

Effects of Human Interleukin-1 on Natural Killer Cell Activity: Is Fever a Host Defense Mechanism for Tumor Killing?

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Interleukin-1 (IL-1) represents a family of polypeptides with a wide range of biological activities. cDNA from two gene products has been cloned; there are probably more. The human IL-1 family plays an important role in the pathogenesis of many diseases and functions as a key mediator of host response to various infectious, inflammatory, neoplastic, and immunologic challenges. Recombinant mouse (pI 5) and recombinant human (pI 7) IL-1s are being used to confirm the multiple biological properties of IL-1s. Some IL-1 biological activities seem to be involved with mechanisms of host tumor killing. Incubating purified or recombinant human IL-1 with human peripheral blood mononuclear cells in the presence of IL-2 or interferon-alpha results in a synergistic enhancement of certain tumor cells. More recent results indicate that IL-1 exhibits direct cytotoxicity for tumor cells *in vitro*. The peripheral blood mononuclear cells of patients with tumors demonstrate decreased production of IL-1 when challenged with endotoxin and show a comparable decrease in natural killer activity; adding exogenous IL-1 reverses this defect in these patients. However, induction of hepatic acute-phase proteins such as serum amyloid A serves as a negative feedback since the amyloid protein suppresses natural killer activity. Moreover, natural killer cell activity in the presence of IL-1 or interferon-alpha is suppressed by incubating temperatures of 39°C. This effect is not reversed by inhibitors of prostaglandin synthesis. IL-1 is clearly important to host defense against malignancy, but some aspects of IL-1 biology seem to exert a contrary influence.

INTRODUCTION

Microbial invasion, injury, immunological reactions, neoplastic changes, and inflammatory processes continually challenge the host's ability to survive. The host, faced with either exogenous or endogenous insults, responds with a series of dramatic changes, most of which are, for the most part, directed at elimination. They are characterized by alterations in metabolic, endocrinologic, neurologic, and immunologic functions. The full spectrum of these acute phase changes includes increases in the synthesis of hepatic acute-phase proteins, leukocytosis primarily of circulating immature neutrophils, decreased plasma iron and zinc levels, negative nitrogen balance, and various endocrinologic changes. Neurologic changes are fever, increased lassitude or sleep, and decreased appetite. Aspects of altered immunoregulation are inconsistent; polyclonal B-cell activation usually occurs but increased suppressor cells and anergy can also be present. Hypergammaglobulinemia and increased hepatic

proteins result in elevated erythrocyte sedimentation rates. Although the most florid presentation of the acute-phase response is observed in patients with bacterial infections, burns, or multiple injuries, clinicians encounter acute-phase changes in patients with occult infections or chronic illnesses. The presence of acute-phase changes can also serve as a silent indicator of local disease and some cancers.

The various acute-phase responses have the outstanding characteristic of being a generalized host reaction irrespective of the localized or systemic nature of the inciting disease. They can be used for diagnosis or monitoring the progression of disease. Perhaps the most fundamental event in the initiation of the response is the production of a mediator called interleukin-1 (IL-1), a family of polypeptides which directly or indirectly induces both laboratory and clinical aspects of the acute-phase response [1]. IL-1 is produced primarily from phagocytic cells, enters the circulation, and affects distant organ systems; in this regard, IL-1 acts as a hormone mediating the host responses to infection and inflammation. The primary sources of IL-1 are blood monocytes, phagocytic lining cells of the liver and spleen, and other tissue macrophages [2]; specialized cells such as B cells, large granular lymphocytes, keratinocytes, gingival and corneal epithelial cells, renal mesangial cells, brain astrocytes and microglia, and synovial lining cells also produce IL-1. The IL-1 produced by these latter cell types probably exerts its primary effects within tissues, and the production of IL-1 by strategically located, specialized cells participates in the mechanism of many disease processes. This situation is particularly the case in the destructive aspects of joint and bone disease. The impact of an IL-1 mediated disease in a closed space is probably underestimated until considerable damage has taken place or indications of organ failure develop. Several investigators have found polypeptide inhibitors of IL-1 activity in plasma, urine, and joint and peritoneal fluid, and it is speculated that these naturally occurring substances play a role in abrogating IL-1 effects.

