

NEUROTROPHINS – MORE THAN NEUROTROPHIC

Shamini Ayyadhury¹ and Klaus Heese^{*,2}

¹University Department of Neuropathology, Division of Neuroscience and Mental Health, Imperial College London and Hammersmith Hospitals Trust, Charing Cross Campus, Fulham Palace Road, London W6 8RF, UK

²Department of Molecular and Cell Biology, School of Biological Sciences, College of Science, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore

*Address correspondence to this author at the Department of Molecular and Cell Biology, School of Biological Sciences, College of Science, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore; Tel: +65-6316-2848; Fax: +65-6791-3856; E-mail: kheese@ntu.edu.sg

Abstract: The Nerve Growth Factor (NGF) is the prototypic member of the neurotrophin (NT) family, which plays an essential role in the development and functioning of the vertebrate nervous system. Although originally defined by their actions on neuronal survival and differentiation in the peripheral (PNS) and central nervous systems (CNS), accumulating data indicate the presence of extensive interactions between the NTs and the immune system. NTs are released normally during lymphocyte and leukocyte development by the bone marrow and the thymus and later by secondary lymph organs to maintain responsiveness of these circulating naïve and memory immune cells. Functional NT receptors have been detected on the cells of the immune system and increased levels of NGF protein are found during the acute phase of various diseases with a significant inflammatory component. Furthermore, in certain conditions such as allergic asthma, the released NTs exacerbate the severity of the inflammation and prolong the diseased state. However, in the CNS, if one can control homeostasis of the internal environment, then the natural response of the infiltrating immune cells to release these NTs can be used to intervene at key points in the disease progression. These wider functions are likely to be of concern in any attempted therapeutic use of NGF or related NTs.

Keywords: Allergy, B-cell, inflammation, nephropathy, nerve growth factor.

INTRODUCTION

The nervous system is an important component of an organism, which allows us to receive and integrate cognitive and physiological information from the surroundings and to transform that processed information into behaviorally relevant responses. Internal organs such as the heart, lungs, endocrine organs, spleen and the thymus are richly innervated by the nervous system, which respond to nervous stimulation by releasing growth peptides and regulatory hormones. The nervous system, which is made up of the central nervous system (CNS, brain and spinal chord) and the peripheral nervous system (PNS), therefore needs to maintain its integrity. This integrity is maintained most strictly in the CNS by the blood brain barrier (BBB) and low expression of MHC type protein thus excluding inflammatory responses [1, 2].

Inflammation does occur when the BBB breaks down or when a remote lesion in the brain allows circulating lymphocytes to infiltrate into the brain parenchyma. Neurodegenerative diseases such as Multiple Sclerosis (MS) are inflammatory diseases whilst Alzheimer's disease (AD) and Parkinson's disease (PD) are often accompanied, as a secondary response, by inflammation. However, immune cells might not often be detrimental as they have been observed to release growth factors that can help to alleviate neurodegeneration and have been considered in immune-modulatory therapies [3, 4].

In addition, normal physiological immune responses are governed by mediators released by neurons and contribute significantly to the immune-response development. One class of peptides which falls into this category of immunomodulators is the neurotrophins (NTs), which include Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3) and Neurotrophin-4/5 (NT-4/5) [5].

The immune system, a network of interconnecting and inter-dependent cells and molecules, is the aintainer of homeostatic balance in higher vertebrates. The sheer elegance of the system expounds the intricacies that nature has perfected to fine-tune this timeless masterpiece, capable of almost indefinitely differentiating between even closely resembling antigens differing in slight conformational or sequential aspects. Without this robust network, life as we know it, would be hugely fragmented and mosaic piece of aberrant disfigurement, ruled by chaos instead of the pliant, auto-regulative and balanced governance that one can see.

The only other system that matches the immune network in its complexities is the nervous system, which in itself is partitioned into sub-groups, with each segment displaying its own unique characteristics.

Anatomical, physiological and functional differences on the surface front impose disparate notions on the roles that the nervous and immune systems play. The immune system, the body's sentinel, is primarily concerned with maintaining homeostasis by eliminating foreign antigens and aberrant cell growth within the host, whilst the nervous system is well adapted to sense environmental changes and in evoking suitable behavioral responses, either kinetic or physiological in the living entity. In particular, the brain has the added responsibility of mediating cognitive responses such as intelligence, memory, emotions, learning and in visceral and tissue homeostasis, such as the control of all (in-) voluntary actions.

However, the two systems are inter-dependant on each other for survival and receive controlled cues from each other to prime immune reactions and modulate neuron-induced hormonal balance. Cells of the immune system potentially infiltrate across brain barriers, taking up residence within the parenchyma, a notion that has only recently been appreciated for its deep-rooted significance in neuro-immune-modulation [6].

THE NERVOUS SYSTEM – AN INTRODUCTION

Basic Anatomy of the Central Nervous System

The neural network is functionally divided into the sensory systems, like vision or hearing, that acquire information from the environment and the motor systems, that allow an organism to respond to this peripheral information by generating movements. Other local circuitries exist, collectively referred to as associational systems, which carry out the more complex and less well-characterized brain functions. The main centre for regulation of nervous inputs and outputs is the brain, which is subserved by the spinal cord. Peripherally located nerve endings stream their collective sensory inputs through the spinal cord, which serves as a conduit for transmission into selective regions in the brain. These sensory informations, after being processed within the supraspinal and at the cortical areas, subsequently foray down along appropriate efferent nerve bundles back into the periphery [7,8].

From an anatomical point of view, the vertebrate nervous system is divided into the central and peripheral components (Fig. 1). The CNS comprises of the brain and the spinal cord, while the PNS includes sensory neurons that link sensory receptors with processing circuits in the CNS. The motor portion of the PNS consists of two components, the somatic motor division and the autonomic motor division. Thousands of nerve impulses pass through the dozens of nerve tracks at any one second and this ceaseless stream of information processing requires an adequate supply of blood, a well organized platform for cell bodies and nerve endings to group and work in consolidation and finally, a stringent surveillance system to seal off any possible invasion by unwanted pathogens or any other ‘contaminants’ that prove detrimental to the integrity of the CNS. Each of the above components will be briefly described in the below sections.

The Blood Supply of the Brain and Spinal Cord

The entire blood supply of the brain and spinal cord depends on two sets of branches from the dorsal aorta. The vertebral arteries arise from the subclavian arteries and the internal carotid arteries are branches of the common carotid arteries. The internal carotid arteries branch to form two major cerebral arteries, the anterior and middle cerebral arteries forming the anterior circulation that supplies the forebrain. Each gives rise to branches that supply the cortex and branches that penetrate the basal surface of the brain, supplying the deeper structures such as the basal ganglia, thalamus and internal capsule. The basilar artery joins the blood supply from the internal carotids in an arterial ring at the base of the brain called the circle of Willis. The posterior cerebral arteries arise at this confluence, as do two small bridging arteries, the anterior and posterior communicating arteries. The posterior circulation of the brain supplies the posterior cortex, the midbrain and the brainstem. Conjoining the two major sources of cerebral vascular supply via the circle of Willis presumably improves the chances of any region of the brain continuing to receive blood if any one of the major arteries becomes occluded. Among the most important dorsal-lateral arteries are the posterior inferior cerebellar artery (PICA) and the anterior inferior cerebellar artery (AICA), which supply distinct regions of the medulla

and pons. These arteries are especially common sites of occlusion and result in specific functional deficits of cranial nerve, somatic sensory and motor function (Fig. 2).

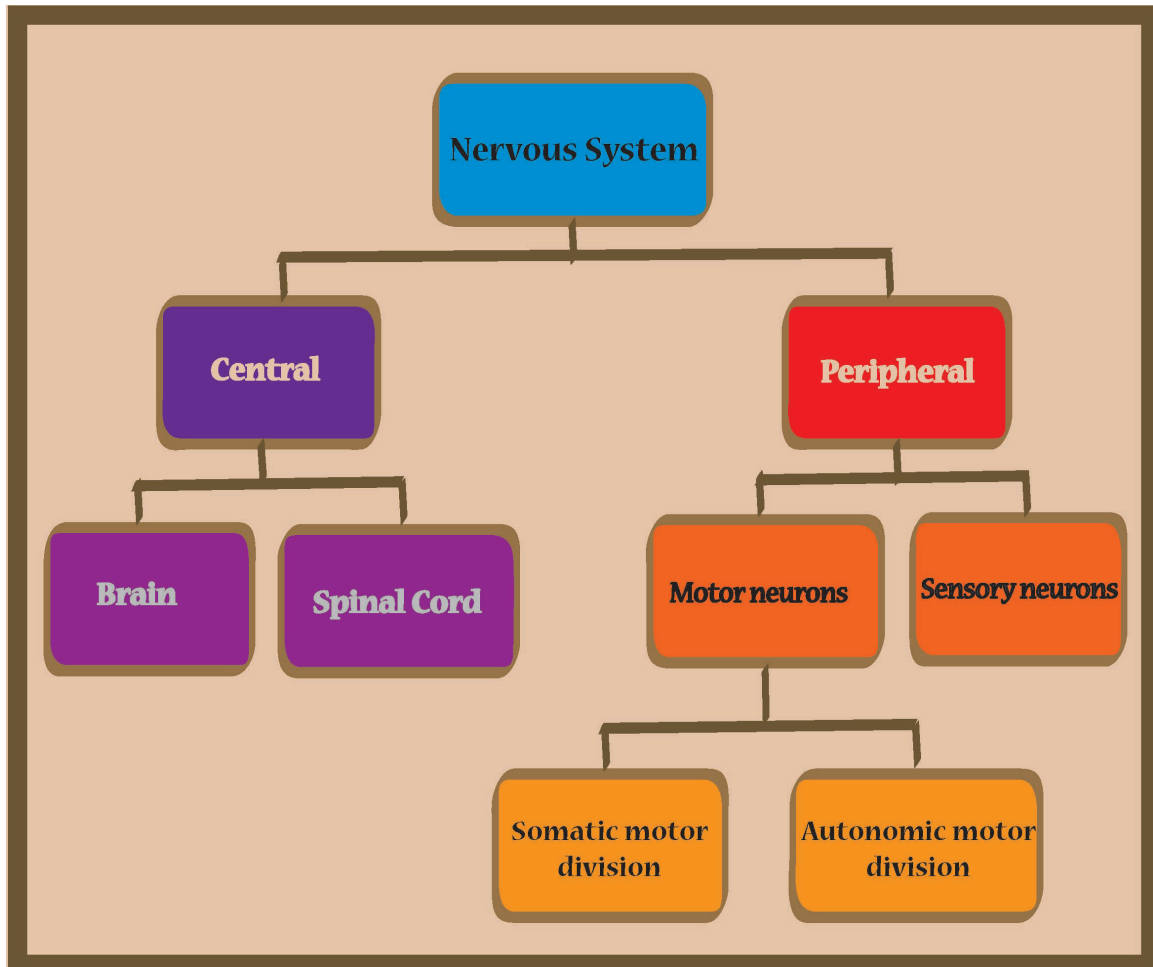


Fig. (1). Schematic subdivision of the nervous system [7, 8]. The nervous system can be divided into the central and peripheral nervous systems, each system further categorized into smaller and more focused functional subunits.

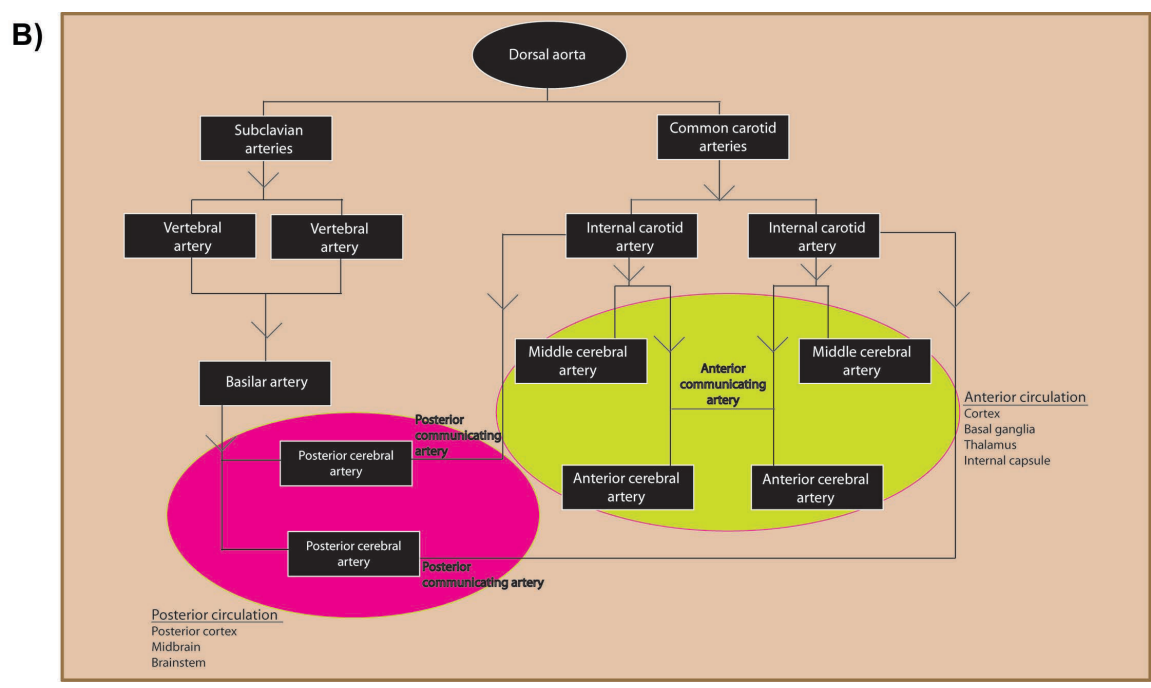
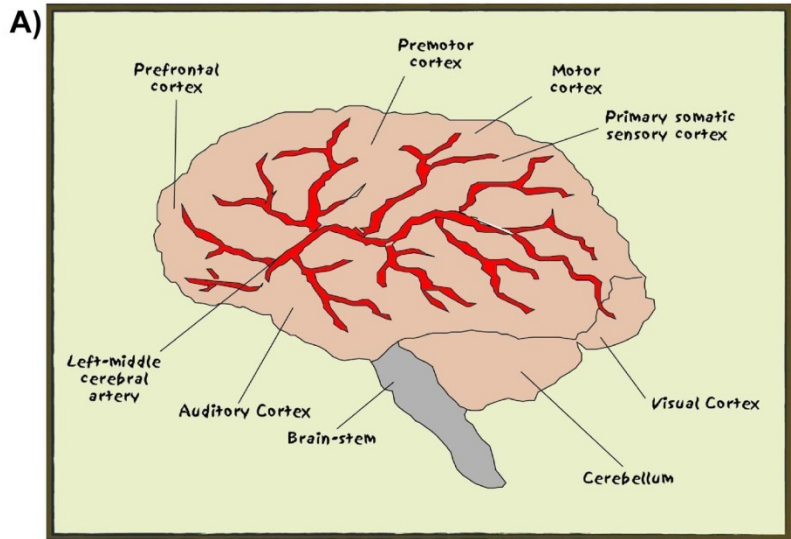


Fig. (2). **A)** Lateral aspect of the brain illustrating major functional regions of the brain and schematically the arterial blood supply through which immune cells – and with them neurotrophins released by them – may enter the brain. **B)** The vertebral arteries arise from the subclavian arteries and the internal carotid arteries are branches of the common carotid arteries. The internal carotid arteries branch to form two major cerebral arteries, the anterior and middle cerebral arteries forming the anterior circulation that supplies the forebrain. Each gives rise to branches that supply the cortex and penetrate deep into the basal surface of the brain supplying the deeper structures such as the basal ganglia, thalamus and internal capsule. The basilar artery joins the blood supply from the internal carotids in an arterial ring at the base of the brain called the circle of Willis. The posterior cerebral arteries arise from this confluence together with the anterior and posterior communicating arteries. The posterior circulation of the brain supplies the posterior cortex, the midbrain and the brainstem. Conjoining the two major sources of cerebral vascular supply *via* the circle of Willis presumably improves the chances of any region of the brain continuing to receive blood if one of the major arteries becomes occluded.

