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The history of discovery of Interleukin-1: A fundamental cytokine of the innate immune response

Veronica Vivona[1*], *Luca Lepore*[1*], *Giorgia Bilato*[2], *Lorenzo Mortara*[1], *Andrea De Lerma Barbaro*[3]

[1]Immunology and General Pathology Laboratory, Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy; [2]PhD student in Experimental and Translational Medicine, Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy; [3]Laboratory of Comparative Physiopathology, Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy. *These authors equally contributed.

Abstract. The discovery of IL-1 marked the beginning of research on soluble mediators of immune responses and initiated studies on inflammatory responses to tissue damage. Initially termed "endogenous leukocyte pyrogen" (derived from the Greek word 'pyr' for fire), due to its role in inducing fever, research on this molecule led to the identification of numerous related soluble factors, collectively named the IL-1 family. Additionally, the study of IL-1 laid the groundwork for characterizing a variety of structurally and functionally related receptors, which are crucial in regulating immune responses. Significantly, the IL-1 family plays a key role in inflammation, response to microbes, and acts as an inducer of both autoimmune and autoinflammatory pathologies, and it is also associated with cancer. In this latter context, both pro-tumor and anti-tumor roles of IL1 family members have been highlighted. Therefore, the IL-1 family molecules present many aspects that are still under active study today. In fact, numerous researchers are engaged in this line of investigation, exploring potential treatments for various diseases.

Key words: Interleukin-1, IL-1, leukocytic pyrogen, endogenous pyrogen, IL-1 family molecules, alarmins

Introduction

The discovery of IL-1 began in the 1940s when Dr. Paul B. Beeson et al. described an endogenous protein able to induce fever in animals, independent of infection. This protein, produced by polymorphonuclear leucocytes (neutrophils), was called endogenous pyrogen or leukocytic pyrogen (1,2). In the same period, Dr. Eli Menkin, a Russian researcher, injected rabbits with supernatants from neutrophils obtained as a result of sterile peritonitis. He assumed that neutrophils released a substance called "pyrexin" that produces fever when injected into rabbits (reviewed in 3). His hypothesis was confirmed in 1948 when Dr. Beeson showed the same results (1), providing evidence for the possibility of fever in the absence of infection. Subsequent attempts to purify leukocytic pyrogen followed, but protein purification was not well optimized during those years, leading to the inevitable loss of most of its biological activity (3).

A breakthrough in the field occurred in 1967 when Drs. Phyllis Bodel and Elisha Atkins from the Department of Internal Medicine, Yale University School of Medicine, demonstrated that human blood monocytes were the main source of leukocytic pyrogen. Their experiments involved the stimulation of the monocytes through exposure to heat-killed *Staphylococcus epidermidis*, also providing clarification about the onset of fever in patients with severe neutropenia (4). This discovery led to a shift in research projects from granulocytes to monocytes.

In the 1970s, a series of works based on biochemical characterizations of IL-1 began. Among the researchers, Dr. Charles A. Dinarello, who would later

become one of the major figures in the field, started purifying IL-1 from human monocytes. At that time, he was a second-year medical student and became affiliated with the laboratory of Drs. Atkins and Bodel, collaborating on their projects related to IL-1 and human monocytes (2). In 1972, Igal Gery and colleagues described an in vitro human and mouse lymphoid cell-derived factor that enhances the proliferative response of mouse thymocytes in response to mitogens such as phytohemagglutinin (PHA) or lipopolysaccharide (LPS). They named it lymphocyte-activating factor (LAF) based on its activity (5). LAF was subsequently shown to promote various biological activities, affecting lymphocytes and a variety of non-lymphocytic cells. Specifically, it seemed to stimulate production of acute phase proteins by hepatocytes, prostaglandin by hypothalamic centre, and to promote antibodies production by B lymphocytes (6).

In 1974, Dinarello and colleagues purified and characterized two distinct molecules with pyrogenic properties from human blood leukocytes derived from normal donors. One was produced by peripheral blood monocytes, whereas the other one was produced by neutrophils (7). These two molecules would later be termed IL-1α and IL-1β.