Multiple Biological Activities of IL-1

It is now clear that there is more than one IL-1 gene from macrophagic cells, and it remains to be shown whether IL-1 produced from specialized cells like keratinocytes, mesangial cells, and the like, is related to the macrophage IL-1 family [2]. IL-1 is more than just an interleukin since, in addition to its effects on neutrophils, T cells, B cells, and natural killer cells, it affects several non-leukocytic targets such as the liver, pancreas, bone, cartilage, muscle, synovial fibroblasts, and brain. IL-1 is a family of polypeptides affecting several unrelated tissues, while IL-2 is a single substance with seemingly a single purpose. IL-1, to make a broad analogy, is similar to the family of interferons in that there are multiple forms which retain a common biological property (in the case of interferons, this is antiviral activity, and in the case of IL-1 this seems to be lymphocyte activation in the presence of antigen or mitogen). Macrophage products with various IL-1 properties had been described for many years under different names. In addition to LAF, initial descriptions included endogenous pyrogen (EP), the mediator of fever [3], and leukocytic endogenous mediator (LEM), the inducer of hepatic acute-phase protein synthesis, neutrophilia, and changes in plasma divalent cations [4]. Since then, other macrophage factors have been isolated and described, such as mononuclear cell factor (MCF), which stimulates prostaglandin synthesis and collagenase production in synovial fibroblasts [5]. The relationship of these various biological activities has been recently reviewed [6]. Catabolin, a family of polypeptides purified from pig blood monocytes, has now been shown to be an interleukin-1 [7]

From over 20 years of investigation, considerable evidence supported the concept that these substances were physically related [6]. The applications of molecular biology and recombinant DNA technology have begun to untangle how the IL-1 family is structurally related and how the biological activities are segregated.

At the onset of infection or injury, blood monocytes and tissue macrophages become activated either by phagocytosis of the invading microbe or by tissue products. Either process results in the synthesis and release of IL-1. Although local effects of IL-1 in the surrounding tissues contribute to certain acute pathological changes such as cellular infiltration and hyperemia, IL-1 also enters the circulation, where it stimulates several distant tissue targets. These include fever and an increase in the number and immaturity of circulating neutrophils. The release of neutrophils is apparently due to the direct action of IL-1 on the bone marrow [4]. There is a rapid drop in plasma iron thought to be due, in part, to sequestration of iron in lactoferrin complexes, and hypozincemia is present and thought to be mediated by hepatic metallothionein [8]. Muscle proteolysis and negative nitrogen balance occur via IL-1 mediated increases in prostaglandin E₂ production [9]. In addition to the direct effect of IL-1 on muscle tissue, there is an indirect effect on muscle as PGE₂ production and muscle degradation are enhanced at febrile temperatures. While the muscles are releasing amino acids into the circulation, hepatic uptake is increased, and there is a dramatic increase in hepatic acute-phase reactants; however, albumin synthesis is reduced [10]. Acute-phase proteins include those normal serum proteins whose concentrations increase several-fold: such proteins as haptoglobin, certain protease inhibitors, complement components, ceruloplasmin and fibrinogen, and those which increase several hundredfold. The latter are true acute-phase reactants and include serum amyloid A protein, which is a precursor of the amyloid fibril deposited in tissues of patients with secondary amyloidosis, and C-reactive protein, which may serve several different nonspecific host defense functions [10]. Although hepatic acute-phase protein synthesis may be under the modulating control of homeostatic hormones, IL-1 is clearly involved in triggering the transcription and synthesis of these proteins [11,12]. Concentrations of these proteins increase so that the sedimentation rates of erythrocytes reach very high levels.

Several systemic changes contribute to the metabolic derangements seen during an acute infection. There are increases in the production of insulin, glucagon, growth hormone, TSH, and vasopressin which are probably influenced by circulating levels of IL-1 [13]. The metabolic changes of the acute-phase response are clearly inefficient, since amino acids from degraded muscle tissue are used for gluconeogenesis and energy, while utilization of fat is reduced. The mechanism of metabolic catabolism during infection and inflammation is distinct from that of starvation [14]. Amino acids are required for the proliferation of lymphocytes and fibroblasts as well as the synthesis of hepatic acute-phase reactants, immunoglobulins, and collagen for repairing damaged tissues. The well-coordinated events of providing ample amino acids for these cellular functions seems to be orchestrated by IL-1. The need for an increased supply of energy substrates and a large supply of amino acids for synthesis of new protein comes at a time when food intake may be severely impaired or appetite reduced. IL-1 depresses appetite in experimental models [15]. Another systemic effect of IL-1 is its ability to induce slow wave sleep following intracerebroventricular or intravenous administration [16], and there is a strong clinical impression that infection is associated with increased sleep. There is also some speculation that the increased sleep

may have an adaptive function in helping the host reserve its energies for fighting infections [17].

