Chapter X

Immunization with Neural-Derived Peptides as a Potential Therapy in Neurodegenerative Diseases

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1. Introduction

There is a nosological dilemma when it comes to classifying what comprises a neurodegenerative disease (NDD). Degeneration – purely speaking – is to go from a higher to a lower level of functioning; it is deterioration from normalcy. Neurons are the functional elements of the nervous system. Then degeneration of the nervous system consists of a decrease or loss in the function of neurons. Not necessarily an atrophy, which consists of the death of a particular population of neurons. Clinically, NDD are comprised of progressive dementias, progressive ataxias, disorders in posture and movement, muscle weakness, and progressive blindness. The common characteristic in all of these pathologies is their chronicity. Each and every one of the aforementioned diseases consists of a chronic progression towards the loss of a particular function. However, this definition does not include a limit on temporality. Nosologically speaking neurodegeneration could include several other pathologies from an acute time frame. NDD can further be divided into an acute and chronic classification. Chronic diseases such as: amyotrophic lateral sclerosis (ALS), Alzheimer disease (AD) and Parkinson disease (PD) were the common conception of NDD. The latter was sustained until acute traumatic injuries to the central nervous system (CNS) were found to cause generalized inflammation and other phenomena that lead to degeneration. Examples of CNS injury that cause this secondary degeneration are: global or focal cerebral ischemia (stroke), spinal cord injury (SCI), and traumatic brain injury (TBI). The similarities in neurodegenerative processes between these and chronic NDD allows us to classify them within acute NDD. Neurodegeneration previously consisted of progressive atrophic disorders but has now expanded into the study of all pathophysiological processes that deteriorate the CNS. As a whole, NDD are the cause of many deaths around the world. In the US, stroke, traumatic injuries (such as: SCI and TBI), AD, and PD occupy the top 15 causes of mortality, averaging 350,000 deaths per year (Xu et al., 2007). Although NDD have an elevated mortality their greatest impact is on morbidity, affecting 50 million Americans each year and generating a large amount of federal spending (Brown et al., 2005). Every year $144 billion USD are spent on AD alone, and that is excluding the spending required for the other 600 neurological disorders that have been described (Alzheimer's Association, 2010; Meek et al., 1998). The elevated prevalence and incidence require a large initiative to research the hallmarks of these diseases. Until now, our understanding of NDD is quite complex but there is still a lot to uncover. Research is normally directed to the NDD with the most impact on society such as: ALS, AD and PD. Due to the increased availability of information on the previous diseases this chapter will only discuss these diseases within the chronic NDD section. In order to find treatment opportunities for each one of these diseases we must first understand the basic pathophysiology. ALS is a progressive degeneration of upper and lower motor neurons in the brain and spinal cord. This atrophy eliminates the brain's control over muscle movements and causes them to weaken and become paralyzed. Progressive muscular paralysis causes the inability to move, swallow, and eventually, breathe (Angelov et al., 2003). AD is a progressive disorder characterized by memory loss and severe cognitive decline. This degeneration is caused by excessive accumulations of extracellular amyloid beta peptide, which forms plaques in the hippocampus and cerebral cortex, leading to neuronal death (Frenkel et al., 2005; Butovsky et al., 2006). PD is a chronic progressive disease characterized by motor symptoms (tremor, rigidity and bradykinesia) and nonmotor symptoms (e.g. autonomic, mood and cognitive). These clinical hallmarks are attributed to the degeneration of nigrostraital dopaminergic neurons and other structures in the brainstem, cortex, and subcortex (Laurie et al., 2007). MS is considered to be an inflammatory autoimmune CNS demyelinating disease that is thought to be perpetrated by myelin-reactive lymphocytes. Demyelination of the CNS causes the loss of function of the affected tract (Stuve et al., 2006). MS is considered an autoimmune disease and not a NDD because there is no direct neuronal death only demyelination. The nosology of NDD excludes MS from our study but it still shares very similar immune pathophysiology and most of the therapies mentioned are derived or designed for use in MS. The inflammatory component of acute injury to the CNS provided new insight into the autoimmune response propagated after a CNS insult. These findings gave immune cells a crucial role in the protection and regeneration of the injured CNS, as well as a role in chronic progressive NDD. Further insight into the immunological component of neurodegenerative diseases provides us with new mechanisms where we are able to intervene in order to resolve these disorders. One of these mechanisms is protective autoimmunity (PA). PA is a new concept where autoreactive mechanisms are being modulated in order to promote neuroprotection. Dr. Michal Schwartz from the Weizmann Institute of Science in Israel originally conceived this concept. Infiltration of immune cells after CNS injury was traditionally regarded as pathological. This view was based on the fact that immune cell-infiltration has been exclusively identified with inflammation, and that inflammation is generally harmful to the injured CNS. However, recent studies indicate that a well-controlled innate and adaptive immune response is essential for the repair of the injured tissue. These results brought about research into immunomodulatory therapies in several NDD. In acute NDD and MS, recent findings have suggested that the inflammatory response is strongly modulated by an autoimmune reaction directed against neural constituents, specifically against myelin basic protein (MBP), one of the most abundant and immunogenic proteins in the CNS (Butovsky et al., 2001; Ibarra et al., 2003; Popovich et al., 1996; Sospedra et al., 2005). Dr. Schwartz started to modulate the action of myelin-specific autoreactive lymphocytes by immunizing with MBP. This strategy improved tissue preservation, neuronal survival and motor recovery after acute SCI (Hauben et al., 2000a; Hauben et al., 2000b). PA also proved to be a T cell-dependent response that is genetically determined (Kipnis et al., 2001) and triggered as a physiological response to CNS trauma (Yoles et al., 2001). However, immunizing animals with self-antigens (i.e. MBP) induced an autoimmune disease known as experimental autoimmune encephalomyelitis (EAE, animal model of MS). Therefore, a different way of eliciting PA had to be obtained in order to prevent this complication. Studies suggested that immunizing with a weaker version of the self-antigen could solve the problem, these type of antigens became known as altered peptide ligands (APL). Vaccinating with APL would generate PA without degenerative autoimmunity. In the study of NDD, APL were derived from neural constituents and were therefore coined under the term neural-derived peptides (NDP). The success in the development of these immunomodulatory peptides has inspired a lot of research into their possible therapeutic applications in both chronic and acute NDD. These applications will be described in detail throughout this chapter.