Over time, several studies demonstrated that many activities considered to be induced by different cytokines were actually different biological functions of the same one (8). In 1979, Lanny J. Rosenwasser and Dinarello showed that human leukocytic pyrogen and LAF appeared to be the same molecule based on shared biochemical characteristics and functions. They worked using supernatants from both mice and humans' macrophages and described that highly purified leukocytic pyrogen was able to act as LAF, stimulating thymocytes activity. Moreover, a large number of similarities, including molecular weights and isoelectric focusing points had been already proved (9). Until that point, cytokines were named based solely on their biological activity in a specific assay, rather than their definitive biochemical and biological properties. This led to the Second International Lymphokine Workshop (Ermantingen, Switzerland, 1979), where a new nomenclature of cytokines was defined and the term Interleukin was proposed. Interleukin means "between leukocytes," emphasizing the ability of these cytokines to act as communication signals between different populations of leukocytes (8). After that, the majority of the scientific community began using the term interleukin-1 (IL-1) to refer to the endogenous pyrogen.

However, at that time, there were many controversies regarding the possibility that a single molecule could be involved in such a wide spectrum of biological activities. Critics focused on the lack of an amino acid sequence and an IL-1 receptor that could justify this conclusion (10). In fact, opponents claimed that the multiple activities of this cytokine were actually due to a mixture of proteins with similar molecular weights or contaminating microbial products. A paper was even published to disprove the idea that LAF and the leukocytic pyrogen were the same molecule (11).

At this point, much of the work on the chemical characterization and purification of IL-1 had been done. Among the researchers, Patrick A. Murphy also made significant contributions by describing two leukocytic pyrogens isolated from rabbit peritoneal exudate cells: a neutral form (IL-1β) and an acidic form (IL-1α) (12,13).

Another breakthrough came in the 1980s when DNA sequencing technologies became available and the cloning era started. Specifically, three crucial articles were published in close succession in Nature.

The first one, dated 1984, was by Peter T. Lomedico et al., which reported the cloning and sequence analysis of an IL-1 cDNA expressed in Escherichia coli and derived from murine macrophages. This cDNA encoded a polypeptide precursor which later turned out to be IL-1α (14).

In the same year, Philip E. Auron and colleagues, including Dinarello, published an article explaining that they had isolated a human IL-1 cDNA from peripheral blood monocytes instead. The encoded protein closely resembled IL-1β, and its nucleotide sequence suggested that human IL-1 is initially synthesized as a precursor and then cleaved to reach the mature form (15,16).

In 1985, Carl J. March and colleagues isolated two distinct cDNAs encoding IL-1-like proteins, named IL-1α and IL-1β, from a macrophage cDNA library (16). These molecules showed limited similarity to each other and were revealed to have different biological functions, dependent on their C-terminal