IL-1 stimulation of lymphocytes is a vital host defense function since this contributes to the initiation of cellular and humoral immune mechanisms directed against the infecting microbe. There is a large body of evidence concerning the functional role of IL-1 for B-cell proliferation and antibody production [22,23] as well as for its role in T-cell activation [24]. The importance of IL-1 for T cells involves the production of lymphokines, the most notable being IL-2 which, in turn, provides the growth signal for clonal expansion of various helper, suppressor, and cytolytic T cells and augments natural killer cell activity [25].

Recombinant IL-1s

The resolution of the present dilemma of how many of the multiple biological activities of IL-1 are, in fact, due to a single substance has begun with the cloning of cDNAs coding for the human monocyte-derived [26] and the murine macrophage-derived [27]. A similar dilemma had existed with the interferons (IFN), initially described as antiviral substances, and serves as an analogy. Alpha (leukocyte)-IFN had other biological properties including fever [28] and increased natural killer activity, and recombinant IFNs have enabled investigators to confirm that IFNs possess more properties than antiviral activity, such as increasing macrophage killing and antigen expression. Recombinant human IL-2 also has biological properties other than its ability to stimulate T-cell proliferation [29]. However, IL-1 is unique in that there is no signal or cleavage peptide sequence. As a result, most of the IL-1 which is translated following stimulation remains intracellular [30], and the amount of IL-1 released into the surrounding medium is dependent on the type of stimulus. Other workers have proposed that IL-1 is bound to the surface of macrophages [31], and the lack of a signal peptide would certainly be consistent with this finding.

There seems little doubt that human IL-1 is a family of polypeptides. Exactly how many different genes code for human IL-1 activity remains to be established. Presently, there seem to be, at a minimum, two distinct IL-1s from human monocytes [32], probably based on the two major isoelectric points of the molecule. The cDNA made to the poly-A mRNA of the pI 7 codes for less than 26 percent amino acid homology with the cDNA from the pI 5 form. Because of the lack of significant homologies and based on restriction enzyme analyses, these two cDNAs probably represent two distinct gene products. Others have suggested that three forms exist, based on cDNA sequences which correspond to the three pIs. No nomenclature presently exists for IL-1 but it seems logical to assign these forms by (a) species of origin, (b) cell source, and (c) pI (or other physical characteristic) until more information is known.

In studying the effects of IL-1 on NK activity or on IL-1 tumor lysis, it is necessary to use purified preparations or recombinant IL-1s. Our initial studies [25] in IL-1 induced changes in NK activity were carried out using highly purified human monocyte IL-1. Recent studies have also employed homogeneous human monocyte IL-1 with a pI of 7 [33]. We have recently used *in situ* labeling of human monocytes and purified the supernatant medium by immunoabsorption, gel filtration, and chromatofocusing [34]. Three homogeneous bands at 7, 6, and 5 corresponded on SDS PAGE to molecular weights of 18, 20, and 22 kd, respectively, each with activity as endogenous pyrogens and lymphocyte activation. Using antibodies to purified porcine

IL-1s with different pIs, antibodies raised to one pI form do not cross-react with the other within the same species, whereas antibodies raised to the same pI form do cross-react between the species [35]. This has also been shown with human IL-1 [36].

The IL-1s are presently defined biologically by their ability to augment mitogen- or antigen-induced T-cell proliferation. Even using large amounts ($\mu\text{g/ml}$) of purified human recombinant IL-1 with a pI of 7, no direct proliferative response was observed on a cloned murine T helper cell line (D.10.G.4) or human peripheral blood T cells unless in the presence of mitogens or antigens. In those incubations, IL-1 is active in sub-nanogram/ml concentrations. The inflammatory property of the molecule is a better indicator of its biological activity, since it is direct (that is, fever, PGE_2 production, increased enzyme release and production). However, to solve the issue of multiple biological activities, each IL-1 needs to be purified to homogeneity, free of endotoxins, and the specific activity (biological units/mg protein) of each form established in the multiple *in vivo* and *in vitro* assays. Both recombinant *Escherichia coli*-derived and recombinant mammalian cell-derived IL-1 will need to be evaluated, since the recombinant bacterial products are not glycosylated. A critical aspect of any recombinant IL-1 study is the formidable problem of contaminating endotoxins. Endotoxins are ubiquitous, and nanogram quantities can mimic IL-1 in various assays. The production of fever or endothelial neutrophil adherence are particularly sensitive to endotoxins. Contaminating endotoxins from *E. coli* products present a unique challenge in this regard.

