INTERLEUKIN 1, A PROTOTYPIC PLEIOTROPIC LYMPHOKINE

P. GHEZZI and A. MANTOVANI

Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy

Summary. - Interleukin 1 (IL-1) is an endogenous mediator produced by monocytes/macrophages, endothelial cells and other cell types. It was originally identified as the main endogenous pyrogen. It was later found that IL-1 was identical to other factors that had been defined leukocytic endogenous mediator, lymphocyte activating factor. It is now clear that IL-1 has two main aspects. On one side it is an immunostimulatory molecule and on the other side is a mediator of shock and inflammation. As an immunostimulating molecule, IL-1 is a potential immunomodulating drug, while with respect to its proinflammatory action it might be important to identify antagonists or inhibitors that could be useful in the therapy of chronic inflammatory diseases.

Riassunto (Interleuchina 1, una linfocchina pleiotropica prototipica). - L’interleuchina 1 (IL-1) è un mediator endogeno prodotto da cellule monocito/macrofagiche, endoteliali e di altro tipo. E' stato originariamente identificato come il principale pirogeno endogeno. Successivamente è stato visto che IL-1 era identica ad altri fattori che erano stati chiamati mediatori endogene leukocitario e fattore di attivazione dei linfociti. E' ora chiaro che l'IL-1 ha due aspetti principali. Da un lato è una molecola immunostimolante e dall'altro è un mediator dello shock e dell'infiammazione. In quanto molecola immunostimolante, l'IL-1 è un potenziale farmaco immunomodulatore, mentre considerando la sua attività proinfiammatoria sarebbe importante identificare antagonisti o inibitori che potrebbero essere utili nella terapia delle malattie infiammatorie chroniche.

Historical perspective

The term interleukin 1 (IL-1) was first proposed in 1979 [1] when it was clear that several monokine activities were actually different activities from what was then thought to be a single protein, originally designed as lymphocyte activating factor (LAF, first described by Gery and Waksman in 1974) [2]. The term IL-1 stressed the fact that LAF was a macrophage product acting on lymphocytes, and therefore acted as a communication signal between leukocytes. Thus, IL-1 was originally described as an immunoregulatory protein of interest mainly, though not only, to immunologists.

In a completely different field, researchers studying the pathogenesis of fever had focused on the role of soluble products derived from leukocytes as possible mediators of fever. In 1943, Menkens suggested that leukocytes released a pyrogenic substance and termed it “pyrexin” (for a review on the history of endogenous pyrogen (EP) see [3]). In 1955 Atkins and Wood [4] found a circulating pyrogenic factor in febrile rabbits that they termed endogenous pyrogen (EP). Production of EP was then demonstrated in human, murine, and rabbit mononuclear cells [3, 5]. EP was suggested to be identical to another macrophage product, known as leukocytic endogenous mediator (LEM), capable of bringing about various acute-phase changes, including hypothermia, hypoxemia and induction of acute-phase proteins like fibrinogen [6]. Human EP was then biochemically characterized and purified to apparent homogeneity by Dinarello et al. in 1977 [7].

Early biochemical characterizations of LAF and EP revealed similarities between the two and in fact some reports indicated that EP and LAF might be the same protein [8, 9]. The fact that all these mediators were in fact the same became clear when, in 1984, the cloning of human IL-1β was published [10]. From then on a number of papers have reported the cloning of various IL-1 proteins from different species [11, 12].

The availability of highly purified recombinant IL-1 preparations permitted extensive characterization of its structure, biological functions and mode and the discovery of other, previously unknown, activities on a variety of cell targets.

Gene and protein structure

cDNA cloning revealed the existence of two forms of IL-1, in agreement with previous biochemical characterization of EP/LEM, which were shown to exist as two forms.
IL-1 α and β both have a molecular weight of 17 kDa but differ in their isoelectric point (pI 5.2 for IL-1α and 7.0 for IL-1β). The two forms are encoded by different genes and show very little homology. Amino acid sequence homology between human IL-1 α and β is only 26%, while interestingly the degree of homology in the nucleic acid sequence is higher (45%) [12].

