*European Journal of Neurodegenerative Diseases*
ISSN 2974-6345 / 2023 vol. 12, ISSUE N. 1, January-April

*Letter to the Editor*

# HISTORY OF CYTOKINES: MY CONTRIBUTION

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**KEYWORDS:** *cytokine, history, inflammation, immunity, chemokine*

## INTRODUCTION

Professor Pio Conti began scientific research in London in 1977 where he worked in the laboratory of Prof. D.A. Willoughby, studying the mechanisms of chronic inflammation. From 1981-1983, he worked in the USA in Washington D.C. at the Immunology Center at Georgetown University, directed by Prof. J.A. Bellanti. In this lab, he studied the eicosanoids and the effect of lymphotoxin on neutrophils in vitro, in collaboration with Prof. Peter W. Ramwell and Dr. Terry W. Williams, the collaborator of G.A. Granger (University of California), the discoverer of lymphotoxin, which was later named tumor necrosis factor (TNF). In 1985, P. Conti and T.W. Williams published an interesting article highlighting that lymphotoxin damages human neutrophils, causing vacuolization and increasing thromboxane. Later, this discovery proved to be the basis for myocardial infarction. In 1984, P. Conti was invited to Boston (USA) to carry out research on the cytokine IL-1 in the laboratory of Prof. Charles A. Dinarello, the purifier and cloner of IL-1 and the discoverer of various cytokines (IL-18, IL-33, IL-37, IL-38, IL-1RA). His work here led to the publishing of a pioneering article on the effects of IL-1 on natural killer cells and tumor killing with J.W. Mier (who discovered IL-2 with Robert Gallo from NIH). From 1985-86, Prof. Conti worked at Harvard Medical School in Boston, where he collaborated with Dr. C.N. Serhan (collaborator of Prof. Bengt I. Samuelsson, Nobel Prize winner), the discoverer of Lipoxins A and B, and published an original paper on the stimulation of lipoxin A on the release of thromboxane by neutrophils. From 1986-2022, he studied the pathophysiology of mast cells at the Molecular Pharmacology and Drug Discovery Laboratory at Tufts University in Boston, directed by Prof. T.C. Theoharides. The studies done in this research center led to the publication of a significant number of articles in the best international scientific journals. From 2009 to today, he has collaborated with Dr. Susan E. Leeman (former Nobel Prize candidate), discoverer of the neuropeptide neurotensin and purifier of substance P. In 2020, during the pandemic, Professor Conti published an article on the damage effects of cytokines released in COVID-19 which obtained many citations (1,744).

In 1969, the first lymphokines were isolated in *in vitro* lymphocyte cultures, which were also called interleukins due to their ability to act between cells (1). Subsequently, monokines, which derive from monocytes, were discovered and these proteins together with lymphokines were called cytokines. Cytokines are low molecular weight immunoregulatory proteins that can act at very low concentrations (nanograms). They modulate our body's immune responses, but when their levels exceed physiological limits, they can mediate both acute and chronic inflammation. Here, we report some

Received: 02 April 2023
Accepted: 23 April, 2023

2974-6345 (2023)

articles published in the past 40 years by Professor Pio Conti concerning the activity of cytokines in acute and chronic inflammation. In conducting the experiments reported here, Prof. Conti collaborated with internationally renowned scientists who played a decisive role in the discovery and function of cytokines.

**DISCUSSION**

Prof. Conti began studying chronic inflammation in the laboratory of Professor D.A. Willoughby at Saint Bartholomew Hospital in London in the year 1977. Wistar rats were injected subcutaneously with 0.2 ml of saturated water solution of potassium permanganate crystals diluted at a ratio of 1:40. After a week, calcium granulomas indicating chronic inflammation were obtained. These experiments allowed to study at the molecular level both some anti-inflammatory pharmacological compounds and some inflammatory cytokines/chemokines. In 1981, Prof. Conti started attending Prof. Peter Ramwell's laboratory at Georgetown University Medical Center in Washington D.C., where he studied the arachidonic acid cascade in neutrophil granulocytes isolated *in vitro*. Subsequently, from 1982-83, at the Georgetown Immunology Center directed by Prof. J.A. Bellanti (author of the famous textbook: "Immunology"), Prof. Conti collaborated with Dr. Terry Williams, the collaborator of Prof. G.A. Granger who discovered the cytokine lymphotoxin, which was later named tumor necrosis factor (TNF). The experiments conducted at the Immunology Center highlighted for the first time the damage, in terms of vacuolization and cell death, of lymphotoxin on human granulocytes *in vitro* (2).

