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Invited Review

The Multiple Life of Nerve Growth Factor: Tribute to Rita Levi-Montalcini (1909-2012)

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ABSTRACT

At the end of the 19th century, it was envisaged by Santiago Ramon y Cajal, but not, proven, that life at the neuronal level requires trophic support. The proof was obtained in the early 1950's by work initiated by Rita Levi-Montalcini (RLM) discovering the nerve growth factor (NGF). Today, NGF and its relatives, collectively designated neurotrophins, are well recognized as mediators of multiple biological phenomena in health and disease, ranging from the neurotrophic through immunotrophic and epitheliotrophic to metabotrophic effects. Consequently, NGF and other neurotrophins are implicated in the pathogenesis of a large spectrum of neuronal and non-neuronal diseases, from Alzheimer's and other neurodegenerative diseases to atherosclerosis and other cardiometabolic diseases. Recent studies demonstrated the therapeutic potentials of NGF in these diseases, including ocular and cutaneous diseases. Furthermore, NGF TrkA receptor antagonists emerged as novel drugs for pain, prostate and breast cancer, melanoma, and urinary bladder syndromes. Altogether, NGF's multiple potential in health and disease is briefly described here.

Key Words: Nerve growth factor, TrkA, p75NTR, neuronal cells, non-neuronal cells, disease, therapy

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Introduction

Rita Levi-Montalcini (RLM) was born April 22, 1909 in Turin, Italy, where she received her medical degree from the University of Turin in 1936. The same year she entered the Institute of Anatomy as the postgraduate student of Professor Giuseppe Levi, a well-known neuroanatomist and tutor of two other future Nobel Prize winners in Physiology or Medicine, Salvadore 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 nerve cell death. The results of these studies led her to the hypothesis that the failure of neurons to thrive in the absence of a 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 published work, Levi-Montalcini and Hamburger prospected the hypothesis that the nerve-target interactions are reciprocally competitive in the sense that developing neurons depend on feedback (retrograde) signals from the peripheral tissues that are required for neuronal survival (1). These seminal observations and hypothesis paved the way to the discovery of programmed cell death (in today's context, apoptosis), and later the discovery of nerve growth factor (NGF).

The NGF: A New *Eureka* in Neuroscience

In brief, Rita Levi-Montalcini's unpredictable laboratory protocols included (*i*) the transplantation of mouse sarcoma 180 into chicken embryo leading to the growth of sympathetic and sensory nerves, (*ii*) snake venom used to destroy DNA in sarcoma homogenate leading to higher nerve growth than that induced by the sarcoma itself, and (*iii*) the homogenate of male mouse submandibular glands (the mammalian homologue of snake venom) leading to even higher nerve growth than snake venom. This heuristic cascade of nerve growth was marked by a rare combination of scientific reasoning, intuition, and chance, the latter "favors only the mind that is prepared", to quote Louis Pasteur.

The yet unknown molecule mediating such an effect on nerves was initially named nerve growth-stimulating factor, later termed NGF (1-3). In an 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 (4). 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 (5). Thus male mouse submandibular glands

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appeared to be a new and possibly the largest source of NGF, providing the possibility to isolate and purify consistent amounts of this talented molecule.

The NGF and Its Receptors

Since its discovery, NGF became one of the best-characterized members of the protein family of neurotrophic factors designated neurotrophins, including NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3/4 (NT-3/4), NT-5, NT-6, and NT-7 (6). The biological action of NGF is mediated by (*i*) the high-affinity receptor TrkA (tropomyosin-related kinase receptor A; also known as tyrosine kinase receptor), and (*ii*) the low-affinity receptor termed p75 (pan)neurotrophin receptor ($p75^{NTR}$). In most cases, TrkA expression exerts protective actions on diseases involving degeneration of NGF target cells. In these diseases, the balance of TrkA and $p75^{NTR}$ signaling pathways is altered through different mechanisms including co-receptor modulation. TrkA receptor agonists may provide alternatives in the therapy of neurodegenerative and psychiatric diseases (see below). Of note, the NGF precursor proNGF induces apoptosis through $p75^{NTR}$ (7).

NGF-ome, or the Multiple Life of NGF

At the beginning of this century, the Human Genome Project was finalized, estimating over 30000 genes encoding more than 100000 functionally distinct proteins. As usually happens, one solved problem delivered many unsolved ones. Thus, in the postgenome time, many "-ome" projects have emerged, including proteome, transcriptome, interactome, metabolome, adipokinome and connectome. In this vein, the cumulative data of NGF's multiple potential in health and disease are conceptualized here as "NGF-ome".

