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THE VESSEL WALL AS A SOURCE OF VASOREGULATORY AND IMMUNOSTIMULATORY CYTOKINES. Peter Libby, Stephen J.C. Warner and Louis K. Birinyi. Department of Medicine and USDA Human Nutrition Research Center on Aging, Tufts University Boston, MA, U.S.A.

The cytokines Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF, also known as cachectin) exhibit multiple effects on circulating blood cells and cells of the blood vessel wall. For example, these mediators elicit a coordinated program of functions of endothelial cells (EC) that promotes blood coagulation and thrombosis, and lead to clot stabilization. Furthermore, IL-1 and TNF promote adherence to vascular endothelium of leukocytes of many classes. Thus, these cytokines are likely to be involved in signaling the pathologic changes in blood vessels that characterize a number of inflammatory or infectious processes. These two cytokines were originally isolated from activated human mononuclear phagocytes, hence their common designation as monokines and the terminology "interleukin". However, recent findings have broadened this concept considerably. It is now clear that many cell types can produce IL-1-like activity. Several groups showed that human vascular EC can secrete material that stimulates proliferation of thymocytes incubated with suboptimal doses of the mitogenic lectin phytohemagglutinin, a typical acitivty of IL-1 (thymocyte costimulation). Two related but distinct genes cloned from human peripheral blood monocytes encode IL-1 molecules. In human blood monocytes stimulated with bacterial lipopolysaccharide (LPS) IL-1 beta (pI ~ 7) is the major form expressed while IL-1 alpha (pI ~ 5) is the less abundant species secreted by human monocytes under these conditions. We found that EC and smooth muscle cells (SMC) isolated from adult human vessels can express these same IL-1 genes. LPS, a standard stimulus to IL-1 secretion in the monocyte, caused accumulation of IL-1 beta mRNA in both vascular cell types. Endothelial cells from adult human vessels also contained IL-1 alpha mRNA when treated with LPS in the presence of cycloheximide and LPS-stimulated smooth muscle cells contained RNA that hybridized with an IL-1 alpha cDNA probe as well. Although both vascular cell types can transcribe these IL-1 genes, the time course of this response differs. LPS induced IL-1 beta mRNA production by SMC maximally at 4-6 hr., whereas maximal IL-1 induction by LPS in EC occured 1 day after initiation of the exposure. Actinomycin D (1 ug/ml) blocked ^{3}H-uridine incorporation into macromolecules by > 95% in both EC & SMC, and prevented the LPS-induced increases in IL-1 mRNA levels in these cells. This result suggests that this potentially injurious stimulus causes IL-1 mRNA accumulation by an increase in rates of transcription. These LPS-induced increases in IL-1 mRNA levels corresponded to production of biologically active IL-1 determined as thymocyte costimulation activity. Interestingly, gel filtration experiments revealed a molecular weight of around 22kD for both SMC and EC-derived IL-1 secreted into culture medium in response to LPS. This molecular weight contrasts with the 17 kD species which is the fully processed product secreted from activated human monocytes. A possible explanation for this disparity is that the vascular cells secrete a partially processed intermediate form of mature IL-1. Other stimuli for IL-1 mRNA accumulation and secretion of biological activity include TNF and IL-1 itself. Recombinant human TNF ($\geq$ 10 ng/ml) increased IL-1 beta mRNA levels in EC & SMC, and caused the EC & SMC to release IL-1-like thymocyte costimulation activity. Of interest is the recent observation that IL-1 itself can stimulate expression of IL-1 genes in vascular wall cells. Both IL-1 alpha and beta can increase IL-1 beta mRNA content in EC & SMC. This observation was confirmed with homogenous IL-1 prepared by recombinant DNA technologies (rIL-1). These findings raise the possibility of a novel positive feedback loop in vascular pathophysiology. We also found that rIL-1 alpha or beta also induced the production of prostaglandin E_2 (PGE_2) by both vascular SMC & EC. This prostanoid, induced by IL-1, inhibits thymocyte proliferation. Thus, IL-1 not only induced its own expression but increased production of this immunosuppressive prostanoid. This mechanism provides a potential negative control loop in regulation of the local immune response in blood vessels. We conclude that these cells of the blood vessel wall are a source of the potent vasoregulatory and immune mediators IL-1 alpha and beta. Since IL-1 influences the thrombotic, hemostatic, and fibrinolytic functions of endothelium, as well as other responses to acute injury, our findings suggest novel local control mechanisms that may be important in a variety of pathologic states.