In 1984, Prof. Conti was invited by Prof. Charles A. Dinarello to work in his laboratory of Infection Diseases at Tufts University Medical School in Boston, MA. In his experiments, Prof. Dinarello had just demonstrated that the endogenous leukocyte pyrogen (ELP) of fever was the same molecule as interleukin-1 (IL-1) and was generated by macrophage cells (3). This was a revolutionary concept in medicine that clarified the etiopathogenesis of fever and showed that IL-1 was not a molecule produced by neutrophil granulocytes, a concept reported in many textbooks. The experiments conducted in Prof. Dinarello's laboratory led to the publication of an interesting article authored by C.A. Dinarello, P. Conti and J.W. Mier, in which IL-1 was shown to have an effect on natural killer (NK) cells in tumor activity (4). This was the first evidence of the involvement of IL-1 in tumor pathology. Dr. J.W. Mier was a junior researcher at NIH in Bethesda, who had already been highlighted for his role as a collaborator with Dr. R.C. Gallo in the characterization and purification of T-cell growth factor (TGF), subsequently named IL-2.

Until then, IL-1 was used in purified form, but after a few months, Prof. Dinarello cloned IL-1, an important work published in the journal *The Proceedings of the National Academy of Sciences* (PNAS) (5). Following this discovery, Prof. Conti used recombinant human IL-1 in his experiments on polymorphonuclear leukocytes, highlighting the ability of this cytokine to stimulate the production of thromboxane A2 (TxA2) detected as TxB2 (6).

This was an important finding as IL-1 was implicated in cardiovascular disease where platelet aggregation is mediated by TxA2. The study of the arachidonic acid cascade was one of the main themes in the career of Prof. Conti. In fact, these studies continued from 1985-86 at Harvard, Boston, at the laboratory of Dr. C.N. Serhan, collaborator of Prof. Bengt I. Samuelsson, Nobel Prize winner for his studies on eicosanoids. At Harvard, Prof. Conti began to conduct experiments with lipoxin, a new molecule discovered by Dr. Serhan. Experiments show that lipoxin A (LxA) augments the release of thromboxane from human polymorphonuclear leukocyte suspensions (7). It was also reported that LxA regulates TNF-directed neutrophil action *in vitro* and stimulates the secretion of IL-4 in immune responses (8).

From 1986 until today, Prof. Conti has been studying the pathophysiology of mast cells (MCs) at the Molecular Pharmacology and Drug Discovery Laboratory at Tufts University in Boston, MA, under the direction of Prof. T.C. Theoharides. This long period of work and study has led to the publication of a large number of interesting articles (9-16).

In 1995, at Tufts, he found that monocyte chemotactic protein-1 (MCP-1) and MCP-3, pro-inflammatory chemokines that attract leukocytes, cause clump formations on MCs, a phenomenon absent in untreated cells (controls). These results confirm the chemo-attraction capacity of these chemokines and, when analyzed under an electron microscope, demonstrate communication between the cell membranes and the cytoplasm of adjacent MCs (17). In 1997, also at Tufts, Conti et al. found that the chemokine regulated upon activation normal T expressed and secreted (RANTES) attracted basophilic cells in a dose-dependent manner. When this chemokine was injected into rat skin, there was an increase in histidine decarboxylase (HDC) mRNA, demonstrating that histidine genes were activated, leading to histamine synthesis (18). In the same year, still on the study of MCs, Conti et al. found for the first time that RANTES and MCP-1 injected subcutaneously in rats caused the recruitment of MCs and the stimulation of HDC. These studies were published in important international journals and aroused much interest in the scientific community (9-11, 17).