As often occurs, the framework of an initial conception of the physiological role of a newly discovered molecule extends in the light of emerging findings. This was also the case with NGF. During some 25 years after its discovery, for example, there have been few reasons given to indicate that NGF acts on non-neuronal cells. Thus, in 1975, Luigi Aloe and Levi-Montalcini carried out a remarkable experiment demonstrating that treatment of newborn rats with NGF caused a systemic increase in the number of mast cells (8). This seminal finding, published in *Brain Research* in 1977, has triggered the study on neuroimmune connections, leading to today's accumulation of compelling evidence that *NGF is wider than the neuron* (paraphrasing Emily Dickinson's *The brain is wider than the sky*). That is, in addition to its neurotrophic function, this talented molecule influences the survival and activity of a large number of "unpredictable", non-neuronal cells such as immune cells, fibroblasts, epithelial cells, pancreatic beta cells, adipose tissue cells, and cardiomyocytes (6,9-15). Such non-neuronal actions of NGF, and also of other neurotrophic factors subsequently discovered, open novel avenues in the study of neurobiology, namely, neuroimmunology (8) and, recently, neuroadipology (16). Pioneering studies published by Enrico Alleva in collaboration with Levi-Montalcini and Aloe revealed a pivotal role of NGF in aggressive and anxiety-like behaviors (17). Thus, we witness, appreciate, and, hopefully, contribute to an exciting time in the field of integrative physiology (systems biology) of NGF/NGF-ome.

Altogether, "the submerged areas of the NGF iceberg loom very large", Rita Levi-Montalcini (1987) stated in her Nobel Prize lecture reviewing 35 years of research on NGF (18). The involvement of NGF and other neurotrophins, particularly BDNF, in the pathogenesis of various diseases is presented in Table 1.

The Therapeutic NGF

The treatment of diabetic polyneuropathy was one of earliest indication sought for the clinical trial of NGF. 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 led to the hypothesis that NGF may be useful in protecting these neurons that are known to degenerate in the brain of subjects affected b y Alzheimer's disease. While the therapeutic potential of NGF in the treatment of neurotrophic corneal ulcers, skin ulcers, glaucoma and Alzheimer's disease (6,19-21) seems clearly demonstrated, the use of NGF as a drug is, to some extent limited due to its incompletely established pharmacokinetics and the high cost associated with the production of human recombinant NGF. These obstacles have also driven the scientific community toward the identification of small molecules (NGF mimetics) with drug-like properties. These molecules may (*i*) directly bind to NGF receptors causing their activation, (*ii*) enhance the release of endogenous NGF, and/or (*iii*) influence intracellular signaling pathways.

Several therapeutic strategies for delivery of 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 and/or TrkA receptor agonists can prevent or protect against cell degeneration in the nervous system, visual system, cutaneous and myocardial tissue (14,15,19,22-28). It most likely exerts therapeutic effects on cardiometabolic diseases such as atherosclerosis, obesity, type 2 diabetes and metabolic syndrome (29-31). In contrast, TrkA receptor antagonists emerged as novel drugs for prostate (32) and breast (33) cancer, melanoma (34), bladder syndromes (35) and various pain conditions (36). Intriguingly, NGF may contribute to romantic love (37). The experimental approach also includes 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.

Every alternate year, experts in the field of NGF and other neurotrophic factors organize an international scientific meeting in different countries. In 2002, one of us (LA) organized the 7th International Meeting on "NGF and related molecules in Health and Disease" in Modena, Italy (38); in 2004 "Clinical Applications of NGF" Meeting in Rome, and the Rome-2009 meeting to celebrate the 100 years of Levi-Montalcini's life, on 21 April 2009.

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Table 1. Potential role of NGF and BDNF in the pathogenesis of a selected list of diseases

Neurodegenerative diseases
Alzheimer's disease, Huntington's disease, Parkinson's disease
Human immunodeficiency virus-associated dementia
Diabetic neuropathy
Psychiatric diseases
Depression, Schizophrenia
Eating disorders
Anorexia nervosa
Bulimia nervosa
Ocular diseases
Glaucoma, Retinitis pigmentosa, Diabetic retinopathy
Corneal diseases
Peripheral ulcerative keratopathy
Dry eye
Cardiometabolic diseases
Atherosclerosis, Hypertension, Obesity, Type 2 diabetes mellitus, Metabolic syndrome
Heart failure, Myocardial infarction
Sudden cardiac death in diabetes mellitus
(silent myocardial ischemia in diabetes mellitus)
Skin diseases
Diabetic wounds, Pressure ulcers, Chronic vasculitic ulcers
Malignant diseases
Prostate cancer, Breast cancer, Melanoma
Urinary system diseases
Overactive bladder syndrome, Benign prostatic hyperplasia
Chronic pain-associated disorders
Osteoarthritis, Low back or spinal injuries, Cancer
Urological chronic pelvic pain syndromes

The Queen of Modern Neuroscience

Rita Levi-Montalcini has published more than 200 scientific articles which contributed in depth to the excellence of modern neuroscience. She had an unlimited interest both in scientific and human activities and was a member of numerous national and international scientific academies. In 1968, she was elected member of the National Academy of Science of the USA and in 1972 a member of the American Association of Art and Science. In 1986 she was awarded the Albet Lasker Award for Basic Medical Research, a precursor of the Nobel prize she received in 1986.