IL-1 and Tumor Killing

In vitro production of IL-1 from human blood leukocytes of patients with various diseases have demonstrated reduced IL-1 production from circulating leukocytes of malnourished patients and cancer patients with large tumor burdens [6]. Human Epstein-Barr virus B-cell lines produce IL-1 [37], and this fact raises the speculation of IL-1 in chronic viral diseases associated with immunosuppression. Although these data are at present difficult to view in the context of IL-1 as an enhancer of host defense against malignancies, the direct effect of purified IL-1 seems to enhance NK killing. IL-1 is known to synergize with IFN and IL-2 in increased tumor killing by NK cells [25]. A recent report [38] has shown that purified pI 7 human monocyte IL-1 has both direct stimulatory effects on NK cells as well as acting as a direct anti-tumor factor for certain tumor targets. There are also data which suggest that certain patients with large tumor burdens produce less IL-1 and that their NK tumor killing can be normalized by the addition of exogenous IL-1 [39]. Clearly, recombinant human IL-1s provide the necessary materials to advance this important area of clinical research. We have recently expressed the human pI 7 IL-1 and used it to study its effects on human NK activity. The recombinant IL-1 confirms previously published data on the ability of IL-1 to act synergistically with IL-2 and interferon-alpha [25].

Aspirin, acetaminophen, and non-steroidal anti-inflammatory agents are potent inhibitors of brain prostaglandin synthesis and therefore are highly effective antipyretics. These agents also reduce IL-1 induced joint and muscle PGE_2 synthesis, thus helping alleviate the symptoms of arthritis and myalgia. Although non-steroidal anti-inflammatory drugs reduce neutrophil chemotaxis, aggregation, and degranulation caused by a variety of inflammatory mediators, IL-1 induced neutrophil activation appears unaffected by these drugs. Other aspects of the acute-phase response induced by IL-1, such as release of neutrophils from the bone marrow, changes in serum

divalent cations, and synthesis of acute-phase proteins, are also unaffected by non-steroidal anti-inflammatory drugs. Furthermore, IL-1 mediated lymphocyte activation is not diminished by these agents. Antipyretics do not reduce IL-1 induced sleep [16], and this seems to be consistent with clinical observations that patients with febrile diseases have increased sleep regardless of antipyretic therapy. There is, however, increasing evidence that inhibitors of leukotriene synthesis block both IL-1 production and some of its biological activities [40,41]. Although there is a need to develop IL-1 antagonists, some effects of IL-1 on the host play an important and vital role in defense against infection and malignant transformation and therefore, perhaps, total antagonism is contraindicated. The role of acute-phase proteins in host defense and repair is not entirely clear. Studies suggest that the major role of C-reactive protein is to bind serum lipids or opsonize pneumococci, while serum amyloid A is thought to be immunosuppressive. Ceruloplasmin scavenges toxic free oxygen radicals which are induced in neutrophils by IL-1. What is clear, however, is that the production and physical structure of these acute-phase proteins has been conserved through more than 600 million years of evolution, and therefore they have presumably been useful to the host. Reptiles and fish make an IL-1-like molecule, and an IL-1-like substance has been isolated from starfish. This argues that IL-1 and the acute-phase response have played important roles in the survival of many species [42].

Fever and Tumor Killing

Before the development of radiation and chemotherapy, physicians depended upon surgical excision as the primary treatment of most cancers. Infected surgical wounds were not uncommon in the pre-antibiotic era, and in patients who developed post-operative infections, tumor progression and the advent of metastatic disease were often delayed and, in some instances, prevented. This was particularly the case when wounds were infected with erysipelas-producing strains of streptococci or gram-negative organisms. Dr. William Coley, a New York City surgeon, had been sufficiently impressed with this association that he routinely injected his post-operative patients with a mixture of bacterial culture filtrates in an effort to induce the same type of clinical responses that he had observed in infected patients.

