

Rita Levi-Montalcini and the discovery of NGF, the first nerve cell growth factor

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ABSTRACT

The nerve growth factor (NGF) is a signaling protein, discovered by Rita Levi-Montalcini in the early 1950's for its effect on growth and differentiation of specific specific populations of neurons of the peripheral nervous system. Originally identified as neurite outgrowth-stimulating factor, later studies revealed that the purified molecule has a number of target cells in the central nervous system and on non-neuronal cells. Moreover, recent studies showed the potential therapeutic properties of NGF in neuropathies of the central and peripheral nervous system and diseases of the eye and skin. Here I briefly describe the discovery of NGF, the early studies of Rita Levi-Montalcini, a pioneer in modern neuroscience, and my scientific and human experience working in her laboratory for over 40 years.

Key words

NGF • NGF-receptors • Neurotrophins • Ebri • Collaborators

Introduction

Rita Levi-Montalcini was born April 22, 1909 in Turin, Italy, where received her medical degree in 1936 from the University of Turin. The same year she entered the Institute of Anatomy as postgraduate student of Professor Giuseppe Levi, a well-known anatomist and tutor of two other future Nobel Prize winners in Physiology or Medicine, Salvatore Luria (in 1969) and Renato Dulbecco (in 1975). During her early postgraduate years, she studied the relationship between the developing nervous system and its peripheral targets and observed that many sensory neurons died during normal development, and that the limb-bud extirpation caused an increase in the number of immature nerve cell death. The results of these studies lead her to the hypothesis that the failure of neurons to thrive in the absence of peripheral target was because of a degenerative process rather than a failure of differentiation, as had previously been

hypothesized by Victor Hamburger, a well-known neuroembryologist working at the Department of Zoology Washington University in St. Louis, MO. In 1946, Hamburger invited Levi-Montalcini to join his group to reinvestigate their scientific disagreements. In their initial work Hamburger and Levi-Montalcini first published manuscript reported that many sensory neurons die during the course of normal development, and that limb bud extirpation causes an increase of neuronal death. From these results they prospected the hypothesis that interactions with the periphery are reciprocally competitive in the sense that developing neurons depend on feedback signals from the peripheral tissues that are of limited supply and that neuronal targets provide a specific molecules that is required for neuronal survival. These seminal observations and hypothesis paved the way to the discovered of programmed cell death (in the today's context, the apoptosis), and later the discovery of nerve growth factor (NGF).

The road of NGF discovery

The NGF saga, as few years ago defined by Levi-Montalcini, began in 1949, when she first saw the results of Elmer Bueker, a former student of Hamburger showing the effect of transplanting mouse sarcoma tissue into a chick embryo. Bueker observed that upon transplantation, the tumor stimulated the growth of fibers from neurons of the sensory ganglia into the tumor, just as they would have done into transplanted limbs and even more vigorously. Bueker interpreted this result writing that the tumor transplantation effect was greater because the tumor provided a larger target area for the cells to expand than a limb would have. This interpretation did not convince Levi-Montalcini and following the suggestion of Hamburger decided to repeated this experiment. She found the same result, but reached a different interpretation, hypothesizing that the transplanted tumor tissue induced hyperinnervation of internal chick organs by releasing a diffusible agent that stimulated the growth and differentiation of developing nerve cells. Using *in vitro* analysis, she studied the effect of this extract on isolated sensory and sympathetic nerve cells and clearly demonstrated the direct stimulating action effect of the tumor extract on neurite outgrowth and named initially this yet unknown molecule nerve growth-stimulating factor, later termed NGF (Levi-Montalcini and Hamburger, 1951; Levi-Montalcini, 1952; Levi-Montalcini and Booker, 1960; Levi-Montalcini, 1987). In attempt to purify the tumor-derived factor, Levi-Montalcini and Stanly Cohen used snake venom as a rich source of phosphodiesterase, a nucleic acid-destroying enzyme, for the separation of nucleic acids and protein fractions in the tumor material. To their great surprise, the tumor fraction containing the snake venom was several thousand-fold more potent than control tumor homogenate in promoting nerve growth, both *in vitro* and *in vivo*. Further on the road of discovery, Levi-Montalcini and Cohen examined the mammalian homologue of the snake venom, the salivary gland, and found that the male mouse submandibular glands were an even richer source of the same nerve growth-stimulating activity found in both the tumor and the snake venom. Thus male mouse submandibular glands appeared to be a new and possibly largest source of NGF providing the possibility to isolate and purified