Although the extracellular, mature form of IL-1 α and β have a molecular weight of 17 kDa, the corresponding mRNAs (2.2 kB for IL-1α and 1.6 kB for IL-1β) encode translation products of about 31 kDa. While the 31 kDa IL-1α precursor is biologically active and binds the IL-1 receptor, the IL-1β precursor is inactive and does not bind the receptor [13]. These large precursor molecules are then broken down to the 17 kDa, "mature" IL-1 protein, probably by serine proteases related to plasmin and elastase [14, 15]. This processing does not seem to be a classical secretory process through the endoplasmic reticulum, and secreted IL-1 is not glycosylated, despite the presence of potential glycosylation sites. IL-1 α and β, unlike tumor necrosis factor (TNF), lack the secretory signal sequence. Unlike many secretory proteins, processing is not always required for secretion and both the 31 kDa precursor and 17 kDa mature IL-1 are secreted into the culture medium by human monocytes. A membrane-associated, biologically active 22−23 kDa form of IL-1 has also been described. After secretion, mature 17 kDa IL-1 is further degraded and active fragments of 4.2, 4 and 2 kDa were described in human plasma from febrile patients, after exercise and in women after ovulation [16-18].

**Cellular sources and regulation of IL-1 synthesis**

IL-1 was originally described as a monokine, i.e. a product of activated monocytes/macrophages, that are the main sources of IL-1. Subsequent studies revealed that almost all cells produce IL-1, including T and B lymphocytes, NK cells, polymorphonuclear leukocytes, endothelial cells, fibroblasts, asynvalial cells, glomerular mesangial cells, chondrocytes, astrocytes, keratinocytes, Langerhans cells and, in some cases, tumor cells [14, 15]. It is very likely that, with the availability of specific techniques for the detection of IL-1 or IL-1 mRNA, other cells will probably be found to produce IL-1.

The most popular inducers of IL-1 synthesis are bacteria and bacterial products, particularly endotoxin. Several other substances were reported to stimulate IL-1 production [19]: silica particles, phorbol esters, calcium ionophores, complement components, antigen-antibody complexes, neuropeptides like substance P, and other cytokines like TNF [20] and IL-1 itself [21]. Adherence (to plastic, endothelial cells or collagen) was also reported to induce transient expression of intracellular, membrane and secreted IL-1 by macrophages.

**Biological effects of IL-1**

IL-1, like the two other cytokines, TNF and IL-6, is a typical "double faced" molecule. On the one hand it is an immunomodulatory protein, acting as a communication signal between different populations of leukocytes (hence the name "interleukin", between leukocytes). The other face of IL-1 is its activity as an inflammatory mediator, acting on a wide range of cell targets. We will therefore group the different activities of IL-1 in two classes: immunomodulatory and inflammatory. In listing these activities we will also stress the cell targets of IL-1's action and in some cases we will refer to the "old" acronyms (e.g. LAF, LEM, EP) used to identify the different functions of IL-1.

**IL-1 and the immune system**

As indicated by the term LAF, IL-1 enhances the thymocyte mitogenic response to PHA and Con A. IL-1 was also reported to enhance the production of IL-2 by certain subsets of T cells. These two activities of IL-1 (induction of thymocyte proliferation and of IL-2 production) are at the basis of the bioassays used for the detection of IL-1 [19, 22].

The mitogenic action is not unique to IL-1 since, under appropriate experimental conditions, IL-6 and TNF are also active in the thymocyte assay [22]. Based on induction of IL-6 and on the blocking of IL-1 comitogenic action by anti IL-6 antibodies it has been proposed that the effect of IL-1 on thymocytes is indirect and is indeed mediated by induction of IL-6 by IL-1. It is of interest that in endothelial cells, unlike thymocytes, IL-1 induces IL-6 but is not active on these cells via IL-6 [23].

The role of IL-1 in induction of specific immunity, i.e. its role in antigen presentation by accessory cells, has been the subject of considerable debate [19].

IL-1 seems not to be required for activation of antigen-specific T cell clones by antigen presenting cells. In contrast, there is evidence for an important role of IL-1 in activation by antigen of virgin T cells. In the mind of the authors, an obligatory role for IL-1 in antigen presentation has not been established.