2. Role of Immune Cells and their Potential Therapeutic Effect

The CNS has long been considered to be an immunologically privileged location. The blood-brain barrier (BBB) was thought to maintain blood-borne cells of both the innate and adaptive immune system out of the CNS. This hypothesis assumed that microglia were the only innate immune cells of the CNS. During damage, microglia became activated and functioned as destructive inflammatory cells indistinguishable from infiltrating macrophages. Immune cells were thought to contribute to the increase in tissue damage during CNS disease (Bethea et al., 1998; Blight et al. 1992; Dusart et al., 1994; Popovich et al., 1997). The idea was supported by the following: i) CNS trauma activates T lymphocytes against neural constituents, and ii) the passive transfer of myelin autoreactive T cells caused EAE in previously healthy rats (Popovich et al., 1996). The notion was sustained in such a way that the complete inhibition of these responses was proposed as a potential therapeutic intervention, and remains to this day as the predominant clinical approach (Lopez-Vales et al., 2005; Popovich et al., 1999). However, it is now clear that these cells have a pivotal role in CNS repair (Hammarberg et al., 2000; Hashimoto et al., 2007; Hendrix et al., 2007; Moalem et al., 1999; Rapalino et al., 1998; Turrin et al., 2006; Yin et al., 2003). In the healthy CNS the microglia is in a resting state where its morphology consists of a small cell soma and numerous branching processes, known as resting/ramified state. The ramifications are dynamic structures that enable the cell to sample and monitor its microenvironment (Nimmerjahn et al., 2005; Raivich et al., 2005). Resting microglia express CD45 (leukocyte common antigen), CD14, and CD11b/CD18 (Kreutzberg et al., 1996). Under duress, microglial expression patterns are modified from a monitoring role to one of protection and repair. Microglial begin to express key surface receptor such as: CD1, lymphocyte function-associated antigen 1 (LFA-1), intracellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1). Besides changing their surface receptor repertoire they begin to secrete: inflammatory cytokines such as TNFα and interleukins IL-1β and IL-6, chemokines like macrophage inflammatory protein (MIP-1α), monocyte chemoattractant protein (MCP-1), and interferon inducible protein 10 (IP-10). This change in microenvironment changes the resting/ramified state of the microglia into an amoeboid/phagocytic state. The activated state of microglia has beneficial functions during NDD such as: scavenging neurotoxins, removing cellular debris, and the secretion of trophic factors that promote neuronal survival (Frank-Cannon et al. 2009). During CNS injury, if microglia come in contact with products of the adaptive immune response such as interferon gamma (IFN-γ) and IL-4 it will acquire a phenotype that has antigen presenting cell (APC)-like qualities. This phenotype expresses major histocompatibility complex II (MHC-II) and B 7.2 receptors, giving it the ability to interact with elements of the adaptive immune response. As an APC, microglia can hold dialog with T cells and are capable of releasing neurotrophic factors (BDNF, NT-3, NGF) and scavenging toxic neurotransmitters and reactive oxygen species (ROS) that endanger the tissue (Li et al., 2007; Schwartz et al., 2003). However, the chronic and uncontrolled activation of microglia increases the permeability of the BBB and elevates the amount of infiltrating blood-borne immune cells (Schmid et al., 2009). This promotes the activation of microglial cells into a destructive phenotype characterized by the production of high levels of nitric oxide (NO, a potent free radical), as well as TNFα, and cyclooxygenase 2 (COX2) (Franciosi et al., 2005; Lee et al., 2007; Shaked et al., 2004). In this phenotype microglia express low amounts of MHC-II and are thus incapable of communicating with the adaptive immune system, an important condition to promote neuroprotection (Schwartz et al., 2003; Shaked et al., 2004). In addition, T lymphocytes are recruited in small amounts and very late. The lack of T cell-mediated activation of microglia results in an uncoordinated release of additional pro-inflammatory cytokines, exacerbating the damage (Bethea et al., 1999; Lopez-Vales et al., 2006; Pan et al., 2003; Resnick et al., 1998; Schwartz et al., 2003; Vanegas et al., 2001;). The best way to elicit a T cell-mediated activation of microglial cells is through neural autoreactive T cells. This assures that T cells arrive to the CNS and activate microglia into their protective phenotype propagating the beneficial effects mentioned above. PA has proven to yield clinical improvements in the treatment of several NDD.