In summary, adequate blood supply is provided to the brain *via* the internal carotid and vertebral arteries. The circle of Willis, a centrally located blood supply network ensures a well distributed blood flow across the different regions [9].

As a result of the high metabolic rate of neurons, brain tissue deprived of oxygen and glucose as a consequence of compromised blood supply is likely to sustain transient or permanent damage. Thus, ischemia causes neurodegeneration and cell death [9, 10]. Circulating NTs in the blood system may thus contribute to immediate neuroprotective functions during neurodegenerative processes induced, for instance, by ischemia.

The Meninges

The CNS is encapsulated by three layers of membranes, a thin closely apposed translucent layer, the pia mater, followed by a collagenous loosely ensheathing layer, termed the arachnoid mater (below which is the subarachnoid space containing blood vessels and cerebrospinal fluid (CSF)), and a final thick fibrous layer, the dura mater (Fig. 3). These layers present themselves as the first lines of defense, mechanically (the dura mater and the overlaying skull) and physiologically, against possible injuries, infections and harmful immunological inflammation. The three layers or meninges, as they are more commonly known, continue over the spinal cord as well. One other predominant feature of the CNS, which encompasses the brain and the spinal cord, is the inbuilt biochemical barrier that it imposes on itself to preclude any active inflammatory response – in other words, a near total avoidance of an immunological response within the CNS [11, 12].

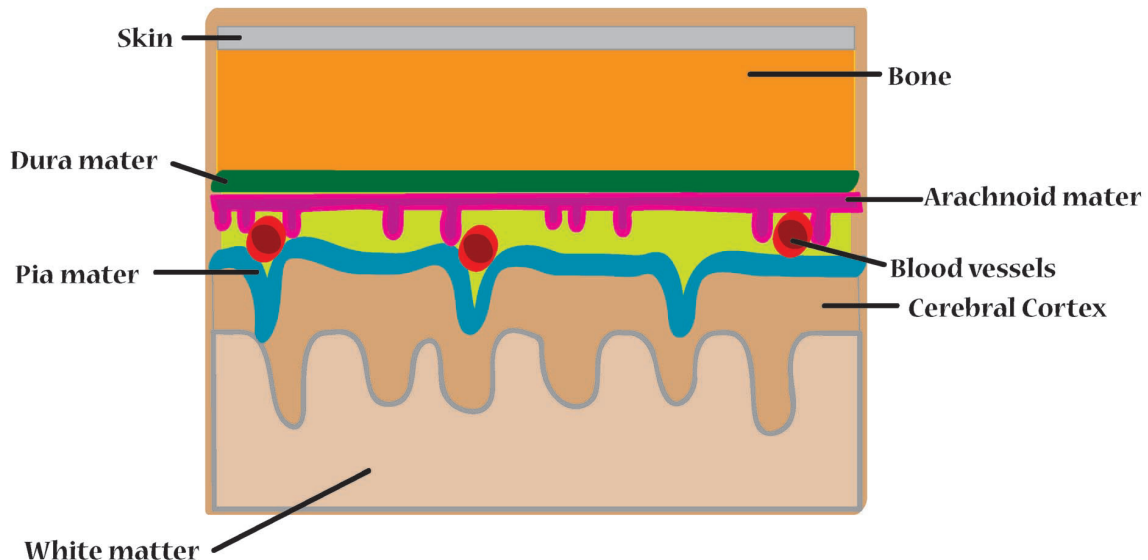


Fig. (3). The meninges which provide the delicate protective coverings apposed close to the cortical mass (the pia mater and the arachnoid mater), and the tougher dura mater which provides a stronger force resistance, assisted by the bone.

The Cerebrospinal Fluid and the Blood-Brain Barrier

The CSF, produced by the choroid plexus, percolates through the ventricular system and flows into the subarachnoid space through perforations in the thin covering of the fourth ventricle; it is eventually absorbed by specialized structures called arachnoid villi or granulations and is returned to the venous circulation. Recent research has focused attention on biochemical diagnostic markers (biomarkers) present in the CSF to increase the specificity and the accuracy of the diagnosis of various neurodegenerative diseases. NGF has been considered as a potential biomarker (in the blood and CSF system) for neuroinflammatory diseases as discussed further onwards [13, 14].

This immune-privileged status, provided by a series of morphological and physiochemical properties, has so far proven to protect the brain from the vast onslaught of the immunological system. This, in retrospect, appears to be a logically constructed fine-tuning, as the CNS is such a delicate area that it is crucial not only to prevent pathogenic infiltration but also to reduce any inflammatory response that would most definitely destroy terminally differentiated nerve cells without any regenerative capability [15, 16]. Furthermore, the brain is at risk from circulating toxins and thus requires specific protection by cellular barrier systems. These protective mechanisms start at the structural level forming robust mechanical walls of barriers throughout the brain network (the BBB or the blood-CSF barrier, etc.) [17,18].

The blood-CSF barrier is the designated region that offers a stringent control of the number and type of particulate matter that can cross into the CSF through the lateral, third and fourth ventricles. One stark difference to note here between the BBB and blood-CSF barrier is that structurally in the blood-CSF, barrier function is attributed to the choroid plexus epithelial (CPE) cells and not to the fenestrated endothelial cells of the choroid blood vessels [19]. The CPE cells form a tight band of cells, clamped tightly by similar junctional complexes as observed even in the BBB surrounding the brain vasculature and brain tissue. Functionally also involved in evolution of the CSF, it provides impedance against substances gaining entry into brain tissue. However, as mentioned above, the barriers are characteristically biochemical in nature rather than structural. Therefore, this barrier is flexible and does indeed allow a restricted movement of immune cells across as necessary.

The BBB is functionally and structurally volatile, resulting in loosening of the tight junctions between the cells and allowing an influx of potential immune mediators [17, 20]. This response deviates away from the conventionally thought of means of lymphocyte entry, which is through a BBB breakdown, an almost irreversible damage to the barriers. The situation could potentially aggravate inflammation-dependent conditions such as MS and as observed in inflammatory model systems such as Experimental Autoimmune Encephalitis (EAE) and others [21, 22]. Excessive inflammation induces a cascading downfall for cells, in general, coming into contact with the vast raid of cytokines, inflammatory immune cells or amino acid excito-toxicity due to damaged cells and release of free radicals [23]. Hence, it must have seemed prudent to establish a barrier excluding immune cells and their mediators from nerve cells.

Controlled Inflammation within the CNS and Neurotrophins

Nevertheless, accumulated data from various research works over the years have shown that exogenously cultured lymphocytes and phagocytes, when activated, are capable of neuro-regenerative functions when delivered near damaged peripheral nerve fibres and brain regions, which leads one to hypothesize on a situation-dependant exclusion principle of immune mediators by the CNS [24]. Depending on the level of inflammatory mediators released within the vicinity of the brain parenchyma and the trophic factors released by the interacting cells, an inflammatory response can turn into a beneficial self-recovery response [25]. For instance, autoimmunity, once solely viewed as destructive to homeostatic balance within the body, could have developed in the brain to kick in a self-recovery mode within damaged nerve tissue. Likewise, though several neurodegenerative diseases such as AD, PD, MS, Amyotrophic Lateral Sclerosis (ALS) or peripheral neuropathy all exhibit some form of immune balance gone wrong, however, it should not be taken as a proof that immunity in its entirety is detrimental to the nervous system [26-28]. It is only detrimental when presented in an aberrant manner, quantitatively or qualitatively.

The nervous system maintains a form of interdependence with the immune system, forming reciprocal quality check. For instance, very often immune cells receive information from nerve endings, which innervate them to stimulate neuropeptide release and nerve endings from pain receptors engage macrophages and mast cells from mucosal epithelia to stimulate the release of prostaglandins and histamine or, local inflammatory conditions force immune cells to release cytokines and other anti-inflammatory components which then stimulate pain receptors within the region. Neurotrophic factors, in particular NGF, play a pivotal role in mediating and modulating pain sensation [29].

Current research now provides a more conclusive insight into the vast interplay between the different neurospecific molecular counterparts and the immune mediators. Many of these molecules are upregulated or downregulated during cellular stress and their relative balance forming a key determinant in initiating pathology.

This review though will specifically focus on one such potential class of candidates which are the NTs – a class of factors, originally discovered as growth stimulants and differentiation factors of the developing and adult CNS and PNS, whose myriad roles in hematopoiesis and the development of the immune system have begun to be appreciated recently. The study of NTs is not only prudent for understanding the mechanisms of action during basal states but also to understand their roles in potentially reversing stressed conditions in cells.

Delivery of NTs and the expression of their respective receptors have exhibited signs of inducing recovery within axotomized nerve fibres and in dying cholinergic and dopaminergic neuronal cells in AD and PD [30]. AD, for instance, is the most common cause of dementia among people aged 65 and older and the treatment of AD remains a major challenge because of the incomplete understanding of the triggering events that lead to the selective neurodegeneration characteristic of AD brains [31, 32]. In AD, the

primary regions affected are the hippocampus, cerebral cortex and amygdala. Neuronal loss includes particularly the cholinergic neurons of the basal forebrain system. NGF promotes survival of those neurons by activating its specific receptor TrkA. Downstream of TrkA, the small G-protein p21ras plays a pivotal role in controlling neuronal survival and differentiation [33-35] and it has been shown that TrkA is down-regulated in AD brains leading to the suggestion that an imbalance of the NT receptor signaling may be involved in AD [36-38]. In addition to an alteration of NTs in AD brains, the CSF shows elevated levels of NGF, turning NGF into a potential inflammatory biomarker for the early diagnosis of AD [39, 40].

Before trying to understand the implications of the crosstalk between the nervous and immune systems, which may play important roles during neurodegenerative processes such as AD, it is important to understand the relative importance of NTs with respect to the nervous system and the associated pathways through which they exert their effects. This knowledge is a prerequisite to delve deeper into the roles that these peptides have on systems outside the nervous system, most importantly, the extent of control they elicit on hematopoiesis, the process of development and maturation of immune cells and in maintaining and activating their functional capacity.

NEUROTROPHINS AND TRK RECEPTORS: STRUCTURE AND FUNCTION IN THE NERVOUS SYSTEM

NTs are polypeptides identified as crucial factors involved in the development of the nervous system, as can be observed in their relative importance as guidance and target-derived factors enabling neurogenesis to culminate into appropriately differentiated cellular phenotypes and to reach target innervation [34-36, 41]. These growth factors have been under the spotlight due to their tremendous beneficiary potential in the CNS and PNS, especially during development and in brain injury. They mainly involve themselves in mediating the proliferation, differentiation and survival of primarily, but not exclusively, sympathetic and sensory neurons during early and late embryogenesis with their roles subverting to maintenance of specific neurons during later adulthood [41-43]. In the PNS, NTs also contribute towards axotomy-induced nerve regeneration as observed in neuromuscular organs and peripheral organs, whilst in the mammalian CNS, NTs participate in nudging appropriate cholinergic and adrenergic neuron population development and moulding synaptic plasticity [44-46].

This multifarious class of protein molecules includes NGF, BDNF, NT-3 and NT-4/5, albeit other less well characterized peptide growth factors and newly discovered neurotrophic factors have been documented in lower vertebrates [48, 49]. The four main NTs described above share similar biochemical properties with considerable peptide sequence homology [34, 35]. Although evolutionarily related, their influences within the microenvironment of the brain vary, with sometimes little overlap functionally and physiologically [34, 35, 41]. This discriminatory potency of the peptides is fine-tuned *via* their interactions with their specific receptor counterparts.

NGF promotes the survival and proliferation of sympathetic and sensory neurons during late embryogenesis and, to a lesser extent, it stimulates the proliferation and differentiation of parasympathetic neuronal tissue [42, 44]. NGF functions not only as target-derived trophic factor but guides the survival and initiates stimulation of nociceptive sensory neurons [29, 41, 47, 50]. It also, in conjunction with neuregulin, participates in mediating plasticity and proper innervation of the motor neuron junctions, *via* its low affinity p75 NT receptor (p75NTR). This is an interesting observation as thus far, motor neuron development and innervation have been implicated as a consequence of BDNF action [51-53].

On a similar note, BDNF and NT-3 stimulate neurite outgrowth of embryonic sensory neurons and mediate survival of retinal ganglion cells, though any effect of BDNF on sympathetic neurons is yet to be clearly established [54, 55]. Rather interestingly, NT-4/5 fails to elicit a positive influence on sensory neurons, even though both BDNF and NT-4/5 act through the same receptor [41]. Post-developmental functional participation of BDNF, NT-3 and NT-4/5 includes modulating synaptic plasticity as mentioned for NGF and to positively or negatively modulate the response of growth cones to guidance cues such as semaphorin 3A [56, 57]. Furthermore, these NTs may have important roles to play in initiating neurite outgrowth in the CNS and PNS after nerve transection [58-61].

The profound width across which NTs exert their effect might seem overwhelming; especially, since these NTs share a high sequence homology with characteristic conservation of hydrophobic residues across the NTs. This conservation of hydrophobic residues across the NTs mentioned above undoubtedly has led to their similar three-dimensional topology, capable of forming both homo- and even hetero-dimers with each other [34, 35, 41]. NGF, BDNF and NT-3 show similar secondary structures consisting of β -sheet moieties and disulfide linkages holding their irregular threedimensional domains together [62-64].

Subsequently, the individuality in their actions derives from their interactions with their specific tyrosine kinase receptors, the tropomyosin-related kinase (Trk) receptors TrkA, TrkB and TrkC and the subsequent activated signaling pathways [33, 47-49]. NGF binds specifically to TrkA, BDNF and NT-4/5 to TrkB and NT-3 to TrkC. Furthermore, adding up to an already complex picture, all four NTs bind, though with lower affinity, to a receptor belonging to the tumor necrosis factor superfamily, the p75NTR [33, 49]. It is now known that pro-neurotrophins bind with high affinity preferentially to p75NTR, a cassanova receptor with a proclivity towards all four NTs, initiating a death pathway [65-68]. Likewise, as mentioned earlier, it was highlighted that BDNF and NT-4/5 do not entirely overlap in terms of function. These observations indicate the presence of stringent and carefully evolved signal transduction mechanisms [56].