IL-1 is also active on the proliferation and differentiation of B cells. The physiological significance of this action is not clear. More important, IL-1 could act on B cells by inducing IL-6, a lymphokine which, among other activities, has been identified as plasmacytoma/hybridoma growth factor and B cell stimulatory factor.

IL-1 has also a colony stimulating factor (CSF) activity. In fact, IL-1 accounts for the activity originally identified as hemopoietin-1 [24]. Among CSFs, IL-1 affects the earliest, most immature bone marrow precursors. In addition to having CSF activity, per se, IL-1 induces production of other CSFs, including G and M, in endothelial cells [25].

The CSF/hemopoietin activity of IL-1 is a subject of considerable interest in the perspective of therapeutic application. In fact, as we will discuss later, IL-1 has...
and protective activity and protects or restores bone marrow function compromised by the cytotoxic chemotherapeutic agents cyclophosphamide and 5 fluorouracil. The potential clinical relevance of these observations is currently being explored. Moreover, certain acute myeloid leukemias express IL-1 mRNA and protein and IL-1 could play a role in autocrine stimulation of neoplastic elements [26, 27]. IL-1 has been reported to stimulate monocytes/macrophages to produce other cytokines. In particular, IL-1 induces the synthesis of TNF, IL-6 and IL-1 itself, also on endothelial cells [20, 21, 23, 28, 29].

These findings are not confined to in vitro systems. Administration of human recombinant IL-1 to rabbits causes a biphasic fever response and, during the second peak of fever, endogenous rabbit IL-1 was detected in the circulation [21]. These self-amplification mechanisms could be particularly important in the pathogenesis of chronic inflammatory diseases.

IL-1 and inflammation

Most of the pleiotropic effects of IL-1 are associated with inflammation and is now regarded as the primary mediator of the acute-phase response. It can be detected in the circulation a few hours after the onset of infection or injury (or after injection of endotoxin) and there is a growing literature reporting the presence of IL-1 in biological fluids in pathological conditions. For instance, IL-1 was detected in patients with rheumatoid arthritis [30], and in sera from human volunteers after exercise or post-vaccination [16-18]. Treatment with IL-1 has been shown to induce hemodynamic shock in rabbits [31].

Effects on the central nervous system. - The thermoregulating neurones of the hypothalamus are the targets responsible for the "oldest" action of IL-1, induction of fever. This pyrogenic activity is mediated by induction of prostaglandin synthesis, particularly PGE2. IL-1 induced prostaglandin release from hypothalamic tissue in vitro and its pyrogenic action is prevented by cyclooxygenase inhibitors [3]. In addition to fever, IL-1 induces slow-wave sleep [32] and anorexia [33]. All these effects can be observed upon intracerebroventricular injection of IL-1 or when IL-1 is given systemically [3, 34].

IL-1 also has important effects on the neuroendocrine system. It stimulates the release of corticotropin releasing factor (CRF) that in turn induces the release of ACTH and corticosteroids [35, 36]. Since corticosteroids are potent inhibitors of IL-1 synthesis, this could constitute an effective feedback system for controlling IL-1 synthesis in vivo.

The origin of IL-1 acting on the brain is still unclear. It seems unlikely that IL-1 can cross the blood-brain barrier, and although it can be produced by astrocytes, fever is also observed when IL-1 is induced by local inflammation in the periphery or is administered intraperitoneally or intravenously. The availability of techniques like immunohistochemistry or in situ hybridization may help answer this question, and the recent findings of IL-1 and its receptor in the brain are particularly important in this respect. In this direction, it is important to note that neuropeptides, like substance P, were reported to induce IL-1 synthesis.

Hepatic effects and the acute-phase response. - The hepatocyte is the main target for induction of the synthesis of acute-phase proteins by IL-1 [37]. These include, among other proteins, C-reactive protein, serum amyloid A, anti-proteases and fibrinogen. In some cases, like serum amyloid A, induced synthesis was observed in cultured hepatocytes stimulated with IL-1 in vitro [38].