3. Modulation of the Immune Response using Neural-Derived Peptides

Immunomodulation is an idea from the past that looks more promising than ever. It is a change in the body's normal physiological immune response to a specific antigen. This modulation changes the way the immune system would normally respond to an event and replaces it with an alternate desired response. The modification of immune responses is different from agents that suppress the immune response (such as corticosteroids). Immunomodulation has already become a reality. For example, IFN-γ is used in patients with chronic granulomatous disease (Farhoudi et al., 2003), IFN-β is used in patients with multiple sclerosis (Kumpfel et al., 2007), and IL-2 in patients with AIDS and metastatic melanoma (Davey et al., 1997; Terando et al., 2003). Aside from this, numerous vaccines use adjuvants to achieve the desired immune response (Partidos et al., 2004; Petrovsky et al., 2004). Modulation of the immune response as a therapeutic strategy is a promising alternative for several diseases. PA allows us to speculate that it is better to modulate the immune response rather than eliminating it. In chronic NDD, patients require a competent immune response to fend off pathogens and evade complications due to infections. The ablation of the immune response is usually done with steroids or immunosuppressants, which severely affect the patient's ability to initiate an adequate immune response. In the acute form of NDD the immune system is vital in the return to homeostasis. Immune cells extract cellular debris, reestablish blood flow, secrete neurotrophic factors and eliminate pathogens. All these beneficial effects are lost when the immune response is inhibited using immunosuppressant therapy. Accordingly, it seems only logical that the immune response is essential in NDD. In line with this, it is realistic to envision that the harmful effects exerted by immune cells could be reverted or changed to promote beneficial actions. In order to achieve this goal, it is crucial to avoid or at least diminish the activation of microglial cells by means of the classic pathway (destructive phenotype). For this purpose, an earlier and larger arrival of T cells to the site of injury should be promoted. The opportune and adequate arrival of these cells will favor the activation of microglia under the bases of a protective phenotype (Shaked et al., 2004). A simple way of making this possible is by immunizing with the same antigen that induces the autoreactive response: neural antigens. With this approach, an important number of microglial cells will acquire the protective phenotype and will then release molecules that instead of increasing damage will promote neuroprotection. Thus, we will obtain the benefits and not the detriments of this immune response. The present strategy proposes the modulation of the immunological response by boosting an autoreactive reaction. This could be a bit conflicting for general understanding since it is common to associate autoimmunity with disease. However, at present, it is very clear that autoimmunity is a physiological phenomenon perfectly compatible with homeostasis (Schwartz et al., 2000). Furthermore, autoimmunity has been proposed as a useful and beneficial event (Hauben et al., 2005). Therefore, PA can be proposed as a protective strategy where autoimmunity is the main player in providing beneficial effects during CNS injury.

