytokines can 'prime' macrophages for enhanced oxygen radical production [16]. Their introduction into organs reproduces the acute inflammatory response, with permeability changes and accumulation of neutrophils [17,18]. Combinations of cytokines have synergistic effects *in vivo* [19]. Perhaps the most important and direct evidence for the role of cytokines in the acute inflammatory response comes from the discovery that antibodies to TNF will ameliorate the acute inflammatory response caused by deposition of immune complexes in the lung (Warren *et al. J Clin Invest*, in press). Although this does not exclude a role for other mediators, as yet undiscovered, it is compelling evidence that cytokines have an important role in the development of inflammatory reactions.

Initial work showed that cytokines are produced by macrophages, but more recently it has emerged that they may be produced by a variety of cell types. Some of these, such as endothelial cells (Libby *et al.*, *Am J Pathol* 1986, 124:179–185), mast cells [20] and smooth muscle cells [21] (Warner *et al.*, *J Exp Med* 1987, 165:1316–1331), are present close to sites of acute inflammation, suggesting that there is a broad repertoire of cells that can contribute to the pathogenesis of the acute inflammatory reaction.

Summary

A major theme in current developments in this field has been the realization that the target tissue, in particular the endothelium, has an active role in initiating and participating in inflammatory reactions and is not a passive target, as was previously thought. Attempts to delineate the mechanisms involved have benefited immeasurably from the application of the modern techniques of molecular and cellular biology, and will continue to do so. It is axiomatic that a fuller understanding of these mechanisms will result in the development of new strategies designed to block the acute inflammatory response more specifically and effectively.

Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

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