consistent amount of this intriguing biomolecule. In effect, such an unpredictable experimental cascade (from tumor via snake venom to salivary glands) lead to the identification of NGF. In other words, the discovery of NGF was marked by a rare combination of scientific reasoning, intuition, and chance, the latter “favors only the mind that is prepared”, quoting Louis Pasteur.

The NGF/NGF receptors and neuronal cells

Since its discovery, NGF becomes one of the best-characterized members of a later identified family of neurotrophic factors, which also include brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), NT-4/5, NT-6 (see Aloe and Calzà, 2004).

The biological action of NGF is mediated by two distinct receptors; a selective receptor tyrosine kinase called TrkA and a low-affinity receptor termed p75^{NTR} (Ebendal, 1992; Meakin and Shooter, 1992). NGF/TrkA expression exerts, in most cases, protective actions on disease involving degeneration of NGF-target cells. Indeed in these diseases this pathways is frequently altered through different mechanisms, such as impaired NGF release, down-regulation of the high-affinity receptor, TrkA and/or up-regulation p75^{NTR}. Thus, TrkA receptor agonist, which does not engage p75^{NTR} of the low-affinity NGF receptor, p75. Thus, TrkA receptor agonist, which does not engage p75 receptor, may provide alternatives in treating degenerative diseases involving NGF-target cells.

While the therapeutic potential of NGF in the treatment of neurotrophic corneal ulcer, skin ulcer, glaucoma and Alzheimer's disease (see Aloe et al., 2004) seems clearly demonstrated, its use as applied drug is to some extent limited for its not fully established pharmacokinetics and the high cost associated with the production of human recombinant molecule. These obstacles have driven the scientific community also toward the identification of small molecules NGF mimetics with drug-like properties. NGF signaling can be influenced by different ways. These small molecules can directly bind to NGF receptors causing their activation, can enhance or potentiate activity or release of endogenous NGF,



Fig. 1. - Rita Levi-Montalcini and Luigi Aloe. Photograph taken during the Rome meeting dedicated to the 100th birthday of Rita Levi-Montalcini.

can regulate signaling pathways that lead to high affinity NGF receptor activation, or down-regulate the p75^{NTR} involved in cell death.

NGF and non-neuronal cells

Studies carried out in recent years demonstrated an unexpected diversity of NGF action, indicating that the effect of NGF on cells of the central and peripheral nervous system is only one of a whole specific spectrum of the NGF biology. Such “unpredictable” cells, such as mast cells, lymphocytes, adipose tissue cells, pancreatic beta cells, and hair follicle cells, became sources of and targets for NGF (Aloe and Levi-Montalcini, 1977; Laurenzi et al., 1994; Aloe et al., 1994; Sornelli et al., 2009; Chalidakov et al., 2009a). Pioneering studies published by Enrico Alleva, R. Levi-Montalcini and Luigi Aloe revealed a pivotal role of NGF in aggressive and anxiety-like behaviors (Alleva et al., 1993).

The clinical NGF

One of earliest indication sought for clinical trial of NGF was the treatment of diabetic polyneuropathy. The first results of these studies are at the moment controversial. Subsequent studies investigating the effect of NGF administration in rodent forebrain cholinergic neurons and behavior and memory performances lead to the hypothesis that NGF may be useful to protect these neurons that are known to degenerate in brain of subjects affected by Alzheimer’s disease (AD). This experimental approach is currently being undertaken to determine whether NGF administration has a potential therapeutic role in preventing and/or reducing the neuronal cell loss and atrophy that occurs in brain of patients with AD.