In 1974, she was the first woman elected member of the Pontifical Academy of Sciences of the Vatican. At that time Dr. Tom Woolsey, the George H. and Ethel R. Bishop Scholar in Neuroscience at the School of Medicine, said: "When Levi-Montalcini was appointed to the Pontifical Academy by Pope Paul VI, the protocol required her to kneel and kiss the Pope's hand. Rita said: "I simply stood and shook the Pope's hand." In her independence and determination, she was a model for all scientists". In 2001, RLM was nominated Senator of the Italian Parliament. She wrote several books: *The Praise of Imperfection* (39), her autobiography; dedicated to her 100th Anniversary, one of us (GNC) wrote *In Praise of Perfection* published in the Bulgarian journal *InSpiro* volume 6, 2009; *The Saga of Nerve Growth Factor*, an anthology of key scientific papers on this subject by herself and others (40); *Ninety Years in the Galaxy of the Mind*, which described her continuing research of the brain and presented a system of ethics for future generations; *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 (41-43).

Truly, RLM's centennial life created the scientific bridge between two millenia, a path followed by many generations.

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References

1. Levi-Montalcini R, Hamburger V. Selective growth-stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Expt Zool 1951;116:321-61. [CrossRef]
2. Levi-Montalcini R. Effects of mouse tumor transplantation on the nervous system. Ann NY Acad Sci 1952:55:330-43. [CrossRef]
3. Levi-Montalcini R, Booker B. Excessive growth of the sympathetic ganglia evoked by a protein isolated from the mouse salivary glands. Proc Natl Acad Sci USA 1960;46:373-84. [CrossRef]
4. Levi-Montalcini R, Cohen S. In vitro and in vivo effects of a nerve Growth-stimulating agent isolated from snake venom. Proc Natl Acad Sci USA 1956;42:695-9. [CrossRef]
5. Levi-Montalcini R, Cohen S. Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of mammals. Ann N Y Acad Sci 1960;85:324-41.
6. Aloe L, Calzà L, editors. NGF and Related Molecules in Healthy and Disease. Prog Brain Res 2004;146:1-312.
7. Fahnestock M, Yu G, Coughlin MD. ProNGF:a neurotrophic or an apoptotic molecule? Prog Brain Res 2004;146:101-10. [CrossRef]
8. Aloe L, Levi-Montalcini R. Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. Brain Res 1977;133:358-66. [CrossRef]
9. Aloe L, Bracci-Laudiero L, Alleva E, Lambiase A, Micera A, Tirassa P. Emotional stress induced by parachute jumping changes blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. Proc Natl Acad Sci USA 1994;91:10440-4. [CrossRef]
10. Aloe L, Chaldakov GN, editors. Nerve Growth Factor in Health and Disease. Biomed Rev 1999;10:1-113.
11. Marinova TT, Velikova KK, Petrov DB, Kutev NS, Stankulov IS, Chaldakov GN, et al. Structural and ultrastructural localization of NGF and NGF receptors in the thymus of subjects affected by myasthenia gravis. Autoimmunity 2004;37:587-92. [CrossRef]
12. Larrieta ME, Vital P, Mendoza-Rodríguez A, Cerbón M, Hiriart M. Nerve growth factor increases in pancreatic beta cells after streptozotocin-induced damage in rats. Exp Biol Med (Maywood) 2006;231:396-402.
13. Sornelli F, Fiore M, Chaldakov GN, Aloe L. Adipose tissue-derived nerve growth factor and brain-derived neurotrophic factor:results from experimental stress and diabetes. Gen Physiol Biophys 2009;28:179-83.
14. Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van Linthout S, et al. Nerve growth factor promotes cardiac repair following myocardial infarction. Circ Res 2010;106:1275-84. [CrossRef]
15. Lam NT, Currie PD, Lieschke GJ, Rosenthal NA, Kaye DM. Nerve growth factor stimulates cardiac regeneration via cardiomyocyte proliferation in experimental heart failure. PLoS One 2012;7:e53210. [CrossRef]
16. Chaldakov GN, Fiore M, Tonchev AB, Aloe L. Neuroadipology:a novel component of neuroendocrinology. Cell Biol Int 2010;34:1051-3. [CrossRef]
17. Alleva E, Aloe L, Bigi S. An updated role for nerve growth factor in neurobehavioural regulation of adult vertebrates. Rev Neurosci 1993;4:41-62. [CrossRef]