Trk receptors share a common structural organization of their extracellular domains, which allows easy distinguishment from other tyrosine kinases [33]. The varied Ntreceptor partnership presented, as described above, ends up in the anterograde and retrograde transport of NT-receptor complexes and initiation of a vast array of signaling messages initiated downstream upon ligand-induced receptor activation, inducing gene

transcription and ionic channel coupling [33, 47-49, 56, 69-74]. The downstream signaling activated by the interactions between NTs and Trk receptors leads to a varied phenotypic expression, which includes induction of long term potentiation, increased synaptic activity, cholinergic production, anti-apoptotic pathways and sometimes even apoptosis itself (Fig. 4) [33, 49, 75-78].

It is almost miraculous considering the varied and specific outcome that Trk receptors and their cognate ligands bring about. However, in light of their capacity to initiate a myriad of signaling pathways, their functional potential might appear under-estimated. The secret lies in the unique binding sites within the intracellular domains of Trk receptors. In addition, different NTs may bind to the same Trk receptor but mediating specific and different effects [46, 47,49].

NTs bind to the extracellular juxtamembrane binding portions of Trk receptors, inducing a conformational change leading to receptor dimerization. The cytoplasmic tails of these dimeric Trk receptors cross-phosphorylate at key tyrosine residues, with phosphorylation being crucial to prime Trk receptors for downstream events [46, 47]. These phosphorylated tyrosine residues form docking platforms for adaptor and signaling molecule recruitment, forming composite signaling complexes. Signaling cascades are initiated leading into physiologically significant or, in some instances, pathological neuronal behavior [79-81].

Trk receptors in general possess an autoregulatory loop within their cytoplasmic domain [33, 46, 47]. Consisting of three important tyrosine residues, phosphorylation of these residues gives rise to enhanced activation of the receptor dimer. The remaining seven phosphorylated tyrosine residues then partake in associating with adaptor and signaling structures. Adapter proteins such as Shc, Grb-2, Crk, or enzymes such as SH2 domain containing tyrosine phosphatase SH-PTP-2 (SH-PTP-2), phosphatidyl-3 kinase (PI3K) and phospholipase (PLC)- γ , dock onto phosphorylated tyrosine residues, activating the Ras/MEK/MAP-Kinase, PI3K/Akt/NF- κ B, IP3/DAG/Protein-kinase-C (PKC) and Cdc/Rac/Rho signaling pathways [33, 46, 47, 82]. The consequence of this activation ends up in accumulation of phosphorylated down stream molecules [83]. The Ras/MEK/MAPK pathway gives rise to proliferation and survival conditions [33, 82]. Activation of the NF- κ B (nuclear factor-kappaB) molecular route also potentiates anti-apoptotic activities [84]. Whilst activation of the small G-proteins belonging to the Cdc/Rac/Rho family and other accessory G-protein coupled receptors (GPCRs) culminate in activation of ion channel gene expression and subsequent modulation of ionic currents through neurons and neurite outgrowth [85-87].

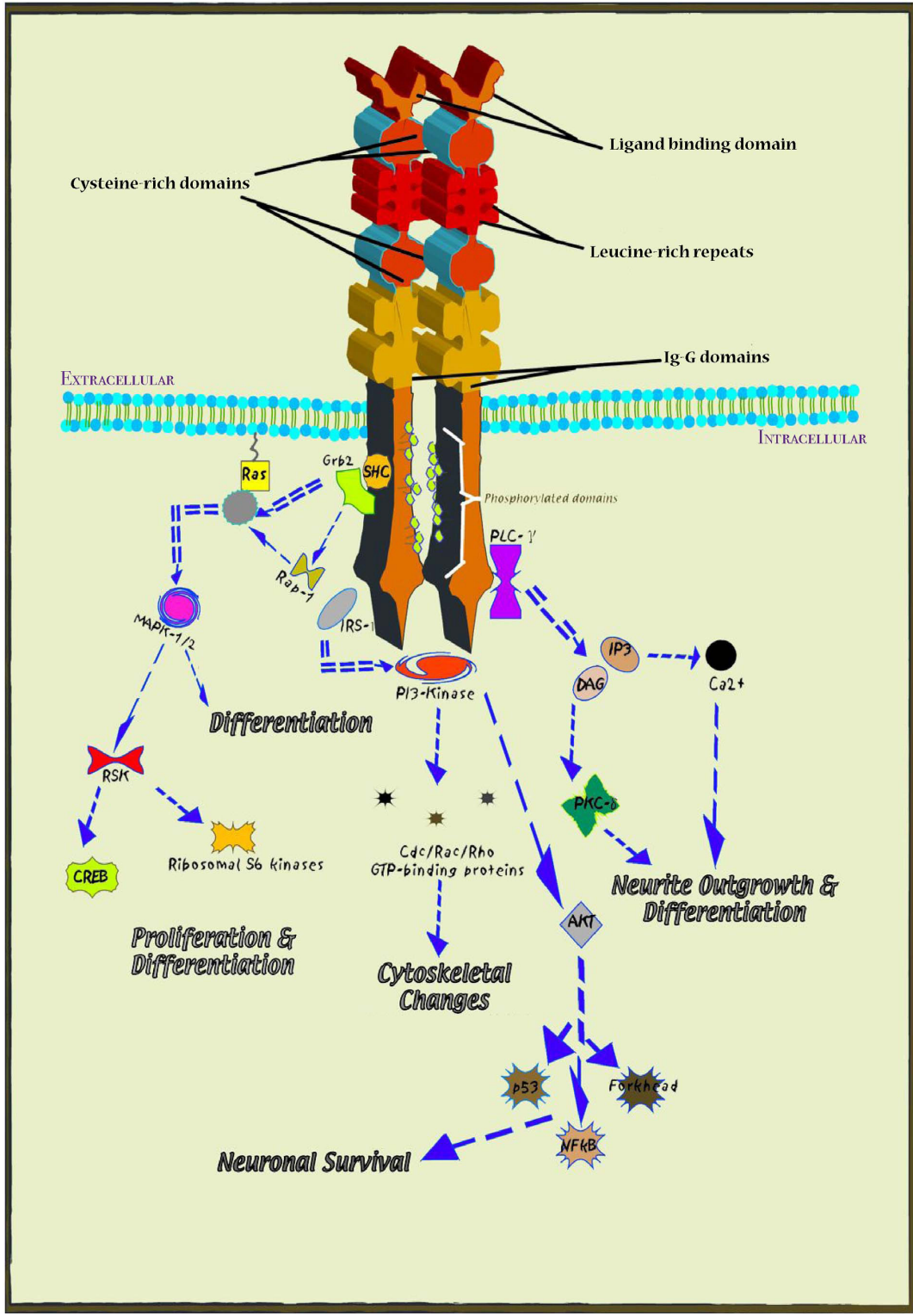


Fig. (4). Intracellular signaling cascades associated with activation of Trk receptors. AKT, protein kinase B; CREB, cyclic-AMP response element binding protein; DAG, diacylglycerol; Ig-G, immunoglobulin-G; IP3, Inositol 1,4,5 triphosphate; IRS-1, insulin-receptor substrate-1; MAPK-1/2, mitogen-activated protein kinase-1/2; NF-κB, nuclear factor kappa B; PI3 kinase, phosphatidylinositol-3 kinase; PLC-γ, phospholipase Cγ; PKC-δ; protein kinase Cδ.

However, what presents itself as the ultimate challenge is for scientists to understand and decipher the unique crossnetworking between the individual signaling pathways because the various interactions often lead to a completely unpredictable pathway and phenotypic expression. For instance, in motor nerves, often the activation of p75NTR or TrkB receptors by BDNF elicits a pro-survival mechanism. However, neuregulin, a molecular trophic factor released by developing target neuromuscular junctions, interacts with Trk activation by inducing an apoptotic pathway, via activation of the c-Jun death pathway. How the activation of the ErbB and Trk/p75NTR pathways interacts is not completely understood [46-49, 51-53, 88, 89].

The p75NTR receptor still elicits much confusion as its precise role in NT signaling is yet to be soundly deciphered, though its predominant influences appear to be involved in initiating the death program [65-67]. Its unique and promiscuous binding partners, which include pro-NGF, neuregulin and noggin, only serve as a reminder to how the varied NTreceptorpartnership leads to diversity in regulation [90, 91].

Thus, the presence of p75NTR on different subsets of neurons and in varying ratios in the presence or absence of alternate splice variants of Trk receptors, depending on the molecular landscape of the characteristic nerve cells contribute to defining how the neural tissue reacts to NTs in the vicinity [49].

Insofar, readers would have gleaned how NTs and their Trk receptors work downstream, leading to the plethora of developmental and post-developmental manifestations. However, the roles of the NTs are not restricted to cells of the nervous system. Recent years have lent credence to research seminal in establishing the neuro-immunomodulatory roles of the NTs and their receptors. These Trk receptors found on immune cells and their accessory organs and tissues are biologically functional, capable of downstream signal streaming.

However, before understanding this common link provided by the NTs, it is rudimentary to appreciate the basic immune network of the vertebrate system and that these key immuno organs involved are also acted upon by the NT system.

THE IMMUNE SYSTEM

A Basic Overview of the Immune System

The immune system is the maintainer of homeostatic balance and is a fundamentally important and welldeveloped contrivance, especially in higher vertebrates. Its main objective lies in assimilating information gathered at the periphery of the body and to initiate a systematically controlled cascading sequence to prevent unwanted pathogens or foreign cell particles from overwhelming the body's systems. Nevertheless, more than often, this fine-tuned surveillance and elimination system fails or ignites into a self-destructive immune response [92].

All the different cellular elements of the immune system, including the red blood cells that transport oxygen, the platelets that trigger blood clotting in damaged tissues, the lymphocytes responsible for adaptive immunity and the myeloid lineages that participate in both innate and adaptive immunity, derive ultimately from the pluripotent hematopoietic stem cells (HSCs) in the bone marrow where many of them also mature and then migrate to guard the peripheral tissues, circulating in the blood and in the specialized lymphatic system [93].

The myeloid progenitor is the precursor of the granulocytes, macrophages, dendritic cells and mast cells. Macrophages and mast cells complete their differentiation in the tissues where they act as effector cells in the front line of host defense and initiate inflammation. Macrophages are one of the three types of phagocytes in the immune system playing a critical part in innate immunity. They are the mature form of monocytes, which circulate in the blood and differentiate continuously into macrophages upon migration into the tissues. They phagocytose bacteria and recruit phagocytic neutrophils from the blood [94, 95]. Dendritic cells are specialized to take up antigens and display them for recognition by lymphocytes. Immature dendritic cells migrate from the blood to reside in the tissues and are both phagocytic and macropinocytic, ingesting large amounts of the surrounding extracellular fluid. Upon encountering a pathogen, they rapidly mature and migrate to the lymph nodes [96-98]. Mast cells also differentiate in the tissues. They mainly reside near small blood vessels and, when activated, release substances that affect vascular permeability to orchestrate the defense against parasites as well as triggering allergic inflammation; they recruit eosinophils and basophils, which are also exocytic. In addition, mast cells also play a major part in protecting mucosal surfaces against pathogens [99-101].

The granulocytes consist of three types, neutrophils, eosinophils and basophils, all of which are relatively short lived and are produced in increased numbers during immune responses, when they leave the blood to migrate as effector cells to sites of infection or inflammation. Neutrophils and eosinophils, together with natural killer cells and mast cells, predominantly engage themselves during early immune events. Neutrophils, which are the third phagocytic cell type of the immune system, are the most numerous and most important cellular component during early innate immune response: hereditary deficiencies in neutrophil function leads to fatal bacterial infection if untreated [102, 103]. Eosinophils are thought to be important in defense against parasitic infections, whilst the function of basophils is similar and complementary to that of eosinophils and mast cells [104-106].

The common lymphoid progenitor gives rise to B-cells which, when activated, differentiate into plasma cells that secrete antibodies; and T-cells, of which there are two main classes. One class differentiates upon activation into cytotoxic T-cells (T_C), which kill cells infected with viruses, whereas the second class of T-cells differentiates into cells that activate other cells such as B-cells and macrophages. A third lineage of lymphoid cells, called natural killer cells, lack antigen-specific receptors and are part of the innate immune system. These cells circulate in the blood as large lymphocytes with distinctive cytotoxic granules. They are able to recognize and kill some abnormal cells such as

tumor cells or virus-infected cells, and are thought to be important in the innate immune defense against intracellular pathogens [107-111].

Lymphoid organs can be divided basically into primary lymphoid organs (the bone marrow and the thymus), where lymphocytes are generated and mature, and secondary lymphoid organs (the lymph nodes, the spleen and the mucosal lymphoid tissues), where adaptive immune responses are initiated and where lymphocytes are maintained. While Bcells mature in the bone marrow, T-lymphocytes have to migrate to the thymus to undergo their maturation. Once they have completed their maturation, both types of lymphocytes enter the bloodstream from which they migrate to the peripheral lymphoid organs. The peripheral lymphoid organs are specialized to trap antigen, to allow the initiation of adaptive immune responses and to provide signals that sustain recirculating lymphocytes. The peripheral lymphoid tissues also provide signals that sustain the lymphocytes that do not encounter their specific antigen (naïve lymphocytes), so that they continue to survive and recirculate until they encounter their specific antigen. In the event of an infection, lymphocytes that recognize the infectious agent are arrested in the lymphoid tissue, where they proliferate and differentiate into effector cells capable of combating the infection. These mechanisms have been put in place to maintain the correct number of circulating T- and B-lymphocytes and ensure that only those lymphocytes with the potential to respond to foreign antigens are sustained. In the lymph nodes, Blymphocytes are localized in follicles with T-cells more diffusely distributed in the surrounding paracortical T-cell zones. The B-cell follicles include germinal centers where Bcells undergo intense proliferation after encountering their specific antigen and their cooperating T-cells. B-cells that encounter an antigen as they migrate through the lymph nodes are sequestered and activated with the help of some of the activated T-cells. Once the antigen-specific lymphocytes have undergone a period of proliferation and differentiation, they leave the lymph nodes as effector cells through the efferent lymphatic vessels [107, 108, 112].

Therefore, immunity is more or less defined as the elimination of foreign pathogens, particles or aberrantly transmogrified neoplastic cells of the host *via* immune responses initiated through a specialized cellular system.

The Innate and Adaptive Immune System

In general, the activated immune system is subdivided into two arms, the innate immune system and the adaptive immune system. These two systems work independently and yet recruit each other's help to potentiate a series of timely sequenced steps [107, 108, 113, 114].

The innate immune system is the first to kick-in during disease pathogenesis and refers conventionally to the white blood cells that carry out this process during the first seventy-two hours of pathogenesis [114]. The innate immunity includes the monocytes, macrophages, mast cells and granulocytes, which are aided by blood derived acute phase proteins, the complement system and blood platelets, which act to supplement and augment the innate immune responses [113-117]. Leukocytes express germ-line encoded surface receptors capable of identifying and attaching to subsets of bacterial and viral

surface epitopes which then transducer activating intracellular signaling to differentiate them into effector cells capable of phagocytosis, to release chemical compounds and free radicals to destroy or opsonize the circulating toxins or antigens to prevent a massive invasion, giving time for the adaptive immune system to get primed and develop [118-120].