In more recent years, a number of basic and clinical studies demonstrated that exogenous administration of purified NGF prevents the progressive neuronal loss, maintains neuronal connections

and function. Several therapeutic strategies to delivery NGF in animal models and in human diseases have been explored and clinical steps have been attempted, while others are currently in progress to evaluate whether NGF can prevent or protect against cell degeneration in the nervous system, visual system (glaucoma, maculopathy, corneal ulcers) and cutaneous tissue (Yuen et al., 1996; Apfel et al., 1998; Lambiase et al., 1998; Bernabei et al., 1999; Chiaretti et al., 2002; Landi et al., 2003; Lambiase et al., 2009a; Lambiase et al., 2009b). This clinical approach includes the use recombinant human neurotrophin application, direct gene transfer using (non-) viral vectors, the implantation of *ex vivo* genetically engineered cells secreting neurotrophic factors, and the grafting of neural stem progenitor cells. Cardiometabolic diseases (atherosclerosis, obesity, type 2 diabetes, and the metabolic syndrome) are also candidates for NGF/BDNF-directed pharmacotherapy (see Chalidakov in this issue of the Journal). Every second years, experts in the field of NGF and other neurotrophic factors organize an international scientific meeting in different countries, to discuss the latest findings on NGF and related factors (Aloe et al., 2004).

Not only a Pioneer in modern neuroscience

Levi-Montalcini has published more than 200 papers and has a deep knowledge of neuroscience. She has an unlimited interest in scientific and human activities and is a member of numerous national and international scientific academies. She was the first woman to be admitted to Italy's Pontifical Academy of Sciences. She is member of numerous national and international scientific academies, including the National American Academy of Sciences and the Italian Academy of Lincei, and in 2001 was nominated Senator of the Italian Parliament. She wrote several books: *The Price of Imperfection* (Levi-Montalcini, 1987), her autobiography; *Senza olio e contro vento* (Levi-Montalcini, 1996); *The Saga of the Nerve Growth Factor* (Levi-Montalcini, 1997); *L'asso nella Manica a Brandelli* (Levi-Montalcini 1998); *Il Cantico di una Vita, Song of Life*, a series of about 200 letters written to her mother during

the years she made her key discoveries (Levi-Montalcini, 2000).

The Queen of the NGF

A number of researchers believed that Rita Levi-Montalcini viewed the NGF molecule as a private property. Ralph Bradshaw, an American senior scientist who collaborated with R. Levi-Montalcini for few years, described Levi-Montalcini as a scientist extremely possessive about NGF. In one interview he remarked that when studies of NGF increased and other scientists became interested in this area, Levi-Montalcini rather than being happy, seemed to be annoyed by the invasion of the NGF field that she saw as her own territory (see Abbot, 2009). And Robert Provine, a young scientist who worked in her laboratory for two years, mentioning the high competition of working with Levi-Montalcini wrote that working for her was like to work for an exploring combination of Marie Curie and dramatic Italian opera star Maria Callas (Provine, 2001).

Rita Levi-Montalcini discovered the NGF by trying to investigate how the nervous system developed, but her study ultimately led to even more than that. Building upon this type of approach, she, her collaborators, and a host of other researchers unearthed a whole class of biological mediators that are intimately involved in every single stage of a cell's or an organism's life from conception to death.

The articles of this Special Issue are dedicated to Rita Levi-Montalcini at her blessed age of 102 years, when she still keep asking about emerging NGF studies. More importantly, all Scientists know her agree that for the discovery of the first growth factor, her scientific achievements, broad cultural knowledge and charming regal dressing even in the laboratory, she is the Queen of the NGF.

The Collaborators

During her long scientific life, numerous young and senior scientists worked in the laboratory directed by Rita Levi-Montalcini. Many of them collaborated with her with long and short periods but contributed very little to the NGF studies. Others like Enrico Alleva in Italy and Ralph Bradshaw and Ruth

Houge-Angeletti in USA left a long lasting contribution to the to the NGF studies. However, reading the “NGF Saga” and her numerous scientific publications, one can come to the conclusion that Levi Montalcini had three major collaborators, Stanley Cohen (1953-1959), Pietro Angeletti (1959-1970), and Luigi Aloe (1968-2010).