4. Modulation of Protective Autoimmunity with no Risk of Autoimmune Disease

As it was mentioned before, the possibility of inducing an autoimmune disease after vaccination with neural constituents is perhaps the main complication of this therapy. In order to solve this issue, immunizations are done with NDP. NDP are analogs of immunogenic epitopes with one (or a few) substitution(s) at specific amino acid positions of MBP. The variation between the amino acid sequence is essential for contact with the T cell receptor (TCR) during antigen processing. This variation allows them to compete for TCR binding and to interfere with the necessary sequence of events required for T cell activation. The interference caused by NDP in TCR antigen recognition could affect T cell differentiation and induce a state of anergy (Nel et al., 2002). The specificity and avidity of the TCR with its ligand is determined by the primary sequence of the antigenic peptide. That particular sequence affects its binding to the complementary-determining regions of the TCR and the peptide-binding groove of the HLA molecule (Garboczi et al., 1996). A small variation in amino acid sequence can alter its ability to interact with either the MHC-II or TCR receptor molecule. This competition thereby converts an agonist peptide into a partial agonist or even an antagonist (Jameson et al. 1995). Agonist peptides engage in high-affinity interactions with the TCR and induce a robust T cell response; whereas partial agonists or antagonists engage in lower affinity interactions that lead to altered or inhibitory responses (Jameson et al., 1995; Kersh et al., 1996). Stimulation of naïve CD4+ T cells with an agonist peptide induces sufficient assembly of signaling complexes to allow activation of the IL-2 promoter and support a Th1 differentiation pathway. In contrast, the signals generated by APL activation are generally insufficient to induce IL-2 synthesis and therefore will not cause activation. That lack of IL-2 production might induce an anergic state or a skewing of the Th1/Th2 differentiation (Nel et al., 2002). Some APL are already being explored for neurological diseases. These peptides are derived from MBP-encephalitogenic epitopes. A group of them (G91, A96 and A91) have already been tested in animal models (Hauben et al., 2001). Importantly, immunized animals did not present clinical signs of EAE. A91 is a peptide derived from MBP (sequence 87-99), where the lysine residue at position 91 is replaced for alanine. This NDP cross-reacts with the original encephalitogenic epitope of MBP but it activates weak self-reactive T cells thus inducing autoimmunity without developing EAE. Immunizing with A91 inhibits EAE but neither causes anergy nor clonal deletion (Gaur et al. 1997). During antigenic presentation, A91 works as a partial agonist that instead of inducing a Th1 response promotes a Th2 differentiation pathway. This preference for the Th2 phenotype may be responsible for the elimination of the Th1-dependent response observed in EAE. Studies also indicate that post-injury injection of bone marrow-derived dendritic cells pulsed with A91, induce the same significant beneficial effects (Hauben et al., 2003). This indicates that the APC properties of the dendritic cell are enough to activate anti-A91 CD4+ T cells that are responsible for the elevated neuroprotection. To further support the use of immunomodulatory NDP, our laboratory examined the effects of combining immunizations with A91 and methylprednisolone (MP). The use of corticosteroids, such as MP, is the only therapeutic agent currently available for the treatment of a variety of NDD, primarily CNS trauma. In our study, a high dose of MP was administered together with an A91 immunization after SCI. As expected, MP eliminated the beneficial effects of A91. Nevertheless, when vaccination with A91 was delayed for 48 h after injury, there was no interference with its effect by the anti-inflammatory action of MP injected immediately after SCI (Ibarra et al., 2004). This finding suggests that vaccination with A91 is neuroprotective even if administered 48 h after injury, and that the effect of MP over the immune system is transient and does not interfere with later therapy even if that treatment is immune related. These results offer another interesting benefit of NDP-induced PA, and that is the clinical plausibility of these therapies. In the clinical setting, CNS trauma and pathology is diagnosed long after the moment of incidence. NDP-induced PA is functional even when administered 48 h after the development of NDD and works as an adjuvant in traditional clinical treatment protocols (MP administration post-CNS trauma). It appears that the beneficial effect of the vaccination with A91 will not necessarily neutralized by concomitant treatment with MP. It is worth mentioning that one of the most prevailing adverse effects observed after NDP immunization is immediate-type hypersensitivity reactions. This undesirable effect is generally associated with the immune deviation toward Th2 phenotype. These observations should stimulate further research into which patients are most likely to benefit from this therapy. Taking into consideration all of the data, therapeutic vaccination with NDP appears to be a promising strategy that could be adapted for treatment in several NDD.

5. Effect of Immunizing with Neural-Derived Peptides

In the study of neuroprotection, the term autoreactivity is immediately associated with increased cell death, inhibition of neuroprotective mechanisms and a worse clinical outcome after CNS injury. However, our understanding of the immune system's role in the pathological CNS has changed drastically in the last couple of years. The old school of thought indicated that the immune response was responsible for the exacerbation of neurodestructive phenomenon, so the first line of defense was immunosuppression. The recent findings of PA suggested that the immune response was not only needed after an insult to the CNS but it also had a beneficial neuroprotective role in most NDD. This radical change in information forces us to reevaluate the existing treatment protocols for all NDD. If PA is present in a number of CNS diseases then the use of NDP immunizations is a reasonable treatment option. The use of NDP-induced PA results in the generation of a prevalent Th2 phenotype. These cell types have shown to have the most overwhelming neuroprotective effect in the CNS. The influential roles that these cells have on the outcome of disease have made them the goal of therapy development. The increase in Th2-inducing interventions has been studied in ALS, MS, AD, PD, SCI, TBI, and stroke; it has even been proposed as a treatment for neurodevelopmental disorders such as Rett syndrome (Ben-Zeev et al., 2011). There are many different approaches to the induction of autoreactive Th2 lymphocytes some of these are: glatiramer acetate (GA, Coplymer-1, Cop-1, Copaxone), A91, poly-YE, p472 (Nogo-A-derived peptide). However, the only FDA-approved use of NDP-induced PA is GA under the brand name Copaxone for the treatment of MS. GA, also known as Cop-1, is the most studied of all APL-based therapies. Cop-1 is a synthetic polypeptide consisting of the amino acids tyrosine, glutamate, alanine and lysine that shows cross-reactivity with MBP (Schori et al., 2001; Kipnis & Schwartz, 2002). While the exact mechanism of Cop-1 is still not clearly elucidated, there is reason to believe that it induces Th2 differentiation, which later goes on to mediate neuroprotection (Aharoni et al., 2003; Aharoni et al., 2000). Although Th2 induction is the primary effect, immunization with Cop-1 also results in a Th1 cell deviation. This effect may seem paradoxical in nature but these pro-inflammatory Th1 cells are responsible for a sustained release of BDNF, NT-3, and NT-4 (Ziemssen et al., 2002; Ziemmssen et al., 2005). This Th1-mediated effect also induces astrocyte and neuronal production of these neurotrophic factors through a bystander effect (Aharoni et al., 2005). However, the effect of Cop-1 is not only mediated by its direct effect on CD4+ lymphocytes but also of its effect on APC, especially dendritic cells (DC). A recent study demonstrated that Cop-1 induced a Th2 response by modulating the APC function of DC. They demonstrated that DC exposed to Cop-1 during maturation had an impaired capacity of secreting IL-17p70 (the main Th1-polarizing cytokine). This effect resulted in the induction of a population with an increased frequency of effector Th2 cells that secreted IL-4 (Sanna et al., 2006; Vieira et al., 2003). Although the main components of NDP-induced PA are superficially understood, more research initiatives should be taken to better understand the therapeutic potential of these peptides. Most of the studies published use Cop-1 as the NDP, but the use of alternate peptide sequences such as A91 must be better understood. Nonetheless, there should be a constant effort to develop shorter, cheaper and more efficacious peptide sequences so that the true potential of NDP can be unlocked. Few studies have been conducted on the use of NDP in NDD.