The adaptive immune system, on the other hand, is considered as the hallmark to immunity [107-111]. The T- and Blymphocytes offer a vast repertoire of surface receptors, which are built upon a similar structural platform but are diverse in specific sequences, which map onto epitope recognizing moieties [121, 122]. The diversity of these epitope-recognizing moieties is almost colossal, far exceeding the diversity presented by the receptors on cells of the innate immune system. Furthermore, the cells of the adaptive arm of immunity reign in the services of cells and proteins belonging to the innate immune system, in a synergistic effort, to rid the body of foreign bodies [107, 108, 123, 124]. Physiologically, this translates into the innate immune system being capable of differentiating and acting towards bacterial or viral antigenic markers belonging to broad groups and the adaptive immune system capable of differentiating between even closely related species or sub-species of pathogens. Consequently, as common knowledge goes, no two individuals are exactly alike and the potential of the adaptive immune system hinges on this testimony.

The immune system is of course by far much more complex in the manners in which it is regulated. The main stages involved in immune maturation are outlined in Fig. 5.

Many of the processes that mould the immune system with time, arise from cues given from outside the immune system such as modulatory inputs from the nervous system. Interestingly, NTs have been identified as naturally occurring peptides released from cells of the bone-marrow, including immune cells themselves [125-131]. They mediate their immunomodulatory effect directly through the expression of the appropriate Trk receptors on immune cells and accessory cells of primary and secondary lymphoid organs, swaying early and late hematopoietic processes [132, 133]. Recent results showed a marked expression of NGF and its specific receptor TrkA in cord blood CD34⁺ cells [134, 135]. These findings suggest that NGF may play a major role in the differentiation of hematopoietic progenitors and indicate a different requirement for NGF by immune cells, depending on their state of maturity [134-137].

There is now increasing evidence that besides NGF, the other members of the NT family have broader roles in modulating immune functions because organs participating in immunological functions, such as the thymus and the spleen, express NGF, NT-3 and NT-4 [127, 138]. In addition, monocytes release BDNF and primary primed T-cells release elevated levels of NT-3 and NT-4/5 [139, 140].

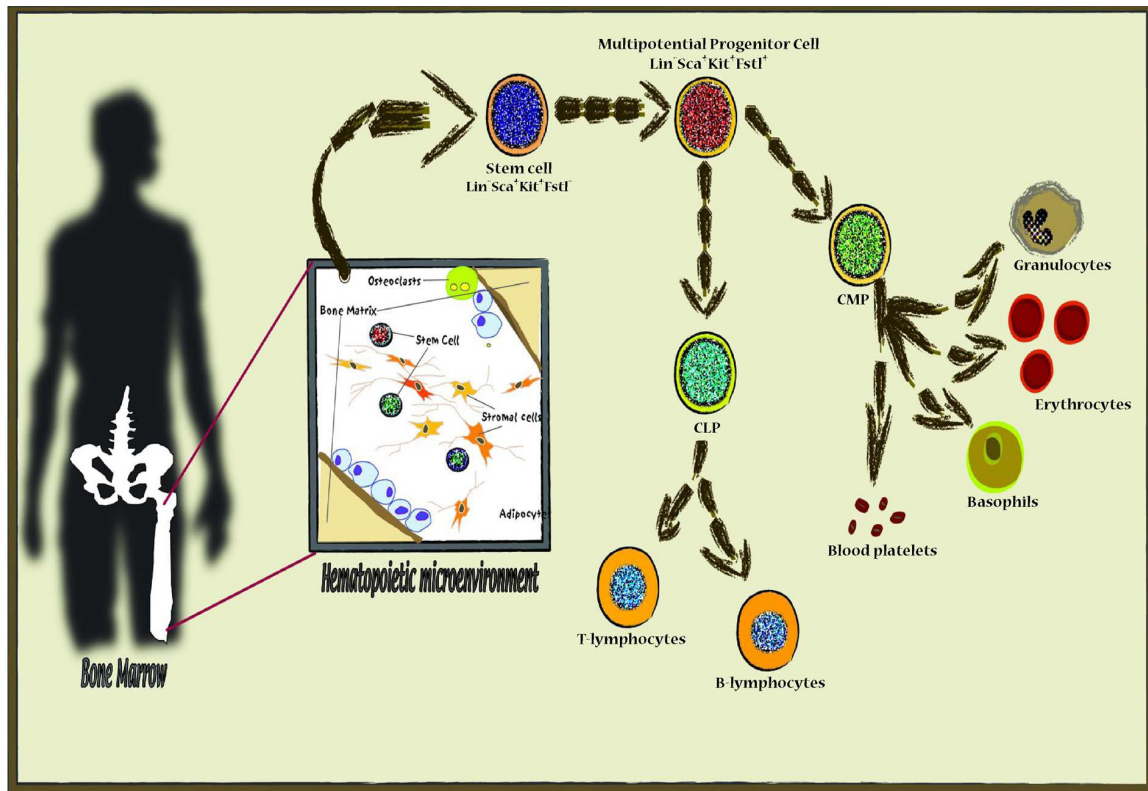


Fig. (5). The bone marrow provides a unique, tightly regulated micro-environment, promoting timely development of the immune cells which then enter the peripheral circulation to carry out effector functions. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; SCA; stem cell antigen.

To bridge the chasm between the nervous and immune systems, the understanding of the functional role of the NTs in physiological immune reactions, including immune development, is essential. This is the ultimate basis that lays the foundation for this huge interplay whereby a neuron-specific trophic factor, such as NGF, dispatches its tools in proliferation, differentiation and gene regulation in a similar manner within the immune system.

NEUROTROPHINS IN IMMUNE DEVELOPMENT AND ACTIVITY

An Interactive Homeostatic Mechanism

NTs, potent regulators of neuronal survival and development, also possess the forte to modulate and tweak immune development and activities. Their ability to act upon these cells is mediated *via* their constitutive and timely expression and secretion at the proper developmental junctions [125, 128, 132-134, 141-143].

Hematopoiesis is a complex process, involving multiple intracellular signaling events and involves the proper recruitment of downstream transcription factors to promote the synthesis of appropriate cell cycle proteins and gene transcription [93, 144]. The bone marrow is where it all begins, the journey of the lone HSC from its multipotent stem cell state *via* an un-differentiated progenitor cell state and finally into the multitude of

differentiated, fully functional immune cell types that can be observed in the final repertoire of cells [93, 145]. These functionally competent immune cells possess the ability to carry out their anti-microbial activities. Development occurs in a tightly regulated microenvironment found within the bone marrow itself. This strict environmental niche is lined with bone marrow stromal cells (BMSC) found in the immediate vicinity with a similar stringent environment for T-cell development and maturation occurring in the thymus [146]. Other important secondary immune organs, such as the spleen, lymph-nodes and lymphoid epithelial tissues provide similarly regulated niches for proper activation and maturation of antigen specific lymphocytes. These lymphoid organs and the resident immune cells presiding within show active NT release and Trk receptor expression [127, 130, 147].

Neurotrophins Action in the Bone Marrow

BMSCs are a heterogenous mixture of cells consisting of adipocytes, fibroblasts, osteoclasts and other accessory cells [148]. Of primary importance are the fibroblastic stromal cells, which release a unique mixture of cytokines, growth factors and adhesion molecules in iota concentrations which work in concert to promote the survival, proliferation and differentiation of the HSCs [149-151]. These HSCs in turn express tyrosine kinase receptors, integrins and cytokine receptors to be able to respond appropriately to the expressed ligands on the stromal cells and furthermore release the necessary factors required for maturation which act upon the developing cells in an autocrine and paracrine fashion [152- 154].

HSCs differentiate to give rise to branches of lineage committed cell lines. The initial stem cell branches out to form two lineage specific cell types, the myeloid progenitor cell line and the lymphoid progenitor cell line. The myeloid progenitor cell line, under the influence of different combinations of factors, works its way towards a characteristic phenotype, as do the cells arising from the lymphoid progenitor cells. The colony-stimulating factors such as c-kitL (also known as stem cell factor (SCF)), interleukin (IL)-3, granulocyte-macrophage colony stimulating factor (GMCSf) and M-CSf are glycoprotein molecules, which partake in lineage proliferation and differentiation of these progenitor cells and have been shown to work in concert with NTs [132-134, 141, 142].

As such, it is interesting to note that BMSCs and early progenitor cells of the developing hematopoietic machinery express and release NTs (Fig. 6). And now their significance in the hematopoietic system and as one of the advocates of the neuro-immunomodulatory network is being increasingly appreciated [155-158].

The absolute significance of the presence of neurotrophic factors in the vertebrate bone marrow is still puzzling. Do they have important roles in depicting the fate of the cells or are they merely present to potentiate intracellular signaling offered by the other tyrosine kinase receptors present? Do their expression profiles change with time as cellular development proceeds? In fact the answer to all these questions posed appears to be a resounding yes, that NTs influence hematopoiesis in a variety of ways.

As aforementioned, stromal cells are important in characterizing the fate of the immune cells and NTs are released by the fibroblastic stromal cells of the bone-marrow. NGF and BDNF are released by BMSCs when incubated with supernatant from injury-induced brain tissues [158], which are rich sources of ILs and other cytokines. BMSCs also stain positive for TrkA, TrkB and TrkC receptors [125, 128]. This, coupled with Trk receptors expressed on early and late phase hematopoietic cells, suggests an autocrine and paracrine mode of stimulation [125]. The functional implications for Trk activation might rhyme in concert with the activities of other tyrosine kinase receptors present.

Various tyrosine kinase receptors are found on the surfaces of BMSCs, which activate signal transduction pathways giving rise to proliferative, differentiation and localization signals important in regulating crucial developmental decisions. For example, vascular endothelial growth factor receptor-1 (VEGFR1) is an important tyrosine kinase receptor, which is expressed on CD34⁺ bone marrow stem cells [159]. They participate in the recruitment of VEGFR1⁺ progenitor cells on site through the autocrine and paracrine release of VEGFR1 ligand molecules [154, 159]. Activation of VEGFR1 ends up in transcription of SCF, which activates its receptor c-kit, leading to mitogenesis of stem cells [160]. SCF works in synergy with NGF to promote proliferation of cells of the erythroblastic lineage, which expresses TrkA receptors. In a similar fashion, CStFs and ILs, which are rudimentary to lineage commitment, work in concert with NTs to expand subsets of progenitor cells [130, 132, 141, 161, 162]. On hindsight, NTs and Trk receptors expressed are not merely incidental but act as one part of a partnership between other factors to potentiate lineage commitment.

The manual by which the synergistic outcome results is however unknown. As indicated, most of the factors act through ligand specific tyrosine kinase receptors, including NTs and employ similar adaptor and signaling molecules downstream [33, 47, 56, 84, 163-165]. NTs might thus not necessarily induce lineage commitment but serve to expand specific subpopulations of progenitor cells when the appropriate CStFs are present and to increase sensitivity of the expanded populations to these CStFs. For instance, CD34⁺38⁺ cells, which give rise to early erythroblasts, respond positively to NGF leading to an expansion of the erythrocyte cell population [166]. These progenitor cells express the TrkA receptor, whereby its activation prompts the release of other CStFs and growth factors to regulate hematopoiesis [134]. As mentioned above, proliferation and stimulation of erythroleukemic cells act through the synergistic effects of NGF and SCF, both factors being released by BMSCs [166]. Strongly validating the above point, also the observation that NGF, when coupled with GM-CStF, promotes the cell lineage differentiation of human basophils whilst coculture of BMSCs with IL-3 and NGF leads to connective tissue-like mast cell colony formation [132, 133,141]. Similarly, HL-60, a human myeloid cancer cell line, requires an initiating signal from NGF before it can respond in a positive manner to GM-CStF to prompt basophilic lineage differentiation [132]. The initiating signals provided by NGF might appear elusive with respect to hematopoietic cell intracellular signaling. Nevertheless, studies from other cell types show that NGF up-regulates transcription and expression of growth factor receptors, transcription factors, adaptor molecules and GTP-binding proteins [167]. Some of the signaling pathways which are important in hematopoiesis include the Ras/MAPK

pathways, Smad signaling pathways and the Ca²⁺/Calmodulin dependent protein kinase pathways. CStFs and interleukins very often work through the complex molecular pathways mentioned above to achieve their signaling goals [165, 167-170].

Calmodulin antagonists, for instance, abolish GM-CSF and IL-3 mediated colony proliferation in hematopoietic cells [171]. NGF though induces an increase in calmodulin cytoplasmic levels in PC12 cells (Pheochromocytoma cells (PC12), has been used as a well known established neuronal model system for the analysis of the effect of NGF on survival, apoptosis and differentiation to elucidate the specific intracellular signaling cascades involved in these responses [172, 173]) and a similar activating signal might occur in hematopoietic progenitor cells, which could serve as the initial priming event required before hematopoietic colonies can respond to GM-CSF and IL-3 [167]. In this manner, NGF might serve as a threshold modulator or priming factor, as it acts on human basophils, raising the threshold concentrations of signaling molecules such as calmodulin or Grb2 [174-177].

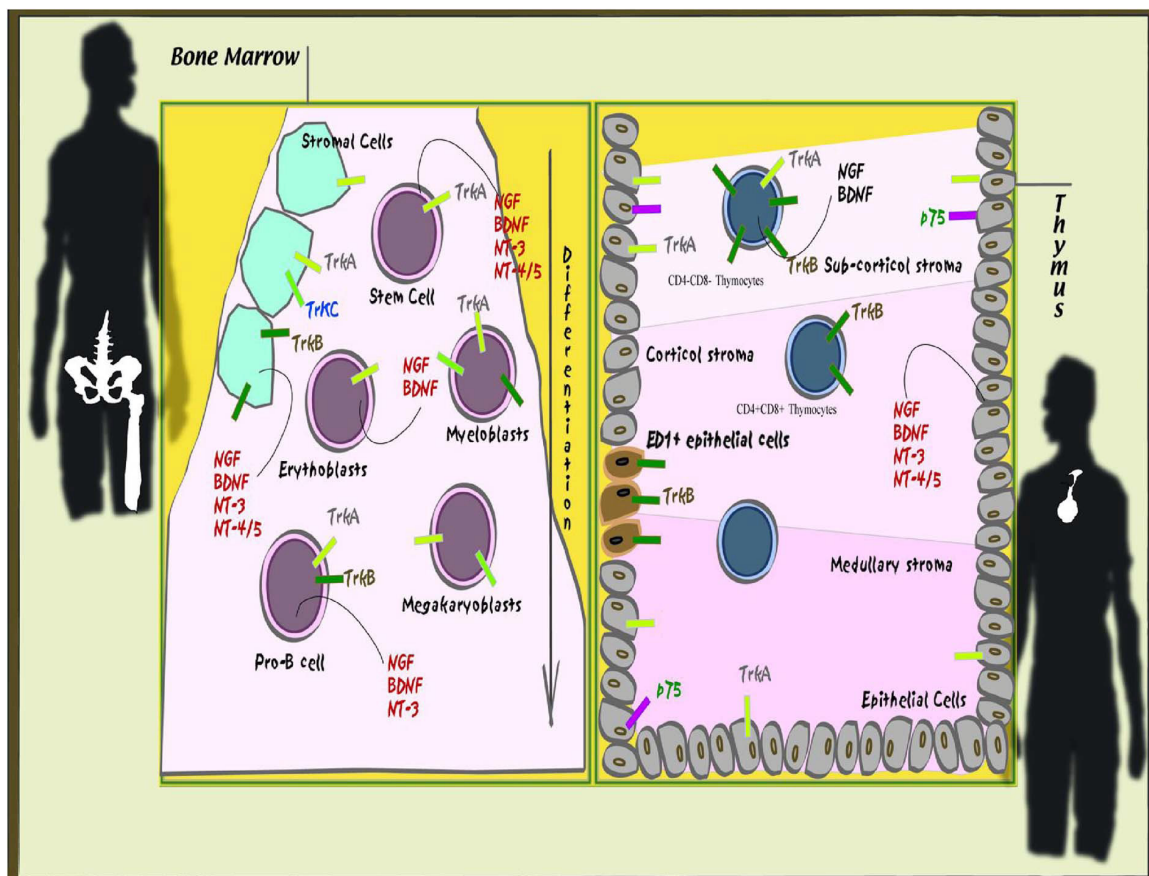


Fig. (6). The neurotrophins NGF, BDNF, NT-3 and NT-4/5 are produced and released by the bone marrow and thymic stromal cells. The cognate Trk receptors are also found expressed not only on the corresponding thymic epithelial cells but also on the developing immune cells as well – therefore, carrying out autocrine and paracrine activations. As the cell lineages diverge out, each progenitor cell line still expresses Trk receptors and releases the corresponding NTs where, in conjunction with lineage specific cytokines, they start to terminally differentiate. The thymocytes undergo a time-dependant decrease in TrkB expression as the thymocytes move in from the sub-cortical regions into the cortical towards the medullary cortical

regions where they mature to express both CD4 and CD8 surface co-receptors. BDNF, brain-derived neurotrophic factor; NGF, nerve-growth factor; NT-3, neurotrophin-3; NT-4/5, neurotrophin-4/5; TrkA, tropomyosin-related kinase A; TrkB, tropomyosin-related kinase B; TrkC, tropomyosin-related kinase C.