For other acute-phase proteins the mechanism is probably more complex. Although administration of IL-1 induces fibrinogen, it does not induce fibrinogen synthesis in vitro on isolated hepatocytes or on hepatoma cell lines. In fact, earlier work indicated that monocytes produced a factor (termed hepatocyte stimulating factor, HSF) that stimulated fibrinogen synthesis by hepatocytes. HSF is distinct from IL-1 and was later found to be identical to IL-6 [39, 40]. Since IL-1 is a powerful inducer of IL-6/HSF [23, 28] it seems likely that induction of some acute-phase proteins is mediated by IL-6. While the synthesis of a large number of specific acute-phase proteins, often with anti-toxic properties, is typical for the IL-1-induced acute-phase response, some negative acute-phase reactants were identified. The levels of these proteins are reduced by IL-1 treatment. One such protein is albumin, whose synthesis is blocked at the transcriptional level by IL-1 [38].

Another protein decreased by IL-1 is cytochrome P-450 (in fact a family of proteins), a key protein in the oxidase system responsible for the metabolism of drugs and detoxification of foreign compounds. Treatment of mice with IL-1 or exposure of hepatocytes to IL-1 in vitro resulted in decreased drug metabolizing activities [41], and this effect probably explains earlier reports of reduced liver drug metabolism associated with infection and inflammation.

The overall pathway of modulation of liver functions by IL-1 is depicted in Fig. 1.

Besides inducing liver acute-phase protein synthesis, IL-1 induces other metabolic changes associated with the acute-phase response, like hypoferremia and hypozincemia.

Fig. 1. - Regulation of hepatocyte functions by IL-1, IL-6 and other cytokines produced by macrophages in response to inflammatory stimuli. Cytokines act on the hepatocyte by increasing the synthesis of acute-phase proteins and decreasing that of negative acute-phase reactants, like albumin and cytochrome P 450 and related drug metabolizing enzymes.
mia [42]. Some of these changes are partially due to induction of metal-binding proteins, including metallothionein, ferritin and ceruloplasmin. Since the hypoferremic response to IL-1 is nearly abolished in neutropenic mice, it is likely that this effect is mediated via neutrophils, possibly through secretion of lactoferrin. In general, hypoferremia is considered part of what is called "nutritional immunity", since iron is an essential factor for many bacteria and its low availability limits for their growth.

**IL-1 and endothelial cells.** IL-1, and the functionally related lymphokine TNF, induces a complex reprogramming of functional status of endothelial cells [43]. It was originally observed that IL-1 induces production of the arachidonate metabolite prostacycline (PGI2) and procoagulant activity (PCA) in endothelial cells [44-46]. These observations were followed by a flood of studies describing a variety of changes in endothelial cell properties after IL-1. These include production of platelet activating factor, of plasminogen activator (PA-1), of leukocyte adhesion molecules and of von Willebrand factor. Moreover, IL-1 stimulated endothelial cells produce various cytokines including IL-6, IL-8 [Sica et al., unpublished] and CSFs. It is of interest that G and GM-CSF have recently been shown to modulate endothelial cell migration and proliferation [47]. All in all, the alterations in endothelial cell function induced by IL-1 cause vasodilation (via PGI2) and render the vessel wall prothrombotic by favouring the activation of the coagulation cascade (e.g. PCA) and inhibiting fibrinolysis (PA-1).

IL-1 treated endothelial cells recruit leukocytes from the blood compartment by expressing adhesion structures and producing chemotactic cytokines (IL-8). Thus, from these and other studies, endothelial cells have emerged as active strategically located compartment of inflammatory and immunological processes (Fig. 2).

**Other effects of IL-1.** Some effects of IL-1 suggest it has a role in the pathogenesis of rheumatoid arthritis. IL-1 induces proliferation of fibroblasts and synovial cells, induces collagenase secretion by synovial cells and chondrocytes, blocks proteoglycans and collagen synthesis and stimulates bone resorption by osteoclasts [48]. IL-1 induces prostaglandin synthesis and proteolysis in muscle. A factor known as PIF (proteolysis inducing factor), detected in the circulation of febrile patients was in fact found to be an active fragment of IL-1 [49].