5.1. Chronic Neurodegenerative Diseases

5.1.1. Amyotrophic Lateral Sclerosis

There have been many attempts to halt the progression of ALS by blocking different mediators of cytotoxicity (Ludolph et al., 2000). Because not all ALS patients have the defective SOD1 gene, motor neuron death is taken as the hallmark of disease because it is common to all cases of ALS. The animal model of ALS is acute peripheral nerve axotomy (Liu et al., 2001; Martin et al., 2000). The only drug currently used to slow down the progression of ALS is riluzole. Riluzole blocks the release of the excitatory neurotransmitter glutamate that can be toxic in elevated concentrations and is fundamental to ALS pathophysiology (Doble & Kennel, 2000; Meininger et al., 2000). In this study conducted by Angelov *et al*, mice treated with Cop-1 (using a different regimen than MS) show more motor neuron survival in the acute and chronic phases of ALS (Angelov et al., 2003). In the study, mice were subjected to a unilateral facial nerve axotomy. They were then immunized with Cop-1 and assessed. The results showed that vaccination with Cop-1 protected against motor neuron death induced after facial nerve axotomy. Transection of the facial nerve in the adult mouse is known to cause an easily visible late degeneration of axotomized motor neurons (Sendtner et al., 1996). Eight weeks after axotomy, mice immunized with Cop-1 had significantly larger numbers of motor neurons compared to PBS-immunized controls. Studies also indicated that immunization with Cop-1 preserved the activity of axotomized motor neurons. The study concluded that there was an elevated preservation of facial nerve motor neurons but the next step was to confirm that these were still functional. Using biometrical analysis of the mice's whisking patterns they found that Cop-1 treated animals exhibited significantly better facial nerve functionality than controls. The previous results demonstrated that Cop-1-immunized ALS mice benefited from improved motor neuron survival and the preservation of their function after facial nerve axotomy. A mice strain that overexpresses human mutant SOD1 gene develops a motor disease that closely resembles human ALS. The loss of motor function eventually causes death because of the lack of muscular respiratory control. Angelov *et al* concluded that treatment with Cop-1 immunizations resulted in an increased survival of the ALS mice. Immunizations with Cop-1 proved to be an adequate and efficacious therapy in an animal model of ALS. A small phase II study was held in human patients with ALS that finished with inconclusive results. Most patients demonstrated adverse reactions at the site of immunization and elevated lymphocyte proliferation. Although the results showed promise, efforts must be taken to increase the sample size and scrutinize the possible mechanisms through which Cop-1 exerts its protective effects (Gordon et al. 2006). These small but conclusive examples of NDP-induced PA in ALS provide us with enough proof to understand the possible therapeutic advantages. The study of Cop-1 in ALS is still in its beginning and should therefore be a priority in the coming years for NDD researchers. The maximal benefits of PA in ALS have not yet been achieved.