18. Levi-Montalcini R. The nerve growth factor:35 years later. Science 1987;237:1154-62. [CrossRef]
19. Aloe L, Rocco ML, Bianchi P, Manni L. Nerve growth factor:from the early discoveries to the potential clinical use. J Transl Med 2012;10:239. [CrossRef]
20. Aloe L, Skaper SD, Leon A, Levi-Montalcini R. Nerve growth factor and autoimmune diseases. Autoimmunity 1994;19:141-50. [CrossRef]
21. Aloe L, Tirassa P, Lambiase A. The topical application of nerve growth factor as a pharmacological tool for human cornea and skin ulcers. Pharmacol Res 2008;57:253-8. [CrossRef]
22. Apfel SC, Kessler JA, Adornato BT, Litchy WJ, Sanders C, Rask CA. Recombinant human nerve growth factor in the treatment of diabetic polyneuropathy. NGF Study Group. Neurology 1998;51:695-702. [CrossRef]
23. Lambiase A, Rama P, Bonini S, Caprioglio G, Aloe L. Topical treatment with nerve growth factor for corneal neurotrophic ulcers. New Engl J Med 1998;338:1174-80. [CrossRef]
24. Bernabei R, Landi F, Bonini S, Onder G, Lambiase A, Pola R, et al. Effect of topical application of nerve growth factor on pressure ulcers. Lancet 1999;354:307. [CrossRef]
25. Landi F, Aloe L, Russo A, Cesari M, Onder G, Bonini S, et al. Topical treatment of pressure ulcer with nerve growth factor:a randomized clinical trial. Ann Intern Med 2003;139:635-7.
26. Chiaretti A, Piastra M, Caresta E, Nanni L, Aloe L. Improving ischaemic skin revascularisation by nerve growth factor in a child with crush syndrome. Arch Dis Child 2002;87:446-8. [CrossRef]
27. Lambiase A, Aloe L, Centofanti M, Parisi V, Mantelli F, Colafrancesco V, et al. Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops:Implications for glaucoma. Proc Natl Acad Sci USA 2009;106:13469-74. [CrossRef]
28. Lambiase A, Coassin M, Tirassa P, Mantelli F, Aloe L. Nerve growth factor eye drops improve visual acuity and electrofunctional activity in age-related macular degeneration:a case report. Ann Inst Super Sanita 2009;45:439-42. [CrossRef]
29. Chaldakov GN, Fiore M, Stankulov IS, Manni L, Hristova MG, Antonelli A, et al. Neurotrophin presence in human coronary atherosclerosis and metabolic syndrome:a role for NGF and BDNF in cardiovascular disease? Prog Brain Res 2004;146:279-89. [CrossRef]
30. Chaldakov GN, Tonchev AB, Aloe L. NGF and BDNF:from nerves to adipose tissue, from neurokines to metabokines. Relevance to neuropsychiatric and cardiometabolic diseases. Riv Psichiatr 2009;44:79-87.
31. Chaldakov G. The metabotrophic NGF and BDNF:an emerging concept. Arch Ital Biol 2011;149:257-63.
32. Weeraratna AT, Arnold JT, George DJ, DeMarzo A, Isaacs JT. Rational basis for Trk inhibition therapy for prostate cancer. Prostate 2000;45:140-8. [CrossRef]
33. Romon R, Adriaenssens E, Lagadec C, Germain E, Hondermarck H, Le Bourhis X. Nerve growth factor promotes breast cancer angiogenesis by activating multiple pathways. Mol Cancer 2010;9:157. [CrossRef]
34. Truzzi F, Marconi A, Lotti R, Dallaglio K, French LE, Hempstead BL, et al. Neurotrophins and their receptors stimulate melanoma cell proliferation and migration. J Invest Dermatol 2008;128:2031-40. [CrossRef]
35. Ochodnický P, Cruz CD, Yoshimura N, Michel MC. Nerve growth factor in bladder dysfunction:contributing factor, biomarker, and therapeutic target. Neurourol Urodyn 2011;30:1227-41.
36. Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology 2011;115:189-204. [CrossRef]
37. Emanuele E. NGF and romantic love. Arch Ital Biol 2011;149:265-8.
38. Chaldakov GN. NGF-2002:more powerful than NGF-1951. Biomed Rev 2002;13:67-9.
39. Levi-Montalcini R. In Praise of Imperfection. Basic book, New York, 1988.
40. Levi-Montalcini R. The Saga of the Nerve Growth Factor. World Scientific Series in 20th Century Biology. Volume 3. World Scient Publish, Singapore 1997.
41. Levi-Montalcini R. Senza Olio e Contro Vento. Baldini e Castoldi Editor. 1996.
42. Levi-Montalcini R. L'asso nella Manica. Brandelli Maldini and Castoldi Editors. 1998.
43. Levi-Montalcini R. Il Cantico di Una Vita. Raffaello Cortina Editore. 2000.