It has also been suggested that IL-1 plays a role in the development of insulin-dependent diabetes, a pathology originating, at least partly, from autoimmune attack destroying insulin-producing β cells of the pancreas. Diabetic islets contain large numbers of macrophages, previously shown to produce substances toxic to β cells. In fact, recombinant IL-1 was toxic to β cells, suggesting it may play a part in the early stages of β cell destruction [50].

**Pharmacology of the IL-1 system**

In outlining the pharmacological relevance of IL-1, we must stress the fact that it can be viewed both as an immunomodulatory agent and as an inflammatory mediator. As an inflammatory mediator, IL-1 could be an important target for pharmacological intervention, with the development of antagonists or inhibitors. Although we are still far from the development of novel antiinflammatory agents acting as anti-IL-1 molecules, some inhibitors have been identified that may help study the IL-1 system.

---

Fig. 2. - The hypothetical central role of IL-1, IL-6 and TNF in communication between endothelium and leukocytes or tissues. Endothelial cells can act as accessory cells (expression of Ia and production of IL-1 and IL-6) and initiate immune responses. These, in turn, via leukocyte IL-1, TNF and γ IFN, can influence endothelial cell function. More in general IL-1 and IL-6 could serve as a communications signal between vessel walls and extravascular tissues. TNF, released by mononuclear phagocytes in tissue, may mediate tissue damage, alter endothelia and recruit leukocytes from the blood compartment.
IL-1 was reported to have some protective and restorative properties as an immunostimulatory agent. Many of these activities have been described in vivo in animal models, and in some cases clinical trials are already in progress.

Adjuvant activity

In agreement with in vitro data on its immunostimulatory activity IL-1 enhances in vivo antibody responses to protein antigens. IL-1 stimulates both the immune response to a T cell-dependent antigen, like sheep red blood cells (SRBC), but also to a T helper-independent antigen, like the poorly immunogenic polysaccharide antigen S III from Streptococcus pneumoniae [51, 52].

Radioprotective and myelorestorative properties

It has long been known that immunomodulatory agents exert radioprotective effects in vivo. The fact that these substances, which include endotoxin, muramyl dipeptide and bacteria, also stimulate IL-1 production suggested that IL-1 could be a radioprotective agent itself.

Pretreatment of mice with recombinant IL-1 did in fact protect against the lethal effects of ionizing radiations [53]. The mechanisms by which IL-1 exerts this effect are not known.

Induction of acute-phase proteins was suggested to play a role, since some of these proteins (e.g. methallothionein and ceruloplasmin) could act as free radical scavengers. Recently IL-1 was reported to induce endogenous manganese-containing superoxide dismutase, an enzyme previously shown to have radioprotective effects [54]. The ability of IL-1 to induce granulocyte-macrophage colony stimulating factor (GM-CSF) could be important in this effect [55].

In vivo, IL-1 promotes earlier hematopoietic recovery after lethal and sublethal levels of irradiation, probably as a consequence of the stimulation of colony forming cells (CFU) into cell cycle prior to irradiation. It has in fact been suggested that CFU in S phase are more resistant to radiation than in other phases of the cell cycle [56, 57].

IL-1 restores T cell functions in mice immunosuppressed by irradiation and has myelorestorative effect in cyclophosphamide treated mice [57]. It prolongs survival if administered before or after otherwise lethal doses of cyclophosphamide, the sequence and timing of treatment being critical for this effect. These studies have prompted clinical trials to test IL-1 as a radioprotective and chemoprotective drug.

Protection from oxygen toxicity

Exposure to a hyperoxic atmosphere causes pulmonary damage and death. The only agent known to induce tolerance to hyperoxia was endotoxin. However IL-1, when administered to rats in association with TNF immediately before exposure to 99% oxygen, markedly enhanced survival and relieved pulmonary toxicity as evaluated by histological examination and hemodynamic parameters [58].

Since free radicals play an important role both in oxygen toxicity and radiation damage, it was suggested that induction of antioxidant proteins, mentioned before, might be at the basis of these protective effects of IL-1.