Neurotrophins in B-Cell Development

Newly produced B-lymphocytes emigrating from the bone marrow mature into cells able to secrete antibodies of high affinity and specificity following interaction with various cell types present in different anatomic compartments. Following antigen recognition, naïve surface immunoglobulin sIgM⁺, sIgD⁺ B-cells and memory sIgD⁻ B-cells proliferate in the T-cell-rich areas of secondary lymphoid organs and mature into plasma cells. This process, called extrafollicular reaction, yields a few B-cell blasts, which enter primary follicles to form the germinal centers. During this reaction, B-cells, in contact with activated CD57⁺, CD4⁺ T-cells, follicular dendritic cells and tingible body macrophages, undergo massive expansion, isotype switching and somatic mutation. Daughter B-cells expressing high-affinity sIg are selected and differentiate into either small memory B-cells or plasmablasts which, following migration to the bone marrow or the mucosal lamina propria, become plasma cells. A very early study on NGF's role in B-cell function has demonstrated that NGF stimulates B-cell proliferation and differentiation as well as regulates the production of IgM and IgA [178].

The additional finding that NGF is a survival factor for memory B-cells confirms that the immune and nervous systems have several features in common [179]. Both systems comprise of a complicated network of cells that are responsible for defending the organism against threats to survival. Interestingly, NGF elicits different responses from naïve and memory B-cells [179]. Neutralization of endogenous NGF leads to apoptotic cell death of resting memory B-cells expressing surface IgG or IgA, whereas proliferation of naïve B-cells in response to mitogens is unaffected. In addition, NGF does not act as a 'switch factor' for B-lymphocytes. In the absence of NGF, B-cells cannot remember the antigen that they have encountered and the memory IgG response to an antigen is abolished. Moreover, Brodie and Gelfand showed that p75NTR and CD40 are expressed in a coordinate fashion on B-cells, but might differ in their activities at different stages of B-cell differentiation with regard to their interactions with other cytokine- and growth factor-systems [180, 181]. Whether p75NTR expression is essential for B-cell functioning remains still unclear. This is, however, of special interest, as p75NTR mediates apoptosis upon activation by proNGF [65-68]. Recently, a research group using a novel 'reverse conditional' gene targeting strategy, which restored normal TrkA expression in the nervous system, thus only eliminating TrkA expression in non-neuronal cells, noted that the immune system developed normally. Though the mice showed B-cell abnormalities such as elevated serum immunoglobulin levels and B-cells, it is important to bear in mind that the evidence thus far gleaned might not necessarily be an absolute reflection of the in vivo conditions of neurotrophic action [182].

Another NT, BDNF, plays a pivotal role in B-cell development. Pro-B cells, the earliest B-lymphocyte committed cells, which give rise to B-cells, appear to depend on secreted BDNF to differentiate further towards a more committed B-cell phenotype and show

alterations in intracellular calcium wave signals when induced with BDNF, therefore augmenting calcium mediated downstream signaling [143]. Schuhmann *et al.* showed that B-cell development in the bone marrow is specifically impaired (but not completely blocked) in BDNF-knock-out (-/-) mice at the Pre-BII stage, which results in a reduced number of B-cells in the periphery while the splenic microarchitecture remained unchanged [143]. The developmental block in bone marrow B-cell development in BDNF deficient mice and the fact that Blymphocytes express NGF, the NGF receptors TrkA and p75NTR as well as the truncated BDNF receptor TrkB_{gp95} points to a crucial role of NGF and BDNF for normal Blymphocyte development and function [143, 182-184].

Neurotrophins in Active Immunity – Neurotrophins and Activated B-Cells

The bone marrow and the thymus are the primary lymphoid organs providing a secluded region for apposite interactions between developing stem cells and stromal cells. This ensures proper interactions as they pass through the various compartments of epithelial cells that release a regulated stream of cytokines and the necessary growth factors[145, 146, 148-150, 152].

Leukocytes upon reaching the final stages cease their immune education. These cells then traverse out from the blood vessels to populate appropriate peripheral organs and tissues. Collectively, leukocytes are released into the peripheral circulation where they populate secondary lymphoid organs (spleen and lymph nodes), lympho-epithelial tissues and circulate through the extravascular spaces of tissues and the blood. Cells of the innate immune system are functionally ready when they are released into the blood from the bone marrow. These cells wait for some form of adhesion to microbial moiety to which they respond by initiating a cascade of downstream signaling events. Lymphocytes on the other hand, functionally competent nevertheless, are immunologically naïve. Lymphocytes are maintained in their resting states via weak recognition of self-antigens and die if they fail to encounter any antigenic epitopes with high affinity. These cells are now deemed competent enough to function in an active reaction against any possible anomaly to the normal environmental homeostatic balance due to an undesired foreign invasion or aberrant cellular proliferation. However, they are presumably arrested in the Go-cell-cycle state. They need to be primed, to execute intracellular programs that will tip the molecular balance towards the G1 stage [185, 186].

There are three separate phases that one has to consider in order to evaluate the crucial moments during which NTs or Trk receptors initiate their key modulatory effects: i) the resting phase, ii) the competence/priming phase, and iii) the activation phase. The resting phase represents the cells in their Go state. Circulating naïve cells are predominantly at this phase, waiting for an activation signal to prime them into a mitotic cycle. In this respect, one can say that they await their final signal to terminal differentiation where they elicit the full extent of their anti-pathogenic functions [185, 186].

The competence or priming phase is the stage where the cells initiate into the G1 phase from the Go arrested phase, which ultimately culminates in the cells entering the G1 → S phase, which kick-starts mitosis. These series of events are crucial to immunity, as

more than often immunity is dependent on specific and rapid expansion of particular subsets of antigen-specific immune cells. Many different adaptor and transcriptional factors are up-regulated or down-regulated following an activating signal [185, 186].

Increasing evidence identifies NGF as a signaling molecule modulating inflammatory processes associated with tissue repair and immunological responses [136, 176]. It is known that levels of immune-cell-derived NGF increase at inflammation sites and numerous pro-inflammatory cytokines such as IL-1 β , tumor necrosis factor (TNF)- α and IL-6 induce NGF synthesis [177]. Production of NGF can be modulated by local levels of cytokines in response to modification of local tissue homeostasis, while NGF itself in turn enhances the expression of cytokines such as IL-6 in thymic stromal cells [187].

B-cells are able to produce cytokines such as IL-4 and IL-6 in an autocrine fashion: these cells, first upregulate the expression of the receptor and then autocrinally produce the cytokine. The activation of human B-lymphocytes has been worked out in some detail to depend on the synergistic effect of IL-4 and CD40 cross-linking [188, 189]. Some of the crucial signals required to activate transcription are a number of transcription factors (STAT-6, NF- κ B, AP1 and B-cell-specific activator protein (BSAP) among others). In looking systematically at the effects of IL-4 on human B-cells, it had been pointed out early on that this cytokine induces not only IgE, but also IgG4. During clonal expansion, switching might occur in successive steps from IgM to IgG4 and IgE. IL-4 causes a striking enhancement of the proliferative responses of B-cells to anti-Ig antibodies and induces the development of IgG1-producing cells from B-cells stimulated with LPS indicating that IL-4 acts as a B-cell proliferation and differentiation factor. In addition to stimulation of cell growth, survival and induction of class switching to IgG1 and IgE, IL-4 mediates a number of other effects on B-cells, including: increasing cell size and increasing the cell surface expression of CD23 (Fc ϵ RII) and major histocompatibility complex class II (MHC-II) molecules [190, 191].

CD40, as p75NTR, is a member of the TNF receptor family that is expressed on B-cells, monocytes, dendritic cells, endothelial cells and epithelial cells as well as on Bcell lymphomas and carcinomas. CD40 has recently been shown to be an important regulator of the production of inflammatory mediators, cell survival, and prolonged antigen presentation by dendritic cells. In addition, development of the acquired immune response is dependent on bidirectional signaling mediated by CD40 and its ligand CD154 (CD40L), which is expressed primarily on activated CD4⁺ T-cells. Accumulating evidence has convincingly demonstrated the pivotal role of CD40 in B-cell activation, antibody production against thymus-dependent antigen, isotype switching and the generation of memory B-cells and germinal center B-cells. And no compensatory mechanism appears to replace CD40's function [192, 193].

Although continuous studies have revealed CD40 signaling pathways that ultimately result in B-cell activation, it still remains to be solved how these signaling events are initiated after receptor ligation and how the CD40- and NGF-receptor systems interact with each other.

New data have shown that B-cells seem to be the predominant source for NTs [194] and early reports have demonstrated p75NTR on resting B-cells and that its expression significantly increases when B-cells get activated [195]. Early studies have also shown that B-cells are able to respond to NGF by increasing their proliferation rate and their differentiation [178]. NGF promotes both B-cell proliferation as observed during ³[H]-thymidine incorporation analyses and B-cell differentiation as NGF induces tonsillar B-cells to increase their IgM secretion [178]. On the contrary, another study on NGF has shown that it causes a decrease in IgG and IgM production from B-cell lines, an effect, which is reversed when IL-4 is added [180]. Furthermore, IL-4 at the same time has been shown to upregulate CD40 antigen and to downregulate p75NTR on these B-cell lines. This finding leads to the speculation that the balance between CD40 and p75NTR numbers on the cell surface gives rise to a physiological switch depending on the stage of the B-cell [180].

Investigations on BDNF-deficient mice indicate that the NGF-response to B-cell activation is dramatically changed and revealed that LPS-, IL-4- and CD40-mediated NF- κ B activation is a key regulatory event for B-cell-derived NGF production [184]. Activated B-cells release BDNF and express a functional but truncated (TrkB_{gp95}) form of the full length BDNF receptor (TrkB_{gp145}) in B-cells isolated from spleen as well as from bone marrow and thus BDNF may also have autocrine functions [143, 194, 196]. B-cells respond also to NGF by increasing their proliferation rate and NGF is essential for the survival of memory B-cells indicating that there might be a cross-talk between NGF and BDNF during B-cell activation [136, 179, 184, 197]. Blocking TrkA activation *via* NGF leads to massive apoptosis in memory B-cell conjunct with a cytoplasmic disappearance of the Bcl-2 protein, which is a key anti-apoptotic factor [179]. Evidently, NGF-mediated TrkA activation has been shown to rescue Bcells from apoptosis induced by ligation of surface IgM, via a Gab-1 docking protein, which results in the PI3-kinasedependent nuclear translocation of PKC ζ which aids in sustaining the survival signals intrinsic to the cells [198]. Memory immune cells are involved in initiating a more robust secondary response to an immune attack, especially so when encountering a previously encountered pathogen or foreign body. However, unlike normal naïve B-cells, the survival factors delineating the survival properties of memory B-cells are still elusive. It is thus tempting, though yet speculative, to imply that NTs secreted by crucially innervating sympathetic neurons in the immune organs might provide partially this potent survival factor for these memory cells.

Several important questions remain to be answered with respect to the humoral response: is the CD40/IL-4–NTsystems-interaction required for clonal expansion of B-cells, for maintenance of B-cell memory and for affinity maturation? Is the CD40/IL-4–NTsystems-interaction required within the germinal centers to orchestrate the affinity maturation of the immune response? The role of CD40L in the regulation of antigen presenting cell function and the expression of other co-stimulatory molecules focus attention on this molecule as a decisive factor in tolerance versus immunity. Thus, the biological role of the interaction between the CD40- and the NT-systems and its pivotal impact on specific B-cell functions remains to be elucidated (Fig. 7). Further studies will

provide deeper insights into the role of the CD40-NT-crosstalk in B-cell development and activity.

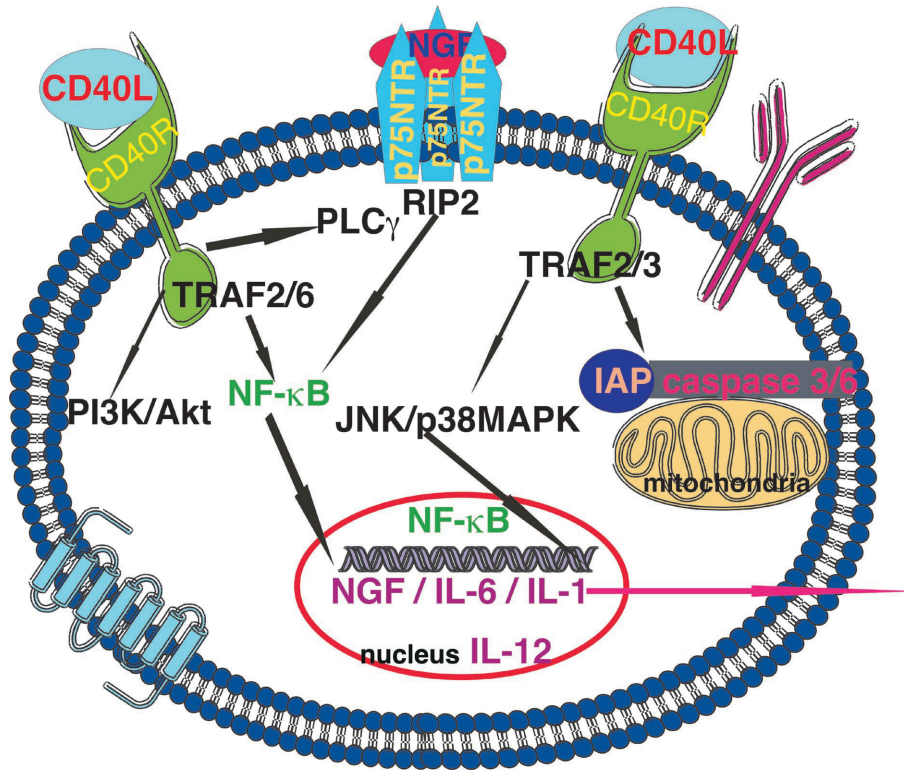


Fig. (7). Schematic illustration of the signaling mechanisms underlying the coupling of CD40 to NGF expression in B-cells. IAP, inhibitor of apoptosis; NF-κB, nuclear factor-kappaB; PI3K, phosphatidylinositol-3 kinase; RIP2, receptor interacting protein 2; TRAF2/3/6, TNF receptor-associated factor 2/3/6 (adapted from Heese *et al.* [184]).