In 2002, Prof. Conti, with the group of Dr. Theoharides, published that IL-6, a crucial cytokine for MC maturation, is important for the maturation of CD34+ cells, human umbilical cord stem cells, in the presence of stem cell factor (SCF) (19). In the 2000s, the study of IL-10 emerged in the scientific community, as it was highlighted that this cytokine is anti-inflammatory and is involved in immunosuppression. In this respect, Conti et al. published an interesting article showing that IL-10 inhibits some inflammatory cytokines, Th2, and natural killer (NK) cells, but not tryptase from MCs (20). The studies done by professors Conti and Theoharides led to the elucidation of the role of MCs in tumor growth, as many articles were contradictory on this topic. In a review on this subject published in the journal *Trends in Immunology*, Conti and Theoharides highlighted that by producing certain compounds, MCs could promote tumor and metastatic growth, but by generating other compounds, they could inhibit tumor development (21). These concepts were highly appreciated and cited by the scientific community. Moreover, in the journal *Trends in Pharmacological Sciences*, Theoharides and Conti reported that the corticotropin-releasing factor (CRF), which leads to the selective release of cytokines and other pro-inflammatory mediators, increases following stress (22).

The studies at Tufts University were very prolific in terms of publication and highlighted various aspects of immunology and MC biology. In 2007, Prof. Conti, together with the group of Dr Theoharides, summarized a part of these studies in an important review which highlighted the mechanisms of MC activation and the relevance of their inhibition (23). Studying the central nervous system (CNS) at Tufts, Prof. Conti performed *in vitro* experiments on MCs in the CNS. This work had a notable development thanks to the collaboration with Nobel Prize candidate Dr. S.E. Leeman, the scientist who had discovered the neurotransmitter neurotensin and purified substance P (24,25).

In the first decade of the 2000s, the group of Prof. Theoharides, including Prof. Conti, started to study autism spectrum disorders (ASD). In 2008, they published an article showing that MCs can also be activated in a non-allergic way and could be involved in the pathogenesis and therapy of ASD (26). In 2010, Conti, Leeman, and Theoharides et al. reported in the journal PNAS that the interaction between SP, IL-33, and MCs leading to VEGF release, contributes to the inflammation involved in psoriasis, a nonallergic neurogenic hyperproliferative skin disease (12). In 2017, Conti et al. reported that SP and IL-33 together markedly enhance TNF synthesis and secretion in human MCs mediated by the interaction of their receptors, highlighting an amplification process of TNF synthesis and secretion, an effect inhibited by methoxyluteolin (15). In light of these studies, in 2018, this research group reported that Substance P and IL-33 administered together stimulate a marked secretion of IL-1β from human MCs, a scientific basis that could lead to new therapies (16).

Meanwhile, studies on autism continued and led to the publication of an article in 2020 which was entitled "IL-38 inhibits microglial inflammatory mediators and is decreased in amygdala of children with autism spectrum disorder". This paper reported the effect of IL- 38, a new anti-inflammatory cytokine that suppresses the IL-1 produced by microglia. The inhibitory effect of IL-38 was more potent than that of IL-37, opening new avenues for ASD therapy (14).

The year 2019 marked the start of the global health crisis of the COVID-19 pandemic caused by SARS-CoV-2. In this period, many elderly patients were hospitalized for lung inflammation and there were many deaths, mainly resulting from respiratory failure. Prof. Conti was one of the first scientists to point out that inflammatory cytokines play an important role in this lung pathology. The article published in a ‘small’ journal was of great scientific interest and the basis of study for other authors, so much so that to date it has received 1,744 citations in the best international journals (27).

## CONCLUSIONS

With this article, Prof. Pio Conti takes the opportunity to thank all his collaborators, (in particular, Dr. T.C. Theoharides and Dr. C.A. Dinarello) and the university of Tufts, Boston, and the "G. D'Annunzio" University of Chieti-Pescara, Italy.

*Conflict of interest*

The author declares that they have no conflict of interest.

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