Induction of nonspecific resistance to infection

Several substances reportedly enhance nonspecific resistance to infections. Once again, these substance (endotoxin, bacillus Calmette-Guérin, muramyl dipeptides) are IL-1 inducers. Using recombinant IL-1 preparations, it has been demonstrated that administration of IL-1 24 h before infection prolonged survival of granulocytic mice lethally infected with Pseudomonas aeruginosa, without affecting the number of bacteria cultured from various organs [59]. The mechanism of this protective effect of IL-1 appeared related to the ability of IL-1 to down-regulate its own effects (IL-1 is a mediator of septic shock) and it was suggested that it was to some extent due to induction of "detoxifying" acute-phase proteins, particularly endotoxin-binding proteins, cytokine inhibitors and others.

Antagonists and inhibitors

The evidence that IL-1 is involved in the pathogenesis of a variety of inflammatory diseases, rheumatoid arthritis and diabetes has made this cytokine a target for possible pharmacological intervention.

One approach would be to develop drugs that block IL-1 production. To date, the best-known inhibitors of IL-1 synthesis and release are corticosteroids [60]. Prostaglandin E2 was also reported to reduce IL-1 production by elevating intracellular levels of cAMP, and this appears to be a major feedback mechanism controlling IL-1 synthesis, since IL-1 is a potent stimulus to PGE2 production [61].

Since processing IL-1 seems to require proteolytic cleavage, inhibitors of the proteases involved in IL-1 processing could stop the release of biologically active IL-1.

Another agent shown to inhibit IL-1 synthesis is interferon gamma (γ-IFN). Gamma-IFN blocks IL-1 induction by itself [62] and could therefore be important in breaking self-amplification of IL-1’s effects in chronic diseases.

Gamma-IFN prevents other effects of IL-1, like induction of prostaglandin production and bone resorption. This prevention of IL-1 synthesis and action could be relevant to the pharmacological effects of γ-IFN, which is now being tested in patients with rheumatoid arthritis [63].
A recent report indicated that dietary supplementation with eicosapentenoic acid ("fish oil") reduces IL-1 production by mononuclear cells of human volunteers by 70% [64]. This finding opens a new field for manipulating IL-1 production through the diet.

A second approach to counteract IL-1’s effects is to develop antagonists of its action. IL-1α and IL-1β bind to the same receptor molecule, which has recently been purified and cloned [65]. It might thus be possible to develop receptor antagonists for IL-1. The biochemical event(s) that follow IL-1/receptor interaction could also conceivably be stopped. However, the second messengers of IL-1 activity are largely unknown, and in some cases receptor-independent activities of IL-1 have been proposed [66].

Since IL-1 has diverse effects on so many cell targets, that different signaling mechanisms may well be involved. Polypeptide inhibitors of IL-1 have been identified, particularly from urine [67]. These proteins inhibit the stimulation of prostaglandin production and mitogenic activity of IL-1. Other proteins, like uromodulin, were found to bind IL-1 and therefore to inhibit its activity. In the field of peptide inhibitors, melanocyte stimulating hormone (MSH) has been reported to block IL-1 activity in vitro and in vivo.

### IL-1 peptides

From the therapeutic point of view, it would be desirable to have molecules that retain IL-1’s immunostimulatory properties but not its proinflammatory action. One way to dissociate these two activities was to identify the parts of the IL-1 molecule responsible for its pharmacological activity. Short fragments of IL-1 have been synthesized on the basis of their predicted exposure on the molecule surface. One of these, the highly hydrophilic fragment 163-171 of human IL-1β, has many of the in vivo immunomodulatory and protective properties of IL-1, including adjuvant action, restoration of immune reactivity in immunocompromised animals and radioprotection [52, 68]. However, this nonapeptide, even at high doses, did not reproduce many of the inflammatory activities of IL-1 such as fever, hypothermia, induction of acute-phase proteins and shock [68]. These findings suggest that the region of the IL-1 molecule responsible for its immunostimulatory activity is distinct from that with inflammatory activity and that the two effects can be dissociated.

### Acknowledgements

The generous contribution of the Italian Association for Cancer Research, Milan, Italy, is gratefully acknowledged.

Review submitted on invitation by the Editorial Board of the Annali. Accepted for publication: December 21, 1989.

### REFERENCES


