Review Article

The Degenerating Substantia Nigra as a Susceptible Region for Gene Transfer-Mediated Inflammation

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Parkinson’s disease (PD) is characterized by the progressive degeneration of neurons in the substantia nigra pars compacta (SN) (reviewed in [1]). The aetiology of the most common forms of PD remains unknown. Current therapeutic treatments comprise pharmacological strategies to compensate for dopamine deficiency or surgical interventions that reduce the hyperactivity of specific regions within the basal ganglia (reviewed in [2]). However, dopamine replacement can lead to undesired side-effects 5–10 years after the beginning of treatment [3]. As no treatment is available that can prevent disease progression, the search for new therapeutic interventions is intense. In particular, gene therapy approaches have successfully reached the clinical trial stage in a number of cases [4]. Approved gene therapy clinical trials are based on restoring the activity of the basal ganglia by providing growth factors, inhibiting hyperactive regions, or enhancing dopamine synthesis [4].

Viral gene delivery seems to be the method of choice for gene therapy for PD due to its high efficiency for gene transduction. A drawback to the delivery of genes via viral vectors comes by introducing an antigenic load into the brain. These antigens will invariably elicit a transient innate immune response [5]. The nature and functional (toxic or protective) consequences of this response will vary depending on a number of variables but of utmost importance is the region of gene transfer, the viral dose used, and the state of microglial activation in that region [5]. Importantly, the SN is highly susceptible to the toxic effects of inflammation [6, 7]. In addition, microglial activation during neurodegeneration in this region possesses particular features that could exacerbate disease progression if a proinflammatory stimulus hits the SN [8].

This paper will focus on the properties of microglial activation in the degenerating SN in PD. In addition, the immune reaction after gene delivery by adenoviral, adeno-associated, and lentiviral vectors in the CNS will be discussed. Finally, a list of risk factors and parameters that could be considered when assessing the possible influence of gene transfer to the SN is presented as well as alternative approaches to circumvent inflammation-mediated toxicity.

1. Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SN) (reviewed in [1]). The aetiology of the most common forms of PD remains unknown. Current therapeutic treatments comprise pharmacological strategies to compensate for dopamine deficiency or surgical interventions that reduce the hyperactivity of specific regions within the basal ganglia (reviewed in [2]). However, dopamine replacement can lead to undesired side-effects 5–10 years after the beginning of treatment [3]. As no treatment is available that can prevent disease progression, the search for new therapeutic interventions is intense. In particular, gene therapy approaches have successfully reached the clinical trial stage in a number of cases [4]. Approved gene therapy clinical trials are based on restoring the activity of the basal ganglia by providing growth factors, inhibiting hyperactive regions, or enhancing dopamine synthesis [4].

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2. Inflammation in the Central Nervous System

Inflammation in the Central Nervous System (CNS) has different features according to (i) the region in which it occurs, (ii) the stimulus, and (iii) the molecular and cellular milieu at the time of the response. For example, inflammation in the brain parenchyma is usually restricted to certain leukocyte populations, harder to initiate, and less widespread than inflammation when it occurs in the ventricles, meninges, and choroid plexus [9]. At these sites the characteristics are more reminiscent of a typical systemic inflammatory response. This difference is mainly due to the absence of dendritic cells, conventional lymphatics, the downregulation of major histocompatibility complex (MHC) molecules within the CNS parenchyma, and the presence of local immunosuppressive factors (reviewed in [4, 9–11]). In addition, the innate inflammatory response in the CNS parenchyma does not always lead to an activation of the adaptive arm of the immune system (reviewed in [12]).

By origin and function, microglial cells can be regarded as the resident macrophages of the brain and are the main innate immune cells in the CNS. Microglial activation is a highly dynamic process [13–15] and involves phenotypic and reversible transitions that have been categorized into at least 4 stages according to Kreutzberg [16] (see Figure 1). Microglial activation is a patho-physiological feature of many brain diseases and for many years its key function was thought to be solely the removal of cellular debris [13]. Overwhelming evidence now shows that microglial activation is a phenomenon actively involved in neurodegeneration or neuroprotection (reviewed in [13, 17]).