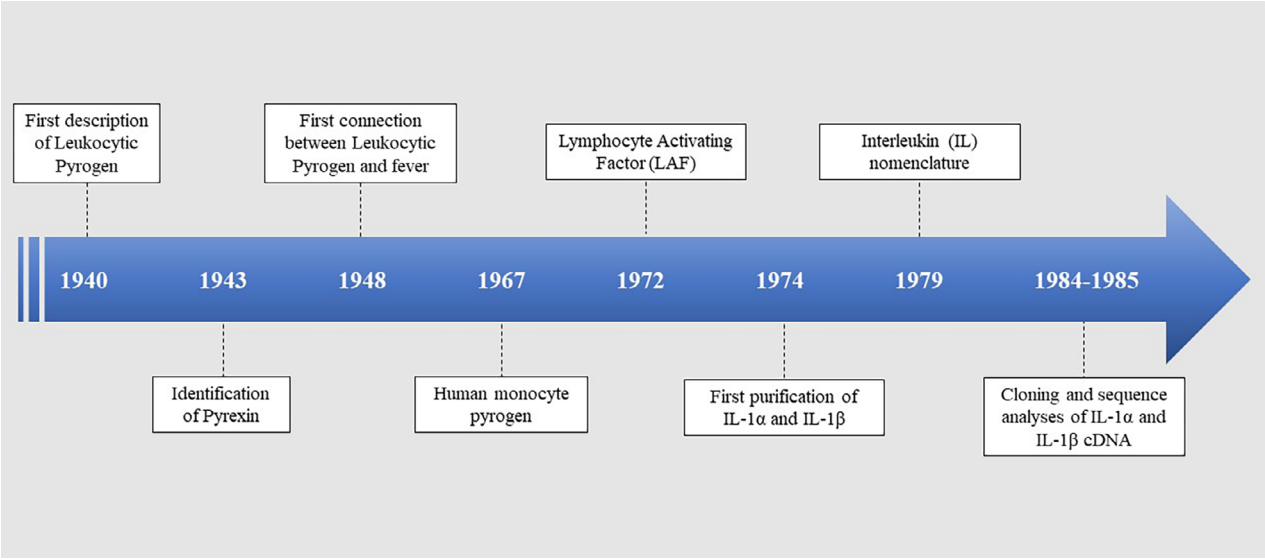

Figure 1. Timeline of IL-1 crucial discoveries.

portion only. Subsequent experiments using recombinant proteins definitively confirmed that IL-1 possesses pyrogenic properties and can induce fever on its own (10). These findings supported earlier research and further expanded the understanding of IL-1 biology.

In summary, the discoveries and characterization of IL-1 from the 1940s to the 1980s paved the way for better understanding its role in many physiological and pathological conditions (Figure 1). Meanwhile, the subsequent identification and description of various IL-1-like molecules resulted in the institution of the IL-1 family, with IL-1α being recognized as its first member.

Dinarello's biography and research

Charles A. Dinarello (born on April 22, 1943 – Boston, Massachusetts) is regarded as one of the pioneers in cytokine research. He successfully cloned and purified one of the major cytokines involved in inflammation, Interleukin-1. Dinarello has made substantial contributions to the field, having published over 600 research articles and 250 reviews and book chapters, primarily focusing on Interleukin-1 and tumor necrosis factor-alpha (TNF-α) (17,18). Notably, Dinarello's research in cytokines extends to their role in cancer, particularly in relation to histone deacetylation. Graduated in Medicine and later specialized in Immunology, he has held the position of Professor of Medicine at the University of Colorado School of Medicine since 1996. Dinarello's crucial contribution was the purification of IL-1, at that time known as "endogenous pyrogen". This huge effort led to the identification of the molecule responsible for inducing fever in animals and humans, confirming that the protein he isolated was indeed the causative agent of fever in both.

Professor Elisha Atkins was a pivotal inspiration for Dinarello's research, especially after a lecture on the "endogenous pyrogen", which deeply fascinated him.

Therefore, he joined Atkins' laboratory to work on his thesis, culminating in his first publication on the endogenous pyrogen in 1968 (19). Following his clinical training, he moved to Boston in 1969 for an internship at the Children's Service of the Massachusetts General Hospital. There, he continued zealously his research regarding the endogenous pyrogen in Sheldon M. Wolff's laboratory.

In 1971, Dinarello and colleagues embarked on the challenging attempt of characterizing and purifying the endogenous pyrogen, culminated in 1977 with its successfully purification published in the Proceedings of the National Academy of Sciences (20).

Dinarello acknowledged the significant contributions of Patrick Murphy, who isolated the leukocyte pyrogen from rabbits, revealing the existence of two

forms of IL-1 in rabbits: IL-1β (neutral form) and IL-1α (acidic form) (12, 13).

The purification of the endogenous pyrogen, albeit without an amino acid sequence, propelled researchers to delve further into isolating the cDNA, even if cloning techniques were still immature in 1982. However, Dinarello started the cDNA isolation project in February 1982, with the precious help of Andrew Webb. By 1984, they managed to isolate the cDNA coding for the IL-1β precursor and determined its amino acid sequence.

