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The pathophysiology of the pro-inflammatory cytokines

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The *in vivo* activities of several cytokines were subject of the Cytokine Workshop held on Hilton Head Island in December, 1989; new and important information on the cytokines as they apply to disease states and possible therapeutic uses were presented. The following is an overview of this subject.

In general, the pro-inflammatory cytokines are polypeptides of 8,000–25,000 Daltons which appear to be synthesized primarily in association with disease states or during host pertubation. Some "normal" physiologic events such as strenuous exercise or normal ovulation result in cytokine synthesis. These polypeptides are very potent molecules which, at picomolar or even femtomolar concentrations, trigger a variety of responses in cells *in vitro* but similar concentrations are effective *in vivo* when one considered the half-life clearance and distribution of parenterally administered cytokines. In fact, current studies indicate that nearly every organ system is affected by cytokines. The cytokines are similar to the classic hormones in that they are synthesized in one cell and act on adjacent or distant cells. But they are distinctly different from hormones in that there does not seem to be a major role for pro-inflammatory cytokines in homeostatic mechanisms. Moreover, their cell sources are not confined to a specific tissue and, with few exceptions, their genes are induced rather than constantly expressed.

A major understanding of the biology of cytokines, in general, is that despite their having their own distinct sequence, structure, and individual receptors, they often share the same biological properties. Nevertheless, cytokines appear to fall into two main groups: cytokines that act primarily as 1) growth factors for a variety of cells and 2) cytokines that possess pro-inflammatory properties. However convenient, this grouping should not be considered exclusive. Examples of cytokines which act as growth factors are interleukin-2 (IL-2) and IL-4; these cytokines stimulate the growth and functional parameters for T- and B-lymphocytes. Colony stimulating factors (CSF) such as IL-3 and granulocyte-macrophage CSF are growth and progression factors for hematopoietic cells. However, some pro-inflammatory cytokines can also stimulate T and B lymphocytes and activate hematopoietic cells, as well, whereas some CSF's possess pro-inflammatory properties.

Tumor necrosis factor (TNF) and IL-1 are two of the most studied pro-inflammatory cytokines [1]. Fibroblast growth factor, transforming growth factor beta (TGFβ) and IL-6 also possess biological activities which are associated with inflammatory states. IL-8 is a monocyte-derived neutrophil activating and chemoattractant protein, inducible by IL-1 and TNF, which likely plays an important role in mediating some of the inflammatory properties thought to be due to IL-1 and TNF.

Attention has focused on the pro-inflammatory cytokines because of their role in the pathogenesis of a variety of acute and chronic diseases. For example, disease states such as local and systemic infection, septic

shock, degenerative arthritis and autoimmune disease such as nephritis, vasculitis and inflammatory bowel disease appear to be mediated, in part, by pro-inflammatory cytokines. In another example, one can re-create hemodynamic changes, organ failure, disseminated intravascular coagulation, capillary leak syndrome and death in experimental animals with infusions of TNF [2]; a combination of TNF and IL-1 appears to be more effective than either cytokine alone. The deleterious effects of Gram-negative sepsis can be abrogated by passive immunization with anti-TNF and circulating levels of IL-1 and TNF correlate with disease activity in patients with sepsis. Acute exacerbations of rheumatoid arthritis are also associated with increased circulating levels of these cytokines. Levels of TNF and IL-1 correlate with fatal outcome in meningitis and the severity of inflammation and necrosis in inflammatory bowel disease. IL-1 and TNF appear to be cytotoxic for the beta cells of the Islets of Langerhans in the pathogenesis of type I diabetes. IL-6 is thought to be a mediator of B-cell proliferation in multiple myeloma.

IL-1, TNF and IL-6 are endogenous pyrogens and produce fever in animals and humans. They also stimulate the liver to express several genes for acute phase proteins. The pro-inflammatory cytokines, particularly IL-1 and TNF, stimulate gene expression for cyclooxygenase and phospholipase A2 and as a result of these effects, prostaglandin and leukotriene synthesis remain elevated in tissues for several hours. IL-1 decreases pain threshold via increased prostaglandin formation. In addition, IL-1 and TNF stimulate the expression of each other's gene as well as those of other cytokines, including CSF's, IL-6 and IL-8. Both IL-1 and TNF inhibit lipoprotein lipase, stimulate fatty acid synthesis and suppress appetite. IL-1, TNF and TGFβ suppress erthropoiesis and may mediate the anemia of chronic disease [3]. IL-1, TNF and TGFβ is a potent activator of osteoclasts and is thought to mediate bone resorption.