5.1.2. Alzheimer Disease

Previous studies proved that immunotherapy in AD via Aβ (amyloid beta) antibodies reduced the levels of Aβ plaques in transgenic mice. However, a human trial with Aβ antibodies caused severe adverse reactions in the form of meningoencephalitis (Nicoll et al., 2003; Orgogozo et al., 2003). A study done by Frenkel et al postulated that meningoencephalitis was very similar to EAE. They decided to test if amyloid precursor protein-transgenic (APP-Tg) mice were more susceptible to develop EAE. They concluded that EAE lowered the levels of Aβ in APP-Tg mice using antibody-independent mechanisms. As a follow-up they decided to see if they could achieve the low Aβ levels without causing EAE. GA or Cop-1 was an FDA-approved treatment for relapsing-remitting MS and was known to cause an autoreactive response without developing EAE. They were able to reproduce the amyloid load achieved in EAE using immunization with GA (Frenkel et al., 2005). Butovsky et al performed a more directed study, towards the analysis of PA in AD. This work found that Aβ activated microglia supports neurogenesis when stimulated by IL-4. This means that a Th2 phenotype will result in the overexpression of IL-4 and increased neurogenesis after microglial activation with Aβ. Vaccination with autoreactive T cells besides aiding in neurogenesis helped in the elimination of the Aβ plaque in AD-Tg mice. The increase in neurogenesis and the removal of the Aβ plaques resulted in the counteraction of the cognitive decline normally seen in AD (Butovsky et al., 2006). The vaccination with NDP has proven to be of paramount importance in the treatment of yet another NDD. This data is also an indicator of the urgency with which these therapies should be developed, standardized, and translated into clinical trials where they can bear fruits to human disease.

5.1.3. Parkinson Disease

Immunological studies in PD are controversial. The animal model is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication (Benner et al., 2004; Laurie et al., 2006). This intoxication depletes dopaminergic neurons in the SNpc, simulating PD. The complication arises because MPTP toxicity also destroys the animal's immune system, causing significant changes in spleen size and diminished numbers of CD3+ T cells 7 days after intoxication (Benner et al., 2004). The alterations in normal immune response impede the researcher's ability to analyze the role of the immune system in PD. However, researchers bypass this complication by cell subset replacements. The use of NDP in PD has been briefly evaluated by several studies from the same laboratory. All studies use the MPTP toxicity model of PD and use adoptive transfer of T cells from Cop-1 immunized mice. In the first study of Benner et al Cop-1 immunity was found to confer dopaminergic neuroprotection after MPTP intoxication. Animals that received the adoptive transfer of Cop-1-reactive T cells exhibited a much smaller reduction in the number of SNpc dopaminergic neurons. For the functional analysis of dopaminergic circuits they quantified tyrosine hydroxylase (TH) density. The loss of TH density was significantly less in Cop-1 immunized mice than in controls. Unfortunately, even in Cop-1 immunized mice, the loss of TH density was up to 72%. However, the conclusion was that Cop-1 reactive T cell passive immunization protected neuronal dopamine metabolism as well as structural neuronal elements and its projections. Complementary analysis stated that transferred lymphocytes were readily observed both in ventral midbrains and striata of MPTP mice. The study was also interested in evaluating microglial activation due to the fact that these cells are considered to be pathological in this NDD. To assess microglial activation they analyzed the Mac-1 gene using real time RT-PCR. Results showed that Cop-1 splenocytes are capable of attenuating MPTP-induced microglial reactions and in turn limiting their neurodestructive processes. In accordance to previously demonstrated concepts, the beneficial effects of Cop-1 immunizations were T cell-dependent. Treatment with the NDP also increased the expression of the neurotrophic factor GDNF. All results demonstrate the beneficial effects of immunizing with Cop-1 in PD (Benner et al., 2004). A similar study by Laurie et al corroborated the results observed by Benner and co-workers. Although similar results were obtained, the latter was able to recollect new data. The study concluded that anti-Cop-1 CD4+ T cell transfer into MPTP intoxicated mice exerted its reparative effects in a dose dependent manner. Also this study attributed the neuroprotection to a particular subset of T lymphocytes, CD4+ T cells. This further implicated T helper cells as the main player in PA. In order to support that PA is T cell-dependent, authors' transplanted Cop-1 specific antibodies to MPTP intoxicated mice to see if this conferred neuroprotection, as expected the effects of Cop-1 are CD4+ T cell-dependent (Laurie et al., 2006). This study reiterates the outstanding potential that NDP-induced PA holds in the outcome of PD. Nonetheless, this topic deserves more investigation as to identify the effect of a normal functional immune system and not just the evaluation through substitution studies.