Neurotrophins Action in the Thymus

The thymus though, which is the primary site for T-cell development, shows a timely regulated expression of NTs and Trk receptors. In terms of NT action, maturation of cells in the thymus is another important aspect of hematopoiesis. The compartmental distribution of Trk receptors and their associative ligands in both the marrow and thymus are not merely bystander participants. They adumbrate a higher functional significance, a pivotal role in initiating a regulated and judicious outcome in immune cell development. Both the bone marrow and thymic microenvironment show a communal use of NTs. This is not surprising as the stroma of both organs not only partake in modulating the immune cells, they conversely depend on the immune cells themselves for associative and secreted signaling to help to mature themselves, especially in the thymus, which undergoes an age-dependent involution process [199].

The thymus is a neuroendothelial organ concerned with the development of the different subsets of T-cells [200]. Progenitor cells with their limited capacity of differentiating into

varied T-cells enter the thymus from the bone-marrow [201]. T-lymphocytes mature to form two main types of cells, cytotoxic-T-(T_C)-cells and T-helper-(T_H)-cells. The T_C-cells are associated on their surfaces with a marker, CD8, whilst the T_H-cells are identified *via* their expression of the CD4 marker. The T_H-cells are further divided into two groups, the T_{H1}- and T_{H2}-cells, each subgroup secreting a predominantly unique cytokine expression profile, which partake in mediating active immunity and as regulators and reciprocal suppressors of T-cell and B-cell activity.

Thymocytes enter the thymus from the blood vasculature into the stromal tissues. Colonization of the thymic epithelial cells is made possible by the presence of chemokine receptors (CCRs) and their ligands (CCLs), such as CCR7 and CCR9 on thymocytes and CCL21 and CCL25 on the epithelial cells or the stromal cells themselves [202]. Some of these cytokine ligand-receptor pairs home in thymocytes to the thymus.

Thymocytes are initially devoid of both CD8 and CD4 accessory surface receptors. In mature T-cells, the presence of CD8 and CD4 is required for proper activation of the Tcell receptor (TCR) complex upon their respective recognition of MHC-I-peptide and MHC-II-peptide complexes. The CD8 and CD4 molecules are rudimentary accessory molecules that help to stabilize the MHC-peptide-TCR linkages and aid in signal transduction for T-cell activation.

Thymocytes therefore interact with the surrounding epithelial cells, resident macrophages and dendritic cells in the thymic environment that provides a unique bilateral interacting environment leading to this attainment of CD4 and CD8 co-receptor molecules. Cell culture studies using thymus-derived neoplastic stromal cell lines and freshly isolated cells of the thymus establish the need for NTs to regulate thymocyte differentiation and survival as the thymocytes make their way through the stromal cell microenvironment [203].

The production of NGF by lymphocytes and the presence of a neuromodulatory loop involving autocrine expression of NGF in thymic stromal cells have been described and the observation that NGF is expressed by lymphocytes and thymic stroma cells suggests that NGF and presumably the related members of the NT family may also broadly influence thymus functions and T-cell differentiation [187, 204, 205]. For instance, BDNF and NT-4 released by thymic stromal cells have been shown to be rudimentary to phasing CD8⁻ CD4⁻ thymocytes into intermediate CD8⁺CD4⁺ thymocytes *via* the activation of TrkB receptors on immature thymocytes [202]. This hypothesis is underlined by the finding that TrkB receptor expression is inversely correlated with the maturation stage and the differentiation potential of thymocytes, being greatly expressed in CD4⁺CD8⁻ immature thymocytes and progressively declining in CD8⁺ and CD4⁺ single-positive and CD4⁺CD8⁺ more mature thymocytes [206].

Exogenous BDNF added to double negative thymocytes induces receptor phosphorylation of TrkB and intracellular upregulation of c-fos signaling protein and exhibits a timeregulated TrkB downregulation as thymocytes pass through to the intermediary double positive CD4⁺CD8⁺ stage [206]. TrkB receptors are also found along

a sub-set of corticomedullary junctional epithelial cells bearing the surface marker, ED-1⁺, a MC-DC (macrophage-dendritic) specific marker, with an increase in receptor number during thymic involution. Transgenic mice expressing mutant dysfunctional TrkB exhibit a significantly smaller thymus with massive lymphocyte cell death as compared to wild-type mice [207,208].

Finally, BDNF is expressed in thymic stroma and is upregulated by signals generated by the thymocyte/stromal interaction, suggesting that BDNF is a survival factor for thymocyte precursors. In addition, the fact that freshly isolated thymocytes express only NT-4/5 but not BDNF, supports the hypothesis of developmentally regulated feedback mechanisms based on autocrine and paracrine NT/NT-receptor interactions that may be involved in the thymocyte differentiation process [143, 203, 206, 208].

Though it may be argued that this could be attributed to a developmental problem as NTs are important trophic factors to mediate proper innervation of peripheral organs, the thymus shows a developmentally regulated expression level of Trk and p75NTR receptors and NTs, which by itself lends credence to a more important role within the thymocyte microenvironment by itself [127, 206, 207].

While the presence of high affinity NGF TrkA receptors has been reported on functionally competent activated CD4⁺ T-cells [209, 210], and several other hemopoietic cells [126], there is now unequivocal evidence of TrkA, TrkB, low levels of TrkC and p75NTR expression in thymic tissues although their cellular localization has not been affirmed concretely [127, 205].

In line with this, p75NTR increases in expression during the development of the thymus, from embryogenesis till adulthood, with its expression primarily confined to the stroma in adult mice [127]. The thymic stroma is composed of epithelial cells, which is anatomically and morphologically divided into three subgroups, the subcortical epithelia, the cortical epithelia and the medullary epithelia. The thymocytes, closely apposed to each of these cell-types, receive their developmental signals as well as neurotrophic signals to guide them through differentiation. Subcortical epithelia, which express both TrkA and p75NTR, and medullary epithelia, which strongly express p75NTR, show a rapid postnatal increase in these receptor expression [211]. Cortisolinduced lymphocyte apoptosis in the thymus shows a downregulated TrkA and p75NTR receptor expression in their native thymic epithelial cells whilst TrkA receptors exhibit an upregulated expression level in cortical thymic epithelia in which they are usually absent [207].

Thus, data so far indicate that NTs such as NGF, BDNF, NT-3 and NT-4/5 are released from both thymocytes and the surrounding stromal tissues, which hypothesizes towards an autoregulatory loop between stroma and T-cells [205, 208, 211].

The influence of NT action is also differentially regulated between different subsets of lymphocytes. T_{H1}- and T_{H2}-cells are important modulators of B-cell and macrophage activation and the relative levels of these cells and their distinctive cytokine profiles allow them to either act as activators or suppressors of immunity. T_{H1}- and T_{H2}-subtypes,

by virtue of their differential cytokine expression profile promote the differential release of Ts. The T_{H1}-cytokine IL-2 stimulates the production of TrkB mRNA and BDNF protein, whilst the T_{H2}-cytokine IL-4 mediates the release of NT-3 but not BDNF [212].

Though NT-3 and NT-4/5 have been noted in tissue samples isolated from the thymus (and the spleen), their levels are comparatively low and the accuracy in determining their cellular source might not be too feasible [205].

Thus, expression of NTs in T-cells still remains a controversial issue. While some authors could detect NTs in T-cells [140, 196, 204, 205, 209, 212-215], other studies show limited or no expression of NTs or their high affinity Trk receptors in T-cells and the question is whether such putative expression levels are high enough for mediating a substantial regenerative biologic effect [140, 179, 194]. Jones and colleagues have provided evidence that autoreactive T-cells infiltrating contused spinal cord may actually impede recovery rather than provide neuroprotection, insofar as their infiltration results in increased levels of proinflammatory cytokines but not NTs in the damaged CNS [216]. In line with this, a recent report indicates that B-cells rather than T-cells are the major, if not the only source of leukocyte-derived NGF and BDNF and as such may provide “protective autoimmunity” in repair and regeneration of the injured nervous system [194, 196]. Science is still far from grafting the neurotrophic map in thymic development. However, progress is underway, which offers the steps necessary towards a better understanding of the definitive roles NTs may play.

Rather than acting in a singular fashion, NGF and cytokines appear to follow a concerted path so as to achieve an array of responses necessary to preserve homeostatic integrity. Cytokines, which are normally present in tissues in extremely low amounts, become more concentrated following traumatic or inflammatory insults. Furthermore, the limiting amounts of NGF in tissue also greatly increase after acute or chronic inflammatory insults. Inflammation-induced increases in NGF might cause this factor to act like a cytokine to modify mast-cell, macrophage and B-/T-cell functions. Crosstalk between the immune and nervous system is also operative during stressful and anxiogenic conditions [142, 177]. NGF and cytokines such as IL-6, can act as mediators of these interactions, in which each molecule maintains its specific functional activity and cellular selectivity while participating as a component of a more general defence program capable of recruiting and integrating endocrine system actions [177, 216]. Acute or chronic dysfunction of this crosstalk, whether due to injury, inflammation, stress or anxiety, can upset the delicate equilibrium regulating intersystemic communication, potentially provoking or exacerbating immune-mediated pathological processes, such as MS [26, 142, 177, 215-217]. Signaling cross-talk is the blue-print towards cellular phenotypic achievement. Serine-threonine kinase receptors are one such class of signaling receptors, which are prudent in marrow stromal cell signaling. The transforming growth factor (TGF) family, which includes TGF- β and BMPs (bone morphogenetic proteins), exerts its effects *via* the Smad signaling pathways and also *via* activating the ras/MAPK pathways [163, 218]. The ras/MAPK signaling route is often one of the main channels through which NGF appears to potentiate its effects. NGF has been shown to increase the cytoplasmic concentration of calmodulin. Calmodulin in turn, *via* its association with

released Ca^{2+} , interacts with Ca^{2+} /calmodulin-dependent protein kinases (CamK), which inhibit the Smad signaling pathways, tipping the balance towards proliferation [219, 220]. CamKIV, which is present in the bone marrow, upregulates BDNF by a CREB (cAMP responsive element-binding protein)-dependent mechanism [221]. Therefore, this could be the beginning of a discovery towards signaling cross-talk between serine-threonine receptors and tyrosine kinase receptors. Cutting the gordian-knot in signaling events is therefore still a virgin endeavor.

Data have suggested that NGF might be viewed as a $\text{T}_{\text{H}2}$ -like cytokine with a regulative role in inflammation, T-cell-dependent tissue remodeling and neuro-regeneration. It also might represent a clinical marker of inflammation [129, 136, 197]. A clearer understanding of the specific roles of TH cells, NGF and the other NTs in this context will lead to new opportunities for pharmacological modulation of neuroimmune dysfunctions and, most likely, such information may yet promote the design of protocols for the induction of allospecific tolerance in transplantation therapy and antigen-specific tolerance in autoimmune diseases in human being [177, 217, 222].

Therefore, the above discussion highlighting the temporal release and expression of NTs and their specific receptors cannot be pushed aside as mere artifacts but in fact, represents important physiological processes which are, however, still partially obscure. Nevertheless, the current research on NT modulation on early hematopoiesis cannot be ignored and deserves further attention and investigation.

NEUROTROPHINS IN INFLAMMATION AND DISEASE

The role of NTs in the development of the immune system has been extensively reviewed till this point. As immune cells acquire competence and start to engage in active responses, sometimes these same inflammatory mediators give rise to pathological conditions. Since NTs are released by the active immune cell, the remaining sections of this review shall focus on selected inflammatory processes and how NTs fit in. As models, two ailments will be used as a foundation to understand the newly discovered roles of NTs in inflammation: allergy and diabetic nephropathy. The discussion will then embark on inflammation in the CNS itself and what are the implications that the points raised in this review have on the future of NTs in neurodegeneration.

Neurotrophins in Allergy

Allergic bronchial asthma is characterized by chronic inflammation of the airways, development of airway hyperreactivity (AHR) and recurrent reversible airway obstruction. Asthmatics develop AHR in response to a range of nonspecific, normally harmless stimuli, including cold air, cigarette smoke and other environmental antigens. During an asthma attack, broncho-constriction occurs and the airways become inflamed, clogged and narrowed, causing breathing difficulties. Clinical symptoms of asthma are usually reversible and biphasic, consisting of an early phase response (occurring immediately) and a late phase response (occurs hours or days after allergen challenge) [223].

There is growing evidence that dysfunction of the neuroimmune system is responsible for airway inflammation and AHR in allergic bronchial asthma. In conditions such as asthma, NTs secretion results in increased hypersensitivity of the inflamed bronchial tissues [137, 140, 224-226]. In asthma and allergic rhinitis, part of the inflammatory response is accentuated by the increased secretion of NGF provided by the infiltrating activated lymphocytes in response to chemotaxis and cytokine release by the lung structural cells during an attack [225-227].

As discussed earlier, several immune cells - such as mast cells, lymphocytes, basophils and eosinophils - produce, store and release NGF [131, 136, 174, 184, 194, 228, 229]. Moreover, NGF's receptors are widely expressed in the immune system, thus indicating the potential of responding to this NT through an autocrine mechanism. NTs may mediate the link between airway inflammation and neuronal hyperreactivity in asthma. They can influence the inflammatory response either directly by regulating immune cell functions or indirectly through the modulation of sensory neuropeptide synthesis. These include substance P (SP) and calcitonin gene-related peptide (CGRP). The increased production of proinflammatory neuropeptides results in neurogenic inflammation. Stimulation of the vagal afferent nerve endings in the airway epithelium of the nonadrenergic noncholinergic (NANC) system by allergens and various inflammatory mediators results in an increased release of neuropeptides and tachykinins including SP, neurokinin A (NK-A), NK-B and CGRP. These cause airway smooth muscle restriction, mucus production, edema and release of inflammatory mediators from inflammatory cells. For example, SP has a degranulating effect on eosinophils and can cause histamine release from human mast cells. It also activates monocytes to release inflammatory cytokines, including TNF- β , IL-1, IL-6 and IL-10. These NANC system-associated neuropeptides with immunomodulatory functions all act *via* G-proteincoupled receptors (GPCRs). NGF also induces the production of proinflammatory peptides like CGRP. In fact, NGF influences development, differentiation, chemotaxis and mediator release of inflammatory cells through a complex network influenced by other pro-inflammatory cytokines [230, 233].