Regrettably, Nature initially rejected the manuscript detailing the cloning of IL-1β by Dinarello and colleagues. However, within a few months, the journal accepted a submission from another group claiming the same sequence, including identical non-coding nucleotide errors, which suggested that they had replicated Dinarello's study (2). In the same year, IL-1α has been cloned from mouse macrophages by Peter Lomedico and Steven Mizel (14).

From one molecule with many roles to a large family involved in different functions

IL-1β, was first cloned from human blood monocytes, and IL-1α was first cloned from mouse macrophages (21,22).

The production of recombinant IL-1β allowed to demonstrate its overlapping activity with the endogenous pyrogen, enabling specific investigations into the numerous activities of IL-1 (23,10).

At that time, it was already known that IL-1 binds to a variety of cells, but the underlying molecular mechanisms were still not identified. The purification and characterization of IL-1 significantly contributed to understanding how this molecule induces fever, especially following Sim's cloning of the IL-1 Receptor (IL-1R) (24) and Greenfeder's discovery of the IL-1R accessory protein (IL-1R3) (25). This protein, an essential component of IL-1R complex, was found to facilitate the binding of both IL-1α and IL-1β. Later, IL-1R3 was identified as the IL-1 receptor accessory protein involved in signaling mediated by six cytokines of the IL-1 family: IL-1α, IL-1β, IL-33, IL-36α, IL-36β and IL-36γ (26,27).

With the IL-1R purification, it became possible to block the receptor and observe the effects of the inhibited IL-1 activity, further expanding understanding of the IL-1 family. Initially, administering IL-1 type I Receptor antibodies in animals reduced inflammation and anorexia by decreasing the production of endotoxins and other inflammation-inducing agents. However, the most significant insights were obtained with the purification of the natural antagonist of IL-1R, IL-1Ra.

IL-1Ra was discovered in 1984 by Dayer and Balavoine, who noted that after IL-1 purification, IL-1 was masked by a factor of approximately 17 kDa, capable of blocking IL-1 activity (28,29). Later, in 1990, IL-1Ra was purified and cloned (30).

Currently, the IL-1 family comprises 11 members, and the IL-1 family of receptors includes 10 members. These regulate a plethora of immune-cell-mediated processes related to inflammation, autoinflammation, autoimmunity, and cancer.

The IL-1 family includes: IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, two receptor antagonists (IL-1Ra, IL-36Ra), and two anti-inflammatory cytokines (IL-37, IL-38). Considering their highly conserved gene structures (which includes identical positioning of certain introns and a more modest conservation of key amino acid sequences determining the folding of each protein into a 12-stranded β-barrel), it is likely that the different members originated from the duplication of a common ancestral gene. Except for IL-18 and IL-33, all the other genes of the IL-1 family are located in a clustered region of approximately 400 kb on chromosome 2 (31).

The various receptors and co-receptors associated with each IL-1 family factor, along their pro-inflammatory or anti-inflammatory functions, are detailed as follows: IL-1α, IL-1β, with their specific receptor IL-1R1 and co-receptor IL-1R3 (pro-inflammatory activity); IL-1β, with its specific receptor IL-1R2 and co-receptor IL-1R3 (anti-inflammatory activity); IL-1Ra (antagonist), with its specific receptor IL-1R1 which acts independently of a co-receptor (anti-inflammatory activity); IL-33, with its specific receptor IL-1R4 and co-receptor IL-1R3 (pro-inflammatory activity); IL-18, with its specific receptor IL-1R5 and co-receptor: IL-1R7 (pro-inflammatory

activity); IL-37, with its specific receptor IL-1R5 and co-receptor IL-1R8 (anti-inflammatory activity); IL-36 Ra, with its specific receptor IL-1R6 and co-receptor IL-1R3 (anti-inflammatory activity); IL-36α, IL-36β, IL-36γ with their specific receptor IL-1R6 and co-receptor IL-1R3 (pro-inflammatory activity); and IL-38, with its specific receptor IL-1R6 and co-receptor IL-1R9 (anti-inflammatory activity).