Despite the many differences among animal models of PD and among PD patients, a common feature found in the SN in PD is the presence of microglial activation (reviewed in [13, 17–20]). Since the first description of microglial activation in the SN of PD brains by McGeer and colleagues in 1988 [21], numerous studies (more than 30) have repeated this observation in animal models and PD patients (reviewed in [13, 19]). Microglial-secreted factors that are associated with PD pathology include Interleukin-(IL) 1β, Tumor necrosis Factor α (TNF), IL-6, IL-2, Interferon-γ (IFN-γ), prostaglandins, and reactive oxygen and nitrogen species (for a comprehensive review see [22]). This is unlike astrogliosis, which is not as pronounced nor so consistently present in PD patients or animal models [23, 24]. Therefore, the discussion will be focused on microglial activation. The role of astrocytes in PD has been recently discussed in [19].

3. Microglial Activation and PD-Animal Models

In the 6-OHDA model of nigrostriatal neurodegeneration, microglial activation in the SN was morphologically defined as stage II and III, but not IV [24], see Figure 1. During the neurodegenerative process, transcription but not translation of key proinflammatory cytokines, was markedly increased [24]. In this way no proinflammatory environment was generated as a consequence of neuronal cell death. Therefore, microglial activation during neurodegeneration is not associated with the production of a proinflammatory milieu, as previously presupposed [8, 24]. This observation concurs with the physiological role of macrophages during the clearance of apoptotic cells in the periphery where such macrophage activation does not promote inflammation [25]. An example of this would be the clearance of neutrophils: it is estimated that $10^{10}$ neutrophils/day enter apoptosis and that macrophages are responsible for removing them in humans. Were this process to be proinflammatory, the human body would be permanently inflamed. In PD, most if not all neuronal loss in the SN is supposed to be apoptotic [26] and thus, even though activated microglia are essential to remove neuronal cell debris, a proinflammatory milieu should not be expected from this activation.

Activated microglial cells with higher proinflammatory cytokine mRNA but not protein expression have been described as being in a “primed” state, ready to produce an outburst of proinflammatory cytokines if a second stimulus appears (see Figure 1). Indeed, it has been demonstrated that if a subtoxic dose of a proinflammatory stimulus, such as bacterial endotoxin, is delivered to the degenerating SN, the translation of increased levels of mRNA coding for IL-1 takes place and an intense proinflammatory environment is generated [8]. Interestingly, this effect can also be elicited systemically by the sustained expression of circulating IL-1 [8]. Of utmost importance was the observation that this displacement of the equilibrium towards a proinflammatory milieu in the SN exacerbated disease progression and triggered earlier and more pronounced motor signs [8] (see Figure 1). Reversing the order of the stimuli could lead to a similar or a different observation. It has been described that previous exposure to LPS rendered the animals more susceptible to the neurotoxic effects of 6-OHDA [27]. On the other hand, the prior inoculation of IL-1 has a neuroprotective effects on the nigral neurons [28]. In addition, if this preexposition to inflammation was performed during pregnancy, the adult offspring were not only more susceptible to 6-OHDA administration, but had fewer dopaminergic cells in the SN at postnatal day 10 compared to controls [29, 30].

Neurons in the SN have been shown to be particularly susceptible to microglial-mediated toxicity in vitro and in vivo [6, 7], and anti-inflammatory interventions have been shown to be neuroprotective in animal models of PD [31–35]. By contrast, some early work has reported neuroprotective effects of inflammatory mediators. Variables such as duration and amount of expression of a specific cytokine seem to be important to anticipate the final effect of a given cytokine on neuronal viability. For example, the acute injection of IL-1β in the SN was not toxic for dopaminergic neurons in vivo if the cytokine was injected alone (10 ng or 1000 Units) or in combination with 1000 Units of TNF and 100 Units of IFN-γ in the SN [36, 37]. If, however, the expression of IL-1 or TNF in the SN was sustained between 14 and 21 days, it caused neuronal death, motor symptoms, and microglial activation to Stage IV [38–40]. Similarly, long-term inhibition of IL-1 or TNF attenuated loss of dopaminergic neurons in PD models [8, 41–43].