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ENDOTHELIAL-DEPENDENT MECHANISMS OF LEUKOCYTE ADHESION: ROLE OF MONOKINES. M.A. Gimbrone, Jr., M.P. Bevilacqua and M.E. Wheeler. Vascular Research Division, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, U.S.A.

Localized adhesion of peripheral blood leukocytes to the vessel wall is an essential component of inflammatory reactions. There is increasing experimental evidence that vascular endothelial cells play an active role in this process. Our laboratory has been especially interested in defining endothelial-dependent mechanisms of leukocyte adhesion, and the role of leukocyte products in their modulation. We have reported[1] that purified natural human monocyte-derived interleukin 1 (IL-1) can act directly on cultured human endothelial cells (HEC) to dramatically increase the adhesiveness of their surfaces for human polymorphonuclear leukocytes (PMN), monocytes and the related cell lines HL-60 and U937. This effect was concentration-, time- (onset ≅ 30 min; peak ≅ 4h), and protein/RNA-synthesis-requiring, and, in selective pretreatment/fixation experiments, was shown to be mediated primarily through the endothelial cell. To better define this inducible endothelial pro-adhesive mechanism, we have developed a series of murine monoclonal antibodies directed against monokine-stimulated HEC surfaces. One of these antibodies (H4/18) recognizes an endothelial cell surface structure which is induced by IL-1 (and certain other cytokines)[2] in a similar fashion (kinetics, concentration-dependence, sensitivity to metabolic inhibitors) as the pro-adhesive surface change for leukocytes. H4/18 partially blocks HL-60 cell adhesion to monokine-treated HEC, and, in vivo, labels human vascular endothelium at sites of experimental delayed hypersensitivity reactions[4]. A second monoclonal antibody (H18/7)[5] significantly blocks the adhesion of both HL-60 cells and PMN to monokine-treated HEC. Monoclonal antibodies H4/18 and H18/7 appear to recognize the same inducible surface structure as assessed by immunoprecipitation of extracts of metabolically labeled, monokine-stimulated HEC. We have designated this monokine-inducible, endothelial-leukocyte adhesion molecule "E-LAM 1". IL-1 treated HEC cultures (in contrast to sham-treated control cultures) generate a soluble leukocyte adhesion inhibitor (LAI)[6,7]. LAI acts on PMN to inhibit their adhesion to hyperadhesive endothelial monolayers as well as to serum-coated plastic surfaces, but does not inhibit PMN activation by chemotactic stimuli (LTB_4, f-met-leu-phe). LAI appears to differentially inhibit adhesion of peripheral blood leukocytes, isolated from the same donor, to hyperadhesive HEC (PMN > monocytes; lymphocytes, no effect), and does not inhibit HL-60 cell-HEC adhesion. Endothelial production of LAI is time-dependent (peak 5-6 h.), and blocked by cycloheximide but not by aspirin. Preliminary characterization indicates that LAI is nonsedimentable (250,000 xg, 45 min), nondialyzable (>10 kD), stable to heat (80°C, 30 min) and acid (pH 2) and is precipitable by ammonium sulphate (60-80% saturation). Thus, this endothelial-derived inhibitory activity, which appears to be distinct from PGI_2 or other cyclooxygenase products, blocks leukocyte adhesion without globally suppressing leukocyte function. Further characterization of the cellular and molecular mechanisms regulating the endothelial expression of E-LAM 1 and LAI should contribute to our understanding of the active role of the vascular wall in the inflammatory process.

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4. Cotran et al. (1986); J. Exp. Med. 164:661.
5. Bevilacqua et al. (1987); Fed. Proc. (in press).
6. Wheeler et al. (1986); Fed. Proc. 45:1725.
7. Wheeler et al. (1987); Fed. Proc. (in press).

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