Importantly, IL-1R2 acts as a decoy receptor, first characterized by Dr. McMahan et al. in 1991 (32). It possesses the binding domain for IL-1β, but lacks the cytoplasmic domain necessary for signaling. Found both as a membrane-bound and soluble form, IL-1R2 sequesters IL-1β in the extracellular environment, thereby preventing its pro-inflammatory function. This inhibitory activity is further enhanced when IL-1R2 binds to the coreceptor IL-1R3, resulting in a competition with IL-1R1 (33,34). The discoveries of the distinct members of the IL-1 family are concisely summarized in the timeline shown in Figure 2.

Moreover, the molecules of the IL-1 family, which include both cytokines and receptors, exhibit functions similar to those of the Toll-like receptor (TLR) families, and they share comparable functional domains. In fact, each member of both the IL-1 receptor and TLR families contains the cytosolic Toll-IL-1-Receptor (TIR) domain. Both the IL-1 family cytokines and TLRs are pivotal in activating innate inflammation: the former via the IL-1 family of receptors, and the latter through interaction with microbial products such as bacteria, viruses, nucleic acids, and damage-associated molecular patterns (DAMPs). Within this context, IL-1α and IL-33 also act as DAMPs. Among the IL-1 cytokines family, only IL-1α and IL-33 function as 'alarmin', as their precursors are biologically active and immediately available for release following cell damages.

Considering the pivotal role of the IL-1 family members in immune-system related functions, deregulation and mutations in these molecules can lead to chronic inflammation, autoimmunity, and allergic reactions (35).

From inflammation to autoinflammation, autoimmunity, cancer and non-immune related diseases

The link between IL-1 and rheumatoid arthritis (RA) dates back to 1977 when Dr. Jean-Michel Dayer and colleagues (36) described a "mononuclear cell factor" able to stimulate in synoviocyte production of matrix metalloproteases and prostaglandin E2. Since then, the role of IL-1 family cytokines in RA has been

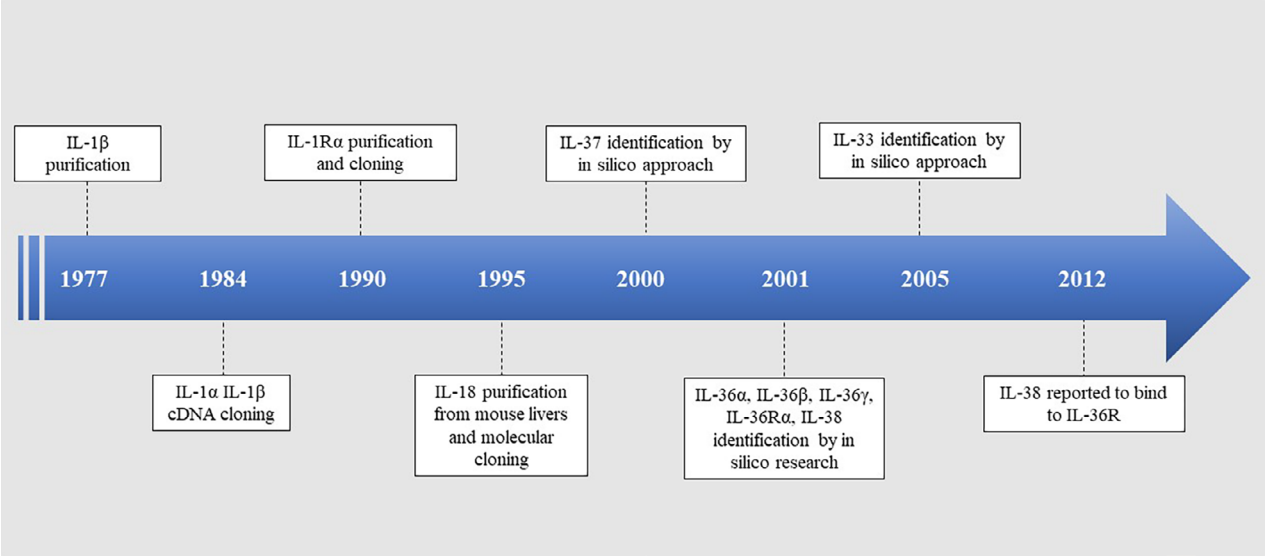

Figure 2. Timeline of discoveries of IL-1 family members.

extensively investigated, leading to a better understanding of their contribution to the pathogenesis of RA and other autoimmune disorders (37-40).