It can be concluded from these data that dosage and duration of expression are important to predict an effect of
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Figure 1: Schematic representation of the interactions between microglial activation and gene transfer. During neuronal death, microglial cells are activated to a “primed” state, where no proinflammatory cytokines are secreted. After receiving an additional (proinflammatory) stimulus, microglia activation changes into an exacerbated proinflammatory state that leads to increased neurodegeneration. Depending on variables such as type and dosage of vector used, inoculation of viral vectors might provide this second proinflammatory stimulus.

4. Microglial Activation and PD—Clinical Data

As explained above, microglial activation has been found in the SN of PD patients (reviewed in [13, 19]) in postmortem tissue sample and also by noninvasive imaging [20, 44]. In addition, higher expression levels of IL-1, IL-2, IL-6, and TNF were found in striatal postmortem samples of PD patients [45–47]. Using the peripheral benzodiazepine receptor as ligand (PK-11195) in a positron emission tomography study, neuroinflammatory processes have been verified in the pons, basal ganglia, and frontal cortex of PD patients [44].

The resolution of this technique has not allowed an accurate study of the SN. It should also be noted that evidence from PD patients indicates that microglial activation is not restricted to the SN, but it can also involve the putamen, hippocampus, brain stem, and cingulate and temporal cortex [44, 48].

5. Possible Effects of Inflammation-Eliciting Gene Transfer on the Degenerating SN

Viral gene delivery will introduce an antigenic load into the brain [49]. These antigens will invariably elicit at least, a transient innate immune response [49]. As stated before, in a naïve CNS, the innate immune response can be dissociated from the adaptive immune response in the brain parenchyma; for example, the systemic immune system can remain ignorant of a first antigenic challenge into the brain parenchyma. However, what happens if this viral load hits a brain region in which on-going inflammation or “primed”
microglial activation (as most likely will occur in the SN of PD patients) is present? Several variables need to be considered to answer this complex question, including vector type, vector dosage, method of delivery, patients’ age, stage of disease progression, and transgene used.

5.1. Vector Type and Dosage. The three main types of vector that emerge as solid candidates for gene therapy clinical trials for PD are adenoviral, adeno-associated, and lentiviral vectors.

Adenoviral vectors are among the most studied vectors for gene delivery in the CNS. In particular, the immune response to this virus in the brain has been extensively investigated. First-generation adenoviral vectors (Fg-Ad) allow transgene expression in the naïve brain for up to one year, which appears to be independent of a transient and initial inflammatory response [8, 12, 38, 39, 49–53]. However, preexisting or subsequent systemic immune response to adenovirus in the host abolish, or at least reduce by half, the transgene expression and can lead to cytotoxicity ([49–52], reviewed in [5, 11]). Direct inoculation of \(5 \times 10^7 - 1 \times 10^7\) infective particles of control adenovectors in the SN, caused between 10–25% of neurodegeneration per se [39, 40], re-enforcing the idea of an increased susceptibility of this region to inflammation. As is the case with any viral vector, dosage determines the response detected after vector administration. A threshold of more than \(10^7\) infective particles of adenoviral vectors in the periphery was determined to be needed to eliminate transgene expression in the brain [52]. This drawback is not seen when high capacity adenoviral vectors (hc-Ad) are used for gene transfer in the CNS. Therefore, hcAd seem to be better vectors for gene transfer in the brain than Fg-Ad. Nevertheless, the viral capsid is identical for both vectors and will elicit a response that, within a degenerating SN may cause an increase in inflammation with toxic effects. In addition, internalized adenoviral DNA activates an inflammasome-dependent maturation of pro-IL-1 to the active form of IL-1 in macrophages. Thus, it is not unlikely that any type of adenoviral vector injected in the degenerating SN will contribute to drive the environment to a proinflammatory milieu [54].