During allergic inflammation, NGF enhances IL-3- or IL-5-mediated histamine release from basophils and mast cells. NGF also increases the release of IL-4 and IL-5 (both of which are T_{H2}-cytokines) from basophils and eosinophils, which is essential for the IgE synthesis by B-cells [175, 225,226]. Specific IgE antibodies are first produced in a process called allergen sensitization. First, allergens are taken up, processed and presented by MHC molecules of antigen presenting cells, which are mainly the myeloid dendritic cells in the respiratory mucosa. A fraction of these cells can differentiate into professional antigen presenting cells and migrate into the paracortical T-cell zone of the draining regional lymph nodes. Dendritic cells may then favor the differentiation of naïve T_{H0}-cells into T_{H2}-cells, which secrete IL-4, IL-5, IL-10 and IL-13 [223, 234, 235]. These secreted cytokines contribute to pulmonary inflammation and AHR by inducing the activation of submucosal mast cells in the lower airways, leading to bronchial smooth muscle constriction and increased secretion of mucus and fluid [223]. Cytokines also induce B-cells to undergo class switching from IgM to IgE, IgG2 or IgG4 [188, 189,235].

The first signal to induce the B-cells to undergo class switching to IgE production is provided by the T_{H2}-cytokines IL-4 and IL-13, which can interact with the receptors on the surface of B-cells and cause a phosphorylation of the transcriptional regulator STAT6 via activation of the Janus family tyrosine kinases JAK1 and JAK3 [190, 191]. The second signal is the costimulatory interaction between CD40L on the T-cell surface with CD40 on the B-cell surface [192, 193]. These lead to the development of (Type-I) hypersensitivity reaction in asthma. Basophils and activated eosinophils express the IgE antigen receptor FcεRI and can further amplify the IgE response. Allergen cross-linking of the IgE molecules bound to the constitutively expressed high affinity IgE receptor FcεRI on the surface of mast cells causes mast cell activation and degranulation[236]. This is associated with an increase in airway smooth muscle tone and NGF secretion by the mast cells. In fact, mast cells play a major role in the asthmatic early phase response. Mediators such as histamine, prostaglandins and leukotrienes cause inflammatory responses in the body whilst IL-4 drives immunoglobulin class switching and IgE production by B-cells [188-191, 234, 235]. Proinflammatory cytokines like IL-1β and TNF-α are elevated in the airways of asthmatics and they stimulate the allergendependent augmentation in the production and secretion of NGF by the lung structural and inflammatory cells. NGF mediates inflammation in an autocrine mechanism and induces the migration and activation of inflammatory and structural cells in the bronchial mucosa. Specifically, NGF stimulates the survival, degranulation and differentiation of mast cells and induces release of inflammatory mediators such as histamine from these cells. NGF is also responsible for the proliferation and differentiation of B- and Tlymphocytes [131, 136, 137, 176, 195, 225]. IL-4 and IL-5 may also play a role in initiating the late phase response [175, 191, 235]. IL-5 produced by T_{H2}-cells is responsible for the development, differentiation and recruitment of eosinophils in the late phase response. Eosinophils generate mediators that contribute to inflammation of the airways in (Type IV) chronic asthma patients. These mediators damage the extra-cellular matrix, epithelium, neurons and inflammatory cells of the airway, stimulate the degranulation of mast cells and basophils and also cause bronchoconstriction [223, 228, 229, 236].

Particularly impressive is the effect of NGF on basophil mediator release. Pre-exposure of mature human basophils to NGF enhances vasoactive mediator release and primes the cells for the formation of large amounts of leukotriene C4 (LTC4) upon stimulation with IgE-independent (and dependent) agonists such as complement factor C5a that by themselves do not promote lipid mediator formation (Fig. 8) [174, 175]. LTs are inflammatory mediators, which exert their effects through GPCRs. GPCRs have been noted to be tied in with NT-Trk receptor signaling also in neurons [87]. Thus, in turn, elevated LT concentrations may lead to an upregulation in NT levels in lymphoid tissues [205].

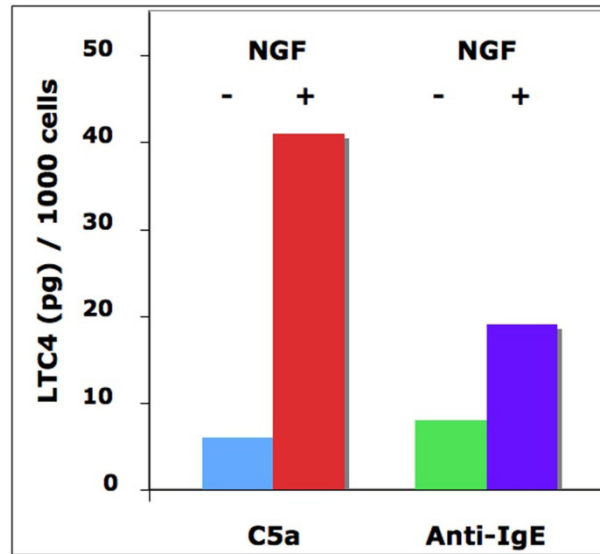


Fig. (8). Basophil priming by NGF: Effect of NGF on basophil mediator release induced by different IgE-dependent and IgE-independent agonists. Cells were pretreated for 10 min with control-buffer (- NGF) and 10 ng/ml NGF (+ NGF), respectively, and then stimulated for 20 min with complement factor C5a (10^{-8} M), or anti-FcεRI monoclonal antibody (29C6; 100 ng/ml), respectively. Leukotriene-C4 was measured in the supernatant according to [174].

Another aspect of asthma and allergy that involves NTs is recurrent reversible airway obstruction. In fact, subsequent response to the same agent causes a biphasic, reversible airway obstruction referred to as the early and late response. The early phase response was mentioned earlier and involves mast cells. Late phase response is characterized by airway narrowing and an influx of NTs, eosinophils and lymphocytes from the blood into the lung parenchyma and airway epithelium [223]. Bronchoconstriction and tissue damage are consequences of mediator release from eosinophils [228, 229]. NGF action on p75NTR seems to potently regulate this allergic late phase response and eosinophils accumulation. There is also evidence that BDNF, NT-3 and NT-4 have a functional role in eosinophil viability and activation [228, 229]. NGF is suggested to play a major role in this bronchial remodeling. It stimulates the contraction and migration of pulmonary fibroblasts as well as their differentiation into myofibroblasts. NGF also induces the release of pro-fibrotic factors, including TGF-β and fibroblast-derived growth factor (FGF)-2 and of cytokines involved in the remodeling mechanisms, including IL-4 from eosinophils and IL-13 from basophils. NGF also stimulates proliferation of bronchial smooth muscle cells and might thus be involved in the smooth muscle hypertrophy observed in asthma [224-227, 237].

It has also been hypothesized that NTs may be involved in the development of eosinophilia and in the activation of these cells [224-229]. Predominantly in allergic asthma, the presence of activated eosinophils is a hallmark in disease progression. NGF, which is released by lung fibroblasts, promotes the survival, proliferation and differentiation of eosinophils, therefore augmenting the pathological condition of the allergy [228, 229, 237]. Furthermore, NGF induces innervation of the bronchial airway

tracts as deduced from transgenic mouse models. Sympathetic innervation could potentially sensitize the lung fibroblasts to allergens in this manner and therefore knowing the regulation of NGF in innervation, would be important in understanding the underlying mechanisms involved in initiating allergic asthma [224, 226, 237]. Thus, the unique ability of NGF to regulate the function of basophils and eosinophils indicates an interaction between the nervous system and the effector phase of allergic inflammation and a potential broader modulatory role of NGF in the various inflammatory cells involved in the allergic inflammatory network, which is reflected by increased NGF serum levels in allergic diseases [174, 227-229, 237-239]. Finally, data suggest that NGF - which is increased in biological fluids of several allergic, immune and inflammatory diseases - might also be viewed as a T_{H2}-like cytokine with a regulative role in allergic inflammation and tissue remodeling. From a clinical point of view, it is of interest that patients with allergic rhinitis and asthma show increased concentrations of NTs in nasal and bronchoalveolar lavage fluids (N/BALF) as well as in their sera indicating the involvement of NTs in the neuro-immune cross-talk as discussed earlier [227, 238, 239]. In addition, neurotrophic factor levels correlate with the severity of the asthma manifestation thus, pointing to another example where NTs are being discussed as potential biomarkers used for the diagnosis of inflammatory diseases [136, 137, 176, 217, 225-229, 237-239].

In addition, increased levels of NGF in the circulation have been reported in systemic lupus erythematosus [238]. Interestingly, NGF also accumulates in the tissues of systemic sclerosis patients and increases in the synovia of patients with chronic autoimmune arthritis [239, 240]. The mechanism that leads to the increase of NGF is not known. One hypothesis is that NGF levels may be regulated by autocrine mechanisms. The increased levels of NGF found in the sera and fluids of patients affected by autoimmune diseases could be explained as an increased production of NGF by immunocompetent B- and/or T-cells. Thus, the activation of these cells after their interaction with antigens and/or after cytokine stimulations could be the cause of this increased production.

NGF and Diabetic Nephropathy

Diabetes mellitus is a global health problem of steadily increasing proportions, with approximately 95% of patients being affected by the type 2 form of the disease. Diabetic nephropathy is one of the most common complications in this disease and has become the main cause of renal failure, but unfortunately, the intimate mechanisms leading to the development and progression of renal injury are not yet fully known [241]. Peripheral neuropathy and specifically distal peripheral neuropathy (DPN), are other frequent complications of this disease [242]. Multiple pathogenic mechanisms are now believed to contribute to this disease and different inflammatory molecules, including chemokines, adhesion molecules, and proinflammatory cytokines, may be critical factors in the development of microvascular diabetic complications, including nephropathy.

Inflammatory mediators found at sites of injury such as IL-1, IL-6, TNF- α and TGF- β have been implicated in inducing NGF synthesis in peripheral organs including mesangial cells [243-244]. A cytokine-NT cascade could therefore be involved in inflammatory processes associated with tissue repair and fibrosis [217, 245]. The NGF

system has been reported to critically participate in kidney development [246]. The p75NTR is expressed during later stages of glomerulogenesis where it is limited to the mesangium. It persists at lower level in adults in glomeruli and a subpopulation of renal interstitial cells. The fact that these receptors are upregulated during inflammatory kidney disease and diabetic nephropathy suggests a pathophysiological role for NTs [247]. An important question is the involvement and specific role of NTs and their receptors in diabetic nephropathy. During kidney inflammation glomeruli have been reported to release chemotactic factors, eicosanoids, nitric oxide, oxygen radicals, growth factors and inflammatory cytokines such as platelet-derived growth factor (PDGF)- β , TGF- β , FGF-2, IL-1 β and TNF- α [248, 249]. These mediators released by invading immune cells and glomerular mesangial cells may further amplify local inflammatory cell functions whereby the mesangial cells play a key role in glomerular inflammatory disease [248]. Interestingly, the inflammatory cytokines IL-1 β and TNF- α elicit a strong increase in NGF expression in these cells, implicating that NGF may also have a pathophysiological role in glomerular diseases [244, 248]. The fact that high glucose levels, comparable to plasma glucose levels found in diabetic patients, up-regulates the NGF-receptor system in mesangial cells, points to an involvement of a cytokine-NGF cascade in diabetic nephropathy [250]. With respect to the role of NGF in human diabetic complications such as diabetic nephropathy [247] or neuropathy [251, 252], it remains to be elucidated whether mesangial cell-derived NGF also acts in the kidney as a proinflammatory cytokine or is a part of a protective defense system (Fig. 9).

Albeit the numerous evidences which define NTs as important in modulating the activities of immune cells – how this is tied in with physiological function is still a blurry avenue. Nevertheless, activated immune cells also have an important role to play in the CNS where a physiological relevance underscores their possible therapeutical intervention.

NEUROTROPHINS AND THE BRAIN IMMUNE SYSTEM

Microglia are the principal immune cells located in the CNS. The functional characteristics of these cells have received increasing attention as they have been found to be the major source of brain immune mediators [253]. Microglial cells, the resident monocytic cells of the brain, are generally considered to be immunologically quiescent under normal, non-pathological conditions. In response to CNS injury or infections, the conversion of the resting, ramified microglia into active microglia occurs in a progressive fashion accompanied by different morphological states, described as activated and reactive microglia, respectively. Microglia are involved in the pathogenesis of diverse neurodegenerative diseases such as AD, PD, prion diseases as well as MS, ALS and AIDS dementia complex. It is widely accepted that microglia contribute to the neurodegeneration through a release of a variety of proinflammatory substances. In fact, they are not the only cells which contribute to immunological processes inside the nervous system. The CNS is composed of different cell populations that respond differentially to pathological factors and influence each other and modulate their reactions. The inflammatory microglial response to CNS damage is characterized not

only by the release of factors that mediate neurotoxicity, but also molecules such as NTs that promote tissue repair and neuronal regeneration [254, 255].

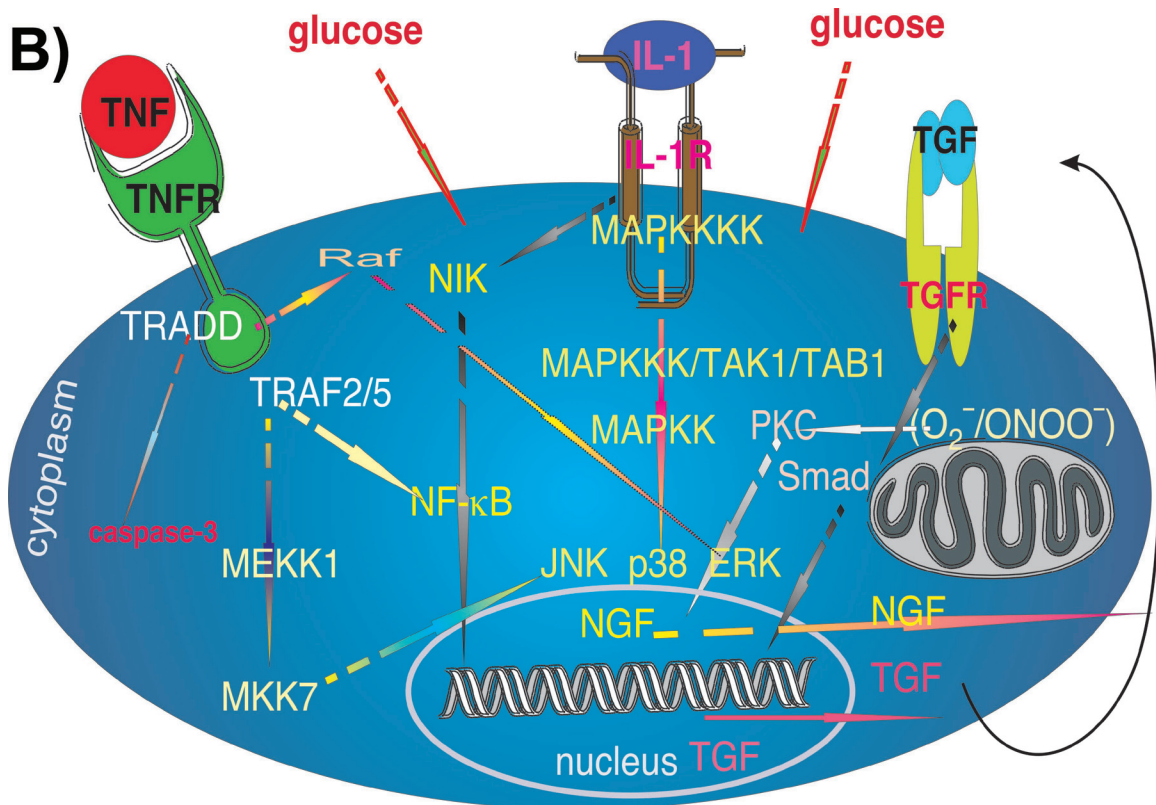
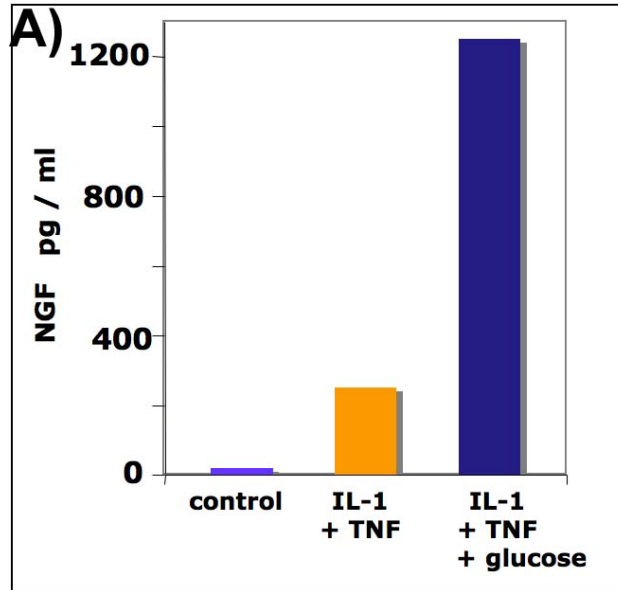