Coley's toxins, a mixture of filtrates from erysipelas strains of *Streptococci* and endotoxin-producing *Serratia marcescens*, were widely used before the introduction of radiation therapy for cancer treatment. The immediate clinical response to the injection of these toxins was fever and, in many patients, hyperpyrexia (fever > 40.5°C). There is little doubt that Coley's toxins were occasionally effective, as several reports have documented tumor regression and cures with toxin therapy [43–45]. Pyrogenic endotoxins have been used in clinical trials in the United States, and a modified version of Coley's toxin is currently being tested in Japan [46].

The mechanisms by which pyrogenic immunostimulants such as bacterial toxins enhance host defense against malignant cells are unclear, but three possible effects are probably involved: (1) the direct effect of hyperthermia on the neoplastic cell growth, (2) the effect of elevated temperature on the cells primarily concerned with host defense, and (3) the ability of the pyrogenic immunostimulants to initiate the production of various cytokines (sometimes called biological response modifiers), which regulate host defense functions, particularly against malignant cells. Elevated temperature has been shown to retard the proliferation of certain tumor cells both *in vitro* [47] and *in vivo* [48], and tumor antigen expression and cell membrane viscosity are altered by temperatures in the febrile range (38.5–40°C) [49].

TABLE 1
Effects of Febrile Temperature on
Tumor-Killing Mechanisms

<i>Increased at Elevated Temperatures</i>
IL-1 induced PGE production
Helper T-cell activation
Thymocyte mitogen responses
Antibody production <i>in vitro</i>
Antibody production <i>in vivo</i>
Generation of cytotoxic T cells
Lymphocyte proliferation to viruses
Cytotoxic T-cell mediated killing
<i>Decreased at Elevated Temperatures</i>
Tumor growth
Viral replication
Lymphokine production
Monokine production
Natural killer cell activity

Another possible effect of the fever induced by these immunostimulants is the direct effect of temperature on cells primarily responsible for various host defense functions, namely neutrophils, macrophages, and lymphocytes. Several studies have shown that cellular functions increase at febrile temperatures (reviewed in [1] and [50]). Recent studies have demonstrated enhanced effects of interleukin-1 (IL-1) on T cells at elevated temperatures [51–55]. Elevated temperature also increases the generation and killing efficiency of specific cytotoxic T lymphocytes. Thus, many components of the host's response to biological response modifiers seem to be enhanced by the elevated temperature (fever) which often accompanies its use. However, another host defense function, namely natural killer (NK) activity [56–59], is apparently decreased by elevated temperatures.

We examined the NK activity of human mononuclear cells following an 18-hour incubation at 34, 37, and 39°C in the presence of IL-1, interleukin-2 (IL-2), and alpha interferon (IFN). IL-1, IL-2, and alpha IFN or combinations of these mediators have been previously shown to stimulate NK cell activity [25]. In clinical trials using IFN or IL-2 in patients with various malignant diseases, the most noted side effect is fever. Homogeneous IL-1, studied experimentally as a potent pyrogenic substance, has yet to be used clinically; however, culture supernates of certain human cell lines which have been injected into humans contain large amounts of IL-1 and have produced dramatic pyrogenic reactions. Data derived from *in vitro* cultures have clearly established that Coley's toxins and other pyrogenic immunostimulants are potent inducers of IL-1 and IFN production.

We therefore studied NK activity following exposure to IL-1, IL-2, and IFN at various temperatures in order to mimic the effects of these cytokines on NK activity during a febrile response. The production of IL-1, IL-2, burst promoting activity (BPA), and granulocyte-macrophage colony-stimulating activity (GM-CSA) by human peripheral blood mononuclear cells *in vitro* was also examined at these same temperatures. In contrast to other immune functions that are enhanced at elevated temperatures, we found that cytokine production and both baseline and cytokine-primed NK activity are markedly inhibited at febrile temperatures (39°C). Since cytokine production and NK activity are closely associated with host defense against

malignancy, these observations suggest that any benefit derived from exogenous IL-1 or IL-1 induced by various toxin therapies takes place despite the detrimental effects of elevated temperatures. These studies support the concept that fever resulting from the injection of an immunostimulant may partially negate any beneficial effects on NK-directed tumor lysis and should be suppressed for optimal clinical results. Table 1 summarizes some effects of elevated temperature on mechanisms involved with host-mediated tumor killing.

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