Targeting IL-1 began in 1993 with the use of anakinra, a recombinant form of the naturally occurring IL-1 receptor antagonist (IL-1Ra), which blocks the function of both IL-1α and IL-1β. Among the various inflammatory pathologies treated with this recombinant inhibitor, the following ones can be reported as an example: RA, ankylosing spondylitis, erosive osteoarthritis, recurrent osteomyelitis, systemic juvenile idiopathic arthritis, adult-onset Still's disease, Behcet's disease, and hereditary systemic autoinflammatory diseases (among which, familial Mediterranean fever, cryopyrin-associated periodic syndrome, TNF-α receptor-associated periodic syndrome) (41).

As opposed to IL-1α, IL-1β is not stored in the cells of healthy individuals, and requires several activation steps in the cytoplasm, involving an inactive IL-1β precursor (proIL-1β). Only active IL-1β can be released from the cell and trigger inflammatory responses. Moreover, the production of IL-1β takes place only in a few and specialized cell types, such as monocytes, macrophages, and dendritic cells. This process requires the activation of the caspase-1 enzyme, a cysteine protease activated by an intracellular multiprotein complex called inflammasome.

So far, we know several autoinflammatory diseases characterized by a high inflammation phase related to the action of cytokines of the innate immune system, particularly IL-1β. This occurs without the induction of autoantibodies or autoreactive T lymphocytes in individuals with a significant predisposition (42). However, in autoimmune diseases a greater involvement of the component of the adaptive immune system is observed. In autoimmune diseases, blocking TNF-α can be a highly effective therapy, whereas, in autoinflammatory diseases, TNF-α neutralization does not work. Classic autoinflammatory diseases are chronic syndromes associated with rare genetic disorders, wherein the clinical signs and abnormalities are common to most inflammatory diseases, characterized by recurrent episodes of systemic and local inflammation. Among them, familial Mediterranean fever is probably the most well-known autoinflammatory disease. In these young patients, there are recurrent bouts of fevers and pain in the lining of the abdomen that mimics acute appendicitis. The genetic mutation was identified in the Mediterranean fever (MEFV) gene, encoding a protein that was given the name pyrin (from the Greek word 'pyr' for fire). Wild-type pyrin is a key protein in the control of the activation state of caspase-1 as well as the processing and release of active IL-1β, so an aberrancy of its function results in a loss of this regulatory step, with an increased release of IL-1β. Higher production of IL-1β has been shown to be associated with other several autoimmune diseases, including Hashimoto thyroiditis (43). Chronic autoimmune thyroiditis also includes hyperthyroidism Graves' disease (44) and, together with Hashimoto's thyroiditis, constitutes approximately 30% of all autoimmune diseases, collectively referred to as autoimmune thyroid disorders (AITD). Interestingly, a key potential immunomodulatory effect on prevention and treatment of AITD is made by vitamin D (45).

The discovery and molecular characterization of inflammasome come from the initial studies of the Dr. Jurg Tschopp's team. In 2002, Dr. Jurg Tschopp proposed the term "inflammasome" to indicate a macromolecular complex that senses 'danger' and trigger the inflammatory response by processing, through proteolytic cleavage, a preformed, intracellular precursor of IL-1β and IL-18. Moreover, his group demonstrated that the spontaneous hyperactivation of the production of IL-1β by mutated forms of the inflammasome scaffolding receptor, named NOD-like receptor family, pyrin domain containing-3 protein or NLRP3, was the basis of several hereditary periodic fever syndromes (46-48). Another important disease topic in which IL-1 plays a relevant role is cancer. In this case, both pro-tumor (negative) and anti-tumor (positive) roles have been highlighted. It is known that chronic inflammation is among the key factors that drive cancer development, indeed it is one of the hallmarks of cancer. Originally, the hallmarks of cancer comprised six biological capabilities acquired during the multistep development of human tumors: 1) cell-sufficiency in growth signals, 2) insensitivity to anti-growth signals, 3) tissue invasion and metastasis, 4) limitless replicative potential, 5) sustained angiogenesis and 6) evading apoptosis (49). However, in 2011, another revision in the Cell journal added