Fig. (9). Effect of high glucose on cytokine-induced NGF expression in renal mesangial cells. A) Schematic illustration that high glucose (4.5 g/L) enhances the pro-inflammatory cytokine-induced (IL-1 β and TNF- α) NGF release. B) The finding that IL-1 β and TNF- α elicit a marked increase in NGF expression implicates that this NT is involved in the pathophysiology of glomerular diseases. The fact that high glucose upregulates the NGF system in these cells points to a critical role of a cytokine-NGF cascade in diabetic nephropathy. Probably oxidative stress, NF- κ B and PKC activation are involved in this effect. In addition, TGF- β is probably one of several players responsible for the immense enhancing effect of high glucose on cytokine-stimulated NGF release in these cells. Cytokine- and glucose-induced superoxide (O $_2^-$ /ONOO $^-$) production followed by an oxidant-mediated PKC activation, may be the main signaling pathway that leads to enhanced NGF expression by mesangial cells (according to [250]).

Brain microglial cells secrete NGF, modulated by the NF- κ B signaling molecule, which has been known to act on several genes for pro-inflammation [184, 256]. IL-1 β , TNF- α and the complement protein C3a stimulate NGF mRNA transcription and protein release from microglia via a NF- κ B-dependent and -independent mechanism [257]. In fact, though the CNS is viewed as a immune-privileged site most of the time, prohibiting the infiltration of immune cells, recent insights in cell culture work and transgenic mouse models have proven that this may not be necessarily so at all times. The barrier system is a result of a dynamic, non-static molecular interaction, which changes to various physiological responses and this as we shall see now offers a vast contributory role of immune cells in modulating brain tissue dynamics, especially in proliferation and regeneration.

The brain parenchyma is an active site for the biological activity of the NTs and exhibits a reduction in neurotrophic factors, either due to age or other factors, which serves as a trigger point in initiating severe diseases such as AD, PD, ALS and MS [215, 258]. Therefore, it is not too hard to fathom the chaotic environment that neuronal cells encounter when this depletion of essential trophic factors or failure of nerve cells to respond to trophic factors is coupled with a localized and concentrated build-up of cytokines, activated microglia and migrant T-cells, effecting the pathogenesis in AD, MS and cancer [253, 259, 260]. NTs are survival factors and their subsequent withdrawal from the local CNS environment could spark off an apoptotic regime and subsequent inflammatory responses leading to the pathological lesions that we observe in conditions such as MS. Therefore, any restorative effort, to bring the reduced levels of NTs to normal, should show some capability in guiding at least the surviving neurons away from cell death. Glatiramer acetate (GA) is a collection of synthetic polypeptides indicated as a therapy for relapsing-remitting MS. As an example, glatiramer-activated T-cells not only mediate their protective and neurogenerative effects in MS by shifting the immunological wave-frequency from T $_{H1}$ - to T $_{H2}$ -type, but by also possibly providing a source of BDNF to damaged and immunologically privileged CNS areas. Both preclinical and clinical studies have demonstrated that peripheral GA administration can enhance central BDNF activity or increase serum BDNF levels [215, 261].

It is also crucial in many instances to gauge the severity and progression of a particular ailment in order to evaluate and screen for suitable treatments. This tracking of progression and remission is especially important in cancer. Recently it has been noted that TrkA and TrkB, the principle tyrosine kinase receptors for NGF and BDNF

respectively, serve as molecular prognostic markers in neuroblastoma cancer prognosis. If this is the case and with other research showing that modulation of Trk receptors is involved in cancer progression, then maybe there might be a possible link between immunological failure and Trk expression during oncogenesis [262].

Thus, as it has been gleaned insofar from the few examples cited, it is quite obvious that the potential influences of NTs and their receptors echo vastly across the immune surveillance system. If this is indeed true and significant, then it would be a worthwhile endeavor to shift focus on ways to compensate or supplement these deficiencies.

The CNS has a relatively low NTs expression. Activated T-cells cross the BBB with much ease. Thus, the compounded effect of increased inflammation allowing lymphocyte entry and the infiltration of NT-rich lymphocytes into the brain parenchyma, could probably lead to a synergistic effort by the body to restore cell viability in damaged neurons. After mechanical CNS nerve injury, artificially injecting myelin basic protein (MBP)-reactive T-cells promotes neuronal repair where the infiltrating T-cells provide a rich source of NTs [24, 214]. In contrast, another study showed that reactive CD4⁺ T-lymphocytes release rather elevated levels of pro-inflammatory cytokines but not NTs and thus impair neuro-regeneration and any possible neuroprotection afforded by myelin-reactive T-cells is likely to be an indirect effect mediated by non-CNS-reactive lymphocytes [216].

Of course the key role in any therapeutic intervention involves a crucial balance between any interacting factors. If any intervention is to be allowed, there must be a balance struck between allowing enough inflammation to activate peripheral T-cells and their subsequent release of NTs and to limit and safeguard the CNS from the potential overflux of activated immune cells, which could aggravate inflammation and nullify the beneficial effects of the released NTs.

Still, the neuroprotective benefits offered are real and vaccination, anti-inflammatory drug treatment, artificial delivery of NTs or NT-releasing immune cells are gaining increased credence as therapeutical avenues in various fields [56, 196, 263]. The production of large-scale recombinant human NGF (rhNGF), which has been shown to have similar potency to the 2.5S native NGF protein, implies far-reaching consequences. When nerve cells get transected there is also considerable loss in vasculature in the proximal regions of injury site. The surrounding vasculature and proper angiogenic factors have to be stimulated as well to promote proper growth and to supply adequate blood to the injured neurons. Infact rhNGF, either alone or associated with 6-hydroxydopamine (6-OHDA) resulted in a significant upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase, neuronal nitric oxide synthase, and VEGF expression in superior cervical ganglion (SCG) neurons. The pleiotropic implications for the possible roles of rhNGF as a regulator of angiogenesis are new and yet a very real prospect [264]. If NGF does indeed show the capacity to upregulate adhesion and migration of endothelial cells via the release of vascular factors then with prudent steps, it is worthwhile to use this for drug development [263]. The development of such a recombinant protein enables the prospective use of this protein as a therapeutic

mediator in human neurodegenerative treatments. However, delivering NGF into the brain in an appropriate manner is still challenging because NGF does not cross the BBB when applied peripherally. Additionally, NGF may cause intolerable side effects (including pain [29]) if administered into the brain ventricular system. Nevertheless, recent experiments show that intranasal administration of NGF rescues recognition memory deficits in an anti-NGF transgenic mouse model, which shows typical features of AD. In addition, a brain sitespecific gene delivery method provides sufficient quantities of NGF to support neuronal survival at restricted sites to avoid adverse side effects. A recent phase-I clinical trial study of NGF gene therapy for AD already provides promising data [265-267].

The above pleiotropic effects of NTs are not just restricted in their participation in restructuring the angiogenic system. Aging is often accompanied by various onslaughts including inflammation and vascular clogging and reduced tissue repairing capabilities. This stresses the physiological homeostatic balance, thus leading to age-related increases in glucocorticoid (GC) levels in the blood. GC increases in aging individuals lead to neuronal damage mediated during hypoglycemia and hypoxia-ischemia, which then stimulates a counterbalancing neurotrophic release of NGF and BDNF [268]. Stress levels have been shown to increase BDNF levels in the paraventricular nucleus (PVN) of the hypothalamus and pituitary glands. NGF has been shown to be released during stress periods where the hypothalamic-pituitary-adrenal (HPA) axis is activated, which in turn leads to GC blood serum level increases [142]. The effects of NTs appear to be a direct activation on the HPA axis. If the body naturally does produce NTs release during stress, it is also possible that the above means of therapy can be used to improve the quality of life in older persons, as it can supplement endogenous levels of NGF already being released.

However, this pleiotrophy more importantly means that any form of therapy has to be carefully modulated and studied, as an over-dosage or improper administration of the drug can actually lead to aberrant activation of stress centers and behaviorally unnecessary hormonal release.

CONCLUSION

NTs are powerful peptides, which have profound influences not just on the nervous system but as well as on the immune system. Changes in stress levels can stimulate NT release and their circulating levels in the blood and thus may most definitely have consequences on circulating immune cells. This might possibly be a naturally occurring physiological response that is now only being understood and could be a potential means of intervention during stressful conditions such as in ageing or traumatic pathological conditions.

NTs influence the immune system in all stages of development and similar processes are depressed during pathological states. If proper intervention is to be mediated, the roles that NTs play during hematopoiesis have to be carefully delineated (Fig. 10).

Furthermore, the brain does allow active infiltration of leukocytes, which provide biologically active NTs to be delivered near damage sites.

It is still too early and most probably not advisable to use these studies on animal models and cell cultures to presume that lymphocytes actively cultured to release NTs could serve as a therapy in neurodegenerative diseases such as AD, PD or MS. However, in the light of upcoming recombinant technologies, which have enabled quantitatively valuable production of rhNGF, these models could be important in helping us to understand at least how NTs' effects are mediated in the context of an inflammatory condition.

This understanding of NTs and Trk receptors and the manner in which they co-modulate and shape the immune system are paramount to setting the understructure, necessary for clinically relevant progression. Together with deeper understanding of the pleiotropic effects of these ligandreceptors the bridge between laboratory studies and clinical testing will hopefully draw closer to be able to use emerging technology driven derivatives of recombinant NTs in drug treatment.

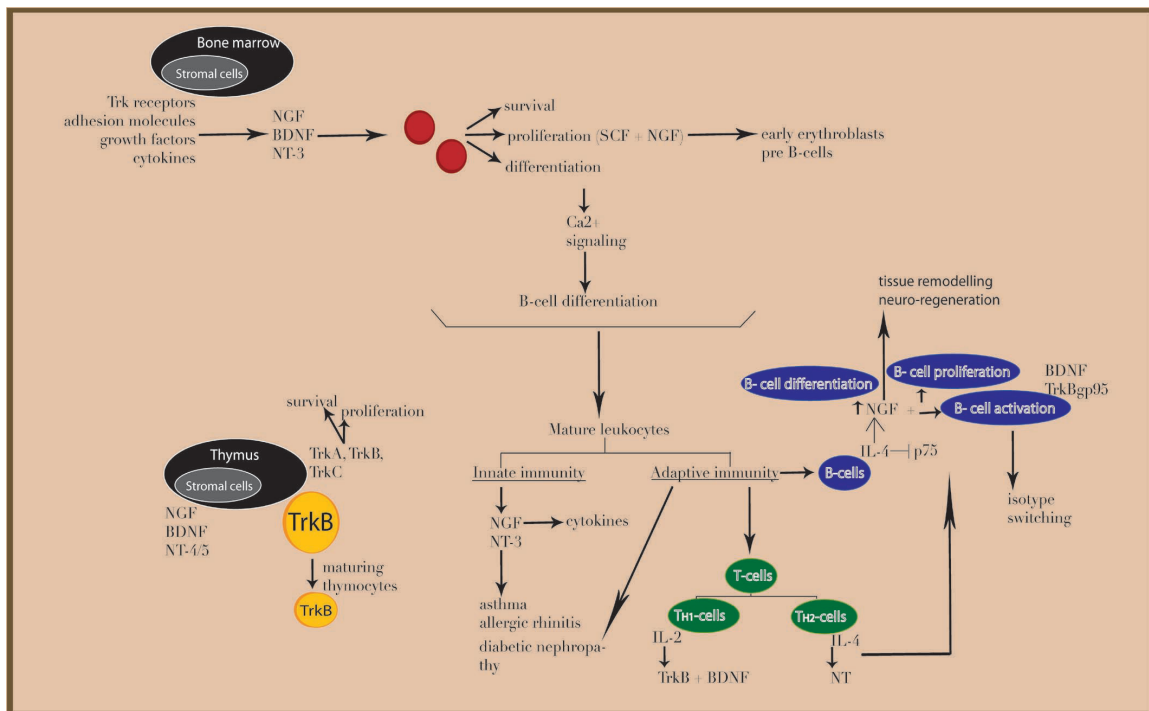


Fig. (10). Summary of NT-mediated immune regulation. The diagram above summarizes the main developmental and regulatory effects of NTs on cells of the immune system. Thymic and bone marrow stromal cells release neurotrophic factors, which go on to affect the proliferation, survival and differentiation of HSCs into specific immune cell lineages. NTs also partake in active immunity and work in concert with various cytokines to ensure that immune cells are properly primed for activation. Their relative influences on the immune cells and their release from these immune cells have wider implications in inflammation and neuronal repair.

ACKNOWLEDGEMENT

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ABBREVIATIONS

AD = Alzheimer's disease
BBB = Blood-brain barrier
BDNF = Brain-derived neurotrophic factor
BMSC = Bone marrow stromal cells
CNS = Central nervous system
CREB = cAMP responsive element-binding protein
CSF = Cerebrospinal fluid
CStF = Colony-stimulating factor
EAE = Experimental autoimmune encephalitis
FGF = Fibroblast growth factor
GC = Glucocorticoid
HPA = Hypothalamic-pituitary-adrenal
HSC = Hematopoietic stem cells
Ig = Immunoglobulin
IL = Interleukin
LT = Leukotrienes
MS = Multiple sclerosis
NADPH = Nicotinamide adenine dinucleotide phosphate
NF-kB = Nuclear factor-kappaB
NGF = Nerve growth factor
NT = Neurotrophin
PD = Parkinson's disease
PDGF = Platelet-derived growth factor
PI3K = Phosphatidylinositol-3 kinase
PKC = Protein kinase C
PNS = Peripheral nervous system
PVN = Paraventricular nucleus
SCF = Stem cell factor
sIg = Surface immunoglobulin
TGF = Transforming growth factor
Trk = Tropomyosin-related kinase
TNF = Tumor necrosis factor
VEGFR = Vascular endothelial growth factor receptor

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