Reducing the synthesis of the pro-inflammatory cytokines by corticosteroids may be, in part, the mechanism by which these agents exert their anti-inflammatory effects. Corticosteroids suppress both the transcription and translation of IL-1 and TNF as well as that of other cytokines. Newer agents are being sought that suppress the synthesis of cytokines without the metabolic consequences of corticosteroid therapy. For example, there is evidence that agents which interfere with the formation of leukotrienes reduce cytokine synthesis. In fact, drugs which have a dual property of inhibition of cyclooxygenase and lipoxygenase on arachidonic acid metabolism are in clinical trials as anti-inflammatory agents. Reducing the formation of PGE2 and LTB4 in the peripheral blood mononuclear cell population by oral N-3 fatty acid supplementation in human volunteers is associated with reduced cytokine synthesis [4]. Other drugs such as pentoxyphylline reduce TNF synthesis in human volunteers given endotoxin. The development of future drugs as anti-inflammatory and immunosuppressive agents will likely take into account effects on cytokine synthesis as a mode of action.

Despite similarities in the biological responses to the various pro-inflammatory cytokines, each has its own receptor molecules. The receptors for the pro-inflammatory cytokines such as IL-1, TNF and IL-6 appear to be multiple in that there are separate genes coding for distinct molecules. In some cases, for example, TNF and IL-6, the receptor ligand interaction appears to be the cross-linking of the two receptor molecules. For TNF, which exists as a trimer, its ability to cross-link the two TNF receptor molecules is sufficient to transduce the signal. The same mechanism has been shown for IL-2 and the CSF's. The IL-1 receptor consists of two separate gene products (p80 and p68) and a similar case may exist for formation of a heterocomplex with the two chains and the IL-1 ligand. A clear pattern appears to be

emerging in that nearly all the cytokine receptors have at least two separate gene products binding the ligand to form a heterocomplex, which is the most efficient way at transducing a signal.

Although antibodies to the pro-inflammatory cytokines themselves have some clinical potential as anti-inflammatory , immunosuppressive or anti-shock agents, the weak affinities of antibodies limit their use, particularly repeated use, in human disease. Similarily, antibodies to cytokine receptors, although effective in reducing and even preventing inflammation, require large amounts and risk the development of immune responses to foreign proteins. On the other hand, the use of soluble receptors or receptor antagonists may be very effective due to the higher (usually 100 fold greater) affinities of ligands for their receptors compared to antibodies. A circulating human, IL-1-specific inhibitor had been described during endotoxemia [5] and in the urine of patients with fever and inflammatory diseases [6]. It shares 40% amino acid homology with IL-1β and binds with nearly the same affinity. Hence this molecule is a receptor antagonist. However, the IL-1 receptor antagonist (IL-1ra) does not trigger signal transduction in cells and hence acts as a competitive inhibitor of IL-1. Certainly an area of future investigation will be to identify comparable inhibitors for other cytokines.

Soluble receptors have also been described and function as inhibitors of cytokine action. This has been shown for TNF [7]. Both receptor antagonists and soluble cytokine receptors have the potential for clinical use with distinct advantages over anti-cytokine antibodies and antibodies to cytokine receptors.

Therapeutic uses for the inflammatory cytokines IL-1, TNF, IL-6 and TGFβ are being tested. IL-1 and IL-6 stimulate bone marrow stem cells and are undergoing trials for protecting bone marrow during chemotherapy or as an adjunct for bone marrow transplantation. IL-1 protects animals from death by radiation and sepsis. TNF upregulates Class I MHC molecules and is being used as adjunct therapy for increasing the generation of cytotoxic T-cells by high IL-2 in cancer patients. IL-6 is a potent B-cell stimulant and being considered as a possible adjuvant for vaccines. Similar to IL-1, IL-6 stimulates stem cells. TGFβ protects experimental animals against lethal sepsis and inhibits the formation of TNF in response to endotoxin. One can speculate that there may be uses for short-term, low doses of the pro-inflammatory cytokines to treat certain human diseases by augmenting host defense systems whereas during some disease states, anti-cytokine therapy is indicated to reduce the unwanted effects of these potent molecules.

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