further new characteristics involved in the pathogenesis of most cancers. Precisely, they defined two emerging hallmarks: reprogramming cellular metabolism and avoiding immune destruction, and two enabling characteristics: genome instability and mutation, and tumor-promoting inflammation (50). Therefore, tumor-promoting inflammation is part of the enabling feature of cancer. Whereas inflammation represents a useful and protective physiological reaction against infections in the repair of tissue wounds, it can result in favoring multiple cancer hallmark capabilities instead. Two pathways of proinflammatory cytokines-mediated effect have been determined: an extrinsic modality, which plays a role in infection, autoimmunity and autoinflammation, and an intrinsic way, involving genetic events associated with the onset of carcinogenesis and development of an inflammatory microenvironment. As a matter of fact, IL-1 has been shown to act on both pathways (51,52). Moreover, IL-1 can play a pro-tumorigenic effect as a driver molecule in orchestrating polarization of innate lymphoid type 3 cells (ILC3) and T helper type 17 (TH17) lymphocytes, i.e. then acting on activation of both innate and adaptive immune cell responses that can favor tumor progression. IL-1 can be associated with the enhancement of carcinogenesis by different mechanisms that play a role in tumor angiogenesis, endothelial cell activation, metastasis, lymphoid cell (TH17) polarization, induction and expansion of myeloid-derived suppressor cells, and expansion of tumor-associated macrophages and tumor-associated neutrophils (53-55). Additionally, IL-1 has demonstrated anti-tumoral effects. For instance, Ghiringhelli's group showed in 2009 that the activation of inflammasome and the secretion of IL-1β in dendritic cells can trigger the production of adaptive anti-tumor interferon-gamma (IFN-γ) by $CD8^+$ T lymphocytes (56).

Furthermore, in some types of human tumors, such as head and neck squamous cell carcinoma, it has been recently shown that IL-1α can increase the anti-tumor effect of the therapeutic monoclonal antibody Cetuximab. However, the use of anti-tumor cytokines in vivo such as IL-1 or others (IL-2, TNF-α IL-12), develops strong toxicity, so multiple strategies are under investigation, including the use of cytokines linked to single chain fragment variable (ScFv) antibodies directed against tumor target or tumor microenvironment (57-59), and cytokine genetically-engineered cells (60).

Finally, IL-1β, and the other members of the IL-1 cytokine family, may play a major role in the pathogenesis of a wide range of acute and chronic diseases traditionally not thought to be related to inflammation. For instance, dysregulation of IL-1β-associated pathways are implicated in the pathogenesis of type II diabetes (61,62) and myocardial infarction (63).

Conclusions

Since its discovery, IL-1 has shown a great diversity of biological functions, making researchers immediately think that this molecule was not actually unique and that related molecules could exist. In fact, early on it was evident that there were two types of leukocytic pyrogens: an acidic form (IL-1α), the first one discovered, and a neutral form (IL-1β). Much research conducted between the 1940s and 1980s, aimed at characterizing the multiple functions of IL-1, was crucial for deeper understanding its role under physiological conditions and in pathological phenomena. The initial discovery of IL-1, with its many roles, led to a large family of correlated molecules involved in diverse processes, including pathogen defense and tissue homeostasis, ranging from inflammation, autoinflammation, autoimmunity and cancer.

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Correspondence:
Lorenzo Mortara
Immunology and General Pathology Laboratory, Department of Biotechnology and Life Sciences, University of Insubria, 21100 Varese, Italy.
E-mail: lorenzo.mortara@uninsubria.it