Stanley Cohen, a biochemist contributed to study the early biochemical properties of the tumor extract and was the first to purify the NGF from the snake venom and from the mouse salivary gland and to produce the anti-NGF antibodies. He shared with Levi-Montalcini of the Nobel Prize in 1986 for the discovered the epidermal growth factor.

Pietro Angeletti, an Italian pathologist with an excellent preparation in biochemistry made a substantial contribution to the study of NGF purification and to analysis of the spectrum of NGF action in the peripheral nervous system and to the mechanism of immunosympathectomy development during early postnatal life.

Luigi Aloe contributed to identification of new NGF target cells within and outside the nervous systems, particularly in the immune and endocrine systems, and recently adipose tissue. In 1977 found that injecting NGF in laboratory animals produced a marked increase in the number and size of mast cells (Aloe et al., 1977). Immunocompetent cells localized throughout the body, including the central nervous system and areas close to peripheral sensory and sympathetic nerve fibers. The discovery that NGF could affect mast cells suggests a connection between the nervous system and the immune system have been intensely investigated by numerous other scientists in recent years, in a field of neuroimmunology. In the last few years, Aloe, in collaboration with clinicians, found that NGF can be an important human therapeutic molecule for healing the human corneal ulcer, pressure and diabetic ulcers, as well as degenerating retinal cells in glaucoma and maculopathy. This latter study has proved to be useful also for Levi-Montalcini who suffers of this ocular deficit.

My early experience

I first met Levi-Montalcini in April 1967, at the Istituto Superiore of Sanità in Rome for an interview, while she was observing under the microscope

the effect of anti-NGF antibody administration on the superior cervical ganglia of mice. The interview lasted about 20 minutes and two weeks later she called me asking if I would accept to work in her laboratory, of the Department of Zoology of the Washington University in St. Louis, Missouri, USA, for 6 months to take care of raising cockroaches for her studies on the insect nervous system. I accepted her proposal, thereafter the 6 months became 12, then two years and after over 40 years.

In 2002, I organized the 7th International Meeting on “NGF and related molecules in Health and Disease” in Modena, Italy, in 2004 “Clinical Applications of NGF” Meeting in Rome, and another to celebrate the 100 years of Levi-Montalcini, on April 2009.

The European Brain Research Institute

One of the most recent initiatives of Levi-Montalcini was to propose, organize and set up a new scientific center, the European Brain Research Institute (EBRI) that in collaboration with the Institute of Neurobiology and Molecular Medicine of the Italian National Research Council and the Santa Lucia Foundation, should become a large scientific institution for basic and clinical studies in neuroscience. In recent years, most probably due to the lack of an innovative scientific approaches and/or lack of sufficient research funding, it seems to face some difficulties. Most probably the staff member and the direction underestimated the importance of pointing to the clinical potentiality of NGF, the production of human NGF recombinant, and even the production of mouse NGF for basic research. Incompetence never comes single and mistakes are always fatherless and, needless to say, no one would blame himself for the present non optimistic situation.

A recent financial and scientific collaboration by a Chinese Biotech Company for the clinical development of NGF seems to offer a more optimistic future for the EBRI that hopefully will lead the EBRI to reach the original aim first indicated by Rita Levi-Montalcini. However, the possibility that the main aim of the Chinese Biotech is not to contribute to improve the quality of the research, but to enter in the European market of growth factors for basic and clinical approaches using the EBRI and the name of the Nobel Prize winner needs to be taken into con-

sideration by the EBRI authority. Indeed, the fact that most, if not all, patents regarding the clinical application of NGF are properties of European and American companies suggests taking into serious consideration such a concern.

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I am grateful to the Chief Editor of *Archives Italiennes de Biologie* who asked me to act as guest Editor of this special issue and accepted my suggestion to dedicate the issue to the Nobel Laureate Rita Levi-Montalcini, who was for a number of years a member of the editorial Board of *Archives Italiennes de Biologie*. I wish to express my gratitude also to all Authors who contribute with their recent studies a valuable occasion for expanding the knowledge of the NGF biologic actions and for its future clinical applications. I hope the data and working hypothesis presented in this special issue will foster the cumulative science on NGF, including basic and translational research and NGF therapeutic applications.

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