Adeno-associated vectors (AAV) are the vectors of choice for the vast majority of gene therapy clinical trials against PD [4]. The intrastriatal injection of relatively low titers of AAV \((2 - 4 \times 10^8\) i.p.) in naïve animals provokes a low innate immune response with no mononuclear cell infiltrate or cuffing of nearby blood vessels [55, 56]. However, at higher doses, a transient but significant astrogliosis can be detected [57]. In addition, in animal models previously exposed to systemic AAV, immunization inhibited AAV serotype 2 (AAV-2) gene transfer in the CNS [56], and readministration of AAV in the brain induced a greater inflammatory response [56, 58].

Results from Phase I and II clinical trials for PD have not reported severe adverse effects related to AAV administration [59–63]. Gene transfer was performed in the putamen or in the subthalamic nucleus. Despite these encouraging results, long-term analysis of a bigger cohort is needed to provide robust data on the degree of safety of these vectors during gene transfer in the brain. Unfortunately, the possible toxic effects of inflammation that could mask the potential beneficial effect of the treatment were apparently not studied. Recently, a Phase I/II clinical trial has been approved to inoculate patients with AAV vectors expressing neurturin not only in the putamen, but also in the SN (http://www.ClinicalTrials.gov identifier: NCT00985517). According to the data on the susceptibility of the SN to inflammation discussed above, it will be valuable to study the inflammatory response to the treatment in each patient to draw conclusions on safety and possibly efficacy in this trial.

Lentiviral vectors have been approved as vehicles for clinical gene transfer of aromatic amino-acid decarboxylase, Tyrosine Hydroxylase (TH) and GTP-cychohydrolase 1, all three genes necessary for dopamine synthesis [64]. Stimulation of dendritic cells by lentiviral vectors is weak compared with other single-stranded RNA viruses [65]. In addition, a reduced immune response has been detected after brain administration of multiple-deleted lentiviral vectors [66]. Worryingly, time-and dose-dependent downregulation of TH, the rate limiting enzyme in dopamine synthesis, has been reported after lentiviral-delivery of neurturin [67]. In addition, in the periphery, lentiviral delivery of reporter genes into the lung triggered T-cell mediated immune responses against the transgene [68]. Results from the above-mentioned clinical trial are awaited to verify the seemingly low-level immune response against lentiviral vectors in the brain. Finally, the phenomenon of gene silencing that depends of vector and promoter used and state of differentiation of the target cell should be considered [69]. For example, it could be tempting to try to compensate loss of expression by gene silencing with increased dosage of vector administered, increasing the probability of eliciting a proinflammatory response and therefore toxic effects on the SN.

5.2. Method of Gene Delivery. Independently of the vector of choice, the method of gene delivery may dramatically influence the magnitude and characteristics of the immune reaction against the gene delivery. For example, it is crucial that the method of vector delivery is accurate enough to prevent antigens from reaching the brain ventricular system and the deep cervical lymph nodes so as to keep the systemic immune system ignorant of the antigenic challenge produced by gene delivery in the CNS [5]. If this cannot be achieved, a systemic immune reaction against the viral vector and/or transgene delivered is likely to be generated [5]. At the very least, it will foreshorten the temporal expression profile and, at worst it will promote neurotoxic immune-mediated effects in the brain (reviewed in [11]). In conclusion, a delivery method of high accuracy is needed to prevent antigen diffusion into undesired brain regions.

5.3. Age and Disease Progression. In parallel, age-related changes in immune reactivity include enhanced Blood-Brain Barrier permeability and increased microglial and astroglial reactivity [70–72]. Therefore, changes in the
immune response against viral gene transfer can be expected in a manner that is dependent on the age of the treated subject.

L-Dopa therapy is quite effective in the early stages of most PD patients. Therefore, most clinical trials, including those related to gene therapy, are usually target to late-stage PD patients. It has been proposed that neuroprotective strategies will not be beneficial to these patients since at that stage of the disease there are limited amounts of dopaminergic neurons to protect. Likewise, late-stage PD patients will have encountered more opportunities for neuroinflammation in the SN to start and therefore have a higher risk of vector-mediated toxicity by gene transfer in the SN.