5.2. Acute Neurodegenerative Diseases

5.2.1. Cerebral Ischemia

Immunization with NDP has also proved to be beneficial in cases of focal and global cerebral ischemia. There have been several studies of oral and nasal tolerization with neural constituents (Becker et al., 1997; Frenkel et al., 2003); however, only a few have resorted to NDP. There are primarily two studies that analyze the effects of this Th2-induced response after middle cerebral artery occlusion. The first by Ziv et al used poly-YE, a high molecular weight (22 to 45 kDa) copolymer that was shown to exert modulatory effects on the immune system (Cady et al., 2000; Vidovic et al., 1988). This peptide demonstrated abilities to downregulate T reg cell functions and allows effector T cell activation. The study showed that a single immunization with poly-YE produced long-lasting clinical and behavioral benefits, along with neuroprotection and increased neurogenesis, starting from the subacute phase. They also found that poly-YE was beneficial even when administered 24 hours after occlusion. The effects of poly-YE immunization were long lasting as animals showed less residual impairment against control even after 6 weeks. Histological analysis indicated that poly-YE attenuated cell loss in the hippocampus where PBS-treated rats showed large numbers of necrotic cells. The reduction in cell necrosis induced by poly-YE was so dramatic that the ipsilateral and contralateral sides were indistinguishable. Immunization with poly-YE had a significant neuroprotective effect after stroke, but authors' also wanted to evaluate its neuroregenerative properties. They found that poly-YE promotes neurogenesis after stroke as they saw an overall increase in the number of newly formed neurons in the dentate gyri of treated animals. The results presented in this study showed that the administrations of poly-YE as late as 24 hours after the induction of ischemic stroke greatly improved subsequent recovery. It had a positive effect on the neurological outcome of stroke, delayed degeneration, and enhanced the repair of damaged structures. Also, the therapeutic window (24 hours) seemed to be significantly wider than most of the current candidate therapies for stroke, giving it much more clinically translational value (Ziv et al., 2007). A separate study in our laboratory examined the effect of Cop-1 immunizations on the outcome of ischemic stroke, using the middle cerebral artery occlusion model. Results suggested that Cop-1 significantly improved the neurological outcome of animals after stroke. Histolopathological assessment also demonstrated a decrease in infarct size and infarct volume in Cop-1 treated animals (Ibarra et al., 2007). The results of both studies do not necessarily elucidate the mechanisms through which NDP-induced PA exerts its protective effects in focal cerebral ischemia but they provide evidence of its neuroprotective, and even neuroregenerative, properties. These studies provide NDP-induced PA with another consequential benefit, and that is the wide therapeutic window. Immunizations with NDP in the treatment of stroke require exhaustive research before they reach clinical trial potential but these preliminary results are an enormous step closer.

5.2.2. Traumatic CNS Injury

Traumatic CNS injury can be broken down into two compartments: TBI and SCI. A study by Kipnis et al found that immunizing with Cop-1 after traumatic brain injury had a better outcome on neurological and histological evaluations after injury (Kipnis et al., 2003). TBI triggers self-destructive processes, like other injuries to the CNS. Kipnis et al. studied mice with closed head injury and determined that the immune system plays a key role in the spontaneous recovery. The trauma-induced deficit was reduced, both functionally and anatomically, by post-traumatic vaccination with Cop-1. Several studies have been published on the use of NDP in SCI. Hauben et al used immunization with a variety of myelin-associated peptides, including those derived from Nogo-A, can be used to evoke a T cell-mediated response that promotes recovery. They show that neuronal degeneration after incomplete spinal-cord contusion in rats was substantially reduced, and hence recovery was significantly promoted, by posttraumatic immunization with Nogo-A-derived, p472 (Hauben et al., 2001). Our laboratory has also demonstrated the beneficial effect of immunizing with NDP (A91) on motor recovery and neuronal survival after SCI (Martiñon et al., 2007). Furthermore, we have determined some of the mechanisms of action of NDP-induced PA. In a recent study we found that immunization with Cop-1 and A91 exerted its neuroprotective effect through the inhibition of lipid peroxidation. Animals were immunized with A91 seven days before injury. With the aim of inducing the functional elimination of CNS-specific T cells, animals were tolerized against SC-protein extract and thereafter subjected to a SC injury. The lipid-soluble fluorescent products were used as an index of LP and were assessed after injury. Immunization with NDP reduced LP after SCI. Functional elimination of CNS-specific T cells avoided the beneficial effect induced by PA (Ibarra et al., 2010). A consequential study hypothesized that lipid peroxidation was caused by an unregulated production of ROS seen after CNS injury. The main ROS produced during the secondary phase of damage after trauma is NO. When NO is produced in an unregulated fashion it can react with other free radicals such as superoxide anion and produce peroxynitrite a powerful neurotoxic substance. We determined that the decrease in lipid peroxidation was caused by an inhibition in the synthesis of NO after immunization with NDP after SCI (unpublished data). Our results supported our hypothesis and allowed us to corroborate the data with expression analysis. We used real time RT-PCR to also demonstrate a reduction in the expression of the enzyme implicated in post-injury synthesis of NO, the inducible form of nitric oxide synthase (iNOS) (unpublished data). By determining that A91 reactive T cells also secrete NT-3 and IL-4 after SCI, making them a Th2 phenotype, we further substantiate the PA hypothesis. Immunizing with NDP deviates the Th response down a Th2 pathway increasing the synthesis of molecules such as IL-4 and IL-10 and secretion of neurotrophic factors like NT-3. Finally, we have found that the severity of injury would determine the strength and the effect of the PA response (unpublished data). This new data adds more factors into the induction of an autoreactive response. Our study noticed that animals that sustained a non-complete injury to the spinal cord had an increased recovery when immunized with A91. These autoreactive T cells also secreted BDNF and had greater recognition for A91 in vitro. On the other hand, animals that sustained complete or severe SCI did not recover even after A91-immunization. Unexpectedly, these animals did not even possess a clonal response to A91, meaning they were not even able to recognize the antigen in vivo, even with an adjuvant. This indicates that animals that sustained a severe or complete injury to the spinal cord are severely immunosuppressed and may therefore not engage a true PA response (unpublished data). This data that has just surfaced indicates that the neuroimmunological components of CNS disease require much more research in order to elucidate this unknown mechanisms. Even further, we must continue to delve into this immunosuppression caused by severe injury. The study of the body's physiology under duress shows us some of the mechanisms it possesses that could help in regenerating CNS during disease. Immunization with NDP has proven to be an excellent therapeutical intervention in SCI and several other NDD, providing it with reasonable necessity to continue research on the topic.