5.4. Transgene Used. Immune responses against the transgene are not infrequent and depend on whether there has been a prior exposure to it and whether it is syn- or xenogenic to the host [11]. In addition, it should also be borne in mind that chronic inflammation can facilitate dendritic cell infiltration into the CNS, which can facilitate antigen presentation to naive T cells [73]. Again this is a plausible situation in the degenerating SN.

6. How Can All These Risks Be Minimized?

A better understanding of the immunological component of the SN in PD patients together with studies on the possible beneficial effects of complementary anti-inflammatory treatments, changes in vector serotype, novel chemical formulations, and novel vector design will all help to design the best scenario to avoid undesired effects of an inflammatory response to gene delivery in the CNS. Alternatively, taking advantage of certain intrinsic properties of viral vectors might help to circumvent the risk of inflammation in the SN. For example, vectors can be used for the retrograde delivery of genes (e.g., adeno viral vectors could be administered in the striatal terminals of nigral neurons to deliver genes in the SN [38]). Certainly, this strategy has the disadvantage that it can reduce the amount of transgene delivered to the SN as seems to be the case in the Phase II trial with AAV-neurturin [63]. Nevertheless, it is a useful strategy to be considered when planning future gene therapy strategies using different vectors or transgenes. In addition, analyzing risk factors for each treatment and patient (age, method of gene delivery, immunogenicity of vector used, immunological status of the brain area to treat, influence of the transgene to be transfer ed, dosage, previous exposure to the virus used as vector) will certainly reduce the risk of immune-derived toxicity during gene transfer protocols against PD. In the future, this immunological risk analysis could even be used as an inclusion or exclusion criterium. Unfortunately, nowadays knowledge is still lacking to define parameters with univocal effects on the immunological response of gene transfer into the SN, and technology is behind to determine the immunological status of the SN at the time of gene transfer. Therefore, the most reasonable measurement is to design a clinical trial protocol to reduce the risk of inflammation-mediated toxicity as much as possible.

For example, we would like to propose that if a constellation of risk factors (increased age, late stage of disease, previous exposed to the virus, high viral dose) is present, an anti-inflammatory therapy could be considered [44]. Anti-inflammatory treatments such as COX-2 inhibition, minocycline, and naloxone have promising effects on animal models of PD [31–35]. In the context of a possible inflammatory reaction in a gene therapy protocol in the SN, these anti-inflammatory treatments may be reconsidered as complementary treatments. In addition, not only conventional anti-inflammatory therapies could be helpful to reduce the inflammatory risk of a PD patient, but anti-inflammatory molecules could be delivered by gene transfer in addition to other therapeutic genes. In particular, the viral delivery of Interleukin-10 or IL-1ra has been shown to be neuroprotective in the 6-OHDA rat model of PD [8, 74].

7. Conclusions

The SN is the main area of neurodegeneration in PD. Microglial activation and proinflammatory cytokine production have special characteristics in the degenerating SN that should not be underestimated when designing a gene transfer protocol in that area. It is expected that “primed” microglia or an on-going inflammatory response will be present at the time of gene transfer in PD patients. Numerous variables are in play that could change the expected outcome of gene delivery. An exhaustive analysis of the status of risks factors known to lead to inflammation at the moment of clinical intervention, leading to complementary anti-inflammatory treatments and/or alternative gene delivery strategies or additional genes delivered (e.g., IL-10) is proposed to reduce undesired, inflammation-driven side effects. Gene therapy against PD has reached maturity with eight clinical trials approved. It is of importance to consider, with the limitations of the available technology and knowledge, all variables affecting the immunological status of the PD patient and the possible interactions with the inflammatory component of gene delivery. This analysis should increase the probability of providing safe gene transfer in the SN and reduce inflammation-biased results that can obscure the efficacy of a given gene transfer protocol.

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References

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