6. Improving the Beneficial Effect of Protective Autoimmunity

Even though the positive effect of immunizing with NDP has rendered significant results, it is possible to potentiate this effect. The improvement of this strategy would yield a better functional recovery and, thereby, a better quality of life for NDD affected individuals. It is clear that several damaging mechanisms take place during the acute phase of injury. Unfortunately, NDP-induced PA develops after a few days of immunization. Before PA sets in, the neural tissue is unprotected; therefore, the best approach is a combination of neuroprotective strategies. A therapeutic intervention tailored to each specific time point of injury pathophysiology. This approach will ameliorate one or more of the destructive events and may improve the functional outcome even more than PA alone. Excessive production of ROS from the beginning of CNS injury causes lipid peroxidation (LP) (Hall, 1994). Peroxidation of membrane lipids affects the integrity of the cell membrane and is the most damaging mechanism. The unregulated synthesis of free radicals offers a potential intervention route for the treatment of NDD. An example of this is the use of glutathione monoethyl ester (GSHE). This cell-permeant derivative of glutathione (GSH) is an antioxidant that limits the effect of ROS on the bi-lipid membrane. GSH has shown neuroprotective properties after SCI (Guizar-Sahagun et al., 2005; Santoscoy et al., 2002). Aside from this effect, GSH supports the proliferation, growth, and differentiation of immune cells. Moreover, GSH is actually required for many specific T cell functions, including DNA replication and IL-2 synthesis (Kidd., 1997). The amount of GSH determines the magnitude of the immunological response (Droge et al., 1994) as well as its depletions inhibits normal function (Kidd, 1997).

According to the data presented above, the addition of GSHE to NDP immunizations could significantly improve neuroprotection. The antioxidant properties of GSH will cover the overproduction of ROS from the beginning of injury while it could also assist in inducing a better PA response. A previous work carried out in our laboratory, examined the effect of this combination and demonstrated that the addition of GSHE to NDP immunizations induced earlier and better motor recovery after SCI compared to immunizations alone (Martinon et al., 2007). This effect was observed in animals subjected to either a contusive or a compressive SCI. The substantial improvement observed in treated animals allowed them to attain weight-supported plantar steps. This recovery is of great relevance when translating this treatment into a clinical setting. Motor improvement significantly correlated with increased axonal myelination as well as a marked survival of rubrospinal neurons. Besides finding adjuvant therapies for NDP-induced PA we wanted to see if multiple immunizations would increase the beneficial effect. We examined the effect of double immunizations and their effect on PA. Contrary to our expectations, double immunizations abolished the neuroprotective effect of single dose NDP-induced PA. The findings support the notion that the second immunization after SCI has a negative effect on PA. Rather than strengthening the protective effect, it eliminated it. This phenomenon was probably secondary to anergy since double immunization did not induce cell death (Martinon et al., 2007). According to the present data, the use of NDP and GSHE in SCI is a promising strategy. Further studies are necessary in order to establish the efficacy of this therapy and its potential applications into other NDD. Another attempt of synergistic therapeutic interventions is the use of GA with IFN-β-1a (Lublin et al., 2001). The development of adjuvant and synergistic therapies will aid in the optimization of NDP-induced PA allowing us to tackle the pathophysiology of several NDD.

7. Conclusion

The concept of PA revolutionized the way we saw the immune system in several different diseases. We figured out that it was more important to modulate the response than to eliminate it. With the logarithmic explosion in knowledge we must now hold these conclusions. The use of NDP and their effect on the immune response have proven to be helpful in several different pathologies, particularly in NDD. Using the information that we have recollected across the years, the mechanisms through which NDP-induced PA exerts its effects is everyday less obscure. Unfortunately, due to hypersensitivity reactions and heterogeneous responses among patients NDP have not been taken to their maximum potential. Unfortunately, PA is developed under the bases that the immune system is healthy and will function normally following an insult to the CNS. However, MS is an autoimmune disease, a case where the immune system is fatally skewed. This paradox forces us to adopt a revolutionary idea such as PA and apply it to NDD. The application of NDP-induced PA to the field of NDD can yield insurmountable results and therefore we urge the scientific community to aid in continuing to shed light on these once obscure mechanisms in order to make this therapeutic intervention efficacious and safe. The ultimate goal is to help the suffering and the complications of human disease.

8. References

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