Etiology of Parkinson’s Disease

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ABSTRACT: Controversy over the etiology and pathogenesis of Parkinson’s disease (PD) has continued for many years and while the details have changed, the uncertainty persists. Although heritability was most emphatically refuted a decade ago by many investigators, recent progress firmly indicates that genetic factors at least play a role, although probably to a variable degree from one individual to another. Evidence for a variety of other etiological factors is amassed from epidemiological studies, animal models, molecular and cellular biology. Genetic factors, infectious and immunological abnormalities, the effects of ageing, toxins (endogenous as well as exogenous) and other environmental factors may all contribute to the development of PD. Loss of nigral dopaminergic neurons may be mediated by varying combinations of oxidative free radical toxicity, impaired mitochondrial function, “weak excitotoxicity” and abnormal handling of cytoskeletal proteins, all of which may shift the balance regulating apoptotic cell death.

SUMMARY: Etiologie de la maladie de Parkinson. La controverse entourant l’étiologie et la pathogénèse de la maladie de Parkinson (MP) dure depuis plusieurs années et, bien que les détails ont changé, l’incertitude persiste. Bien que l’héritabilité de la maladie ait été réfutée avec emphase par plusieurs investigateurs il y a une dizaine d’années, des progrès récents indiquent clairement que des facteurs génétiques sont en cause, probablement à des degrés variables d’un individu à l’autre. Des données sur une variété d’autres facteurs étiologiques, provenant d’études épidémiologiques, de modèles animaux, de la biologie moléculaire et cellulaire, s’accumulent. Les facteurs génétiques, anomalies infectieuses et immunologiques, les effets du vieillissement, les toxines (endogènes et exogènes) et les autres facteurs environnementaux peuvent contribuer au développement de la MP. La perte de neurones dopaminergiques dans la substance noire peut être médidée par différentes combinaisons d’effets toxiques oxydatis dus à des radicaux libres, une fonction mitochondriale altérée, une “faible excitotoxicité” et un métabolisme anormal des protéines du cytosquelette, qui peuvent tous déséquilibrer la régulation de la mort cellulaire par apoptosis.


EPIDEMIOLOGICAL FACTORS – GENERAL CONSIDERATIONS

The overall incidence of Parkinson’s disease, based on multiple studies world-wide, is approximately 10-20 per 100,000 per annum. Estimates of PD prevalence vary considerably, but the currently accepted figure is approximately 200-300 per 100,000 population.1 This is age-dependent, with prevalence rates per 100,000 ranging from approximately 5 for age under 40 to 300-700 in the 7th decade and >700 for age over 70.

Considerations of incidence, prevalence and possible causative factors should take into account the fact that diagnostic accuracy based on initial assessment is probably only about 65% and even when followed over an extended time, this improves to 76%.2 Furthermore, many case-control studies of environmental exposure have included data derived from informants other than the patient. Inclusion of such proxy-derived data may substantially degrade the reliability of the information.3

GENETIC FACTORS

In general, twin studies have shown a very low rate of concordance,4 and this has traditionally been accepted as evidence against a major genetic basis. However, when co-twins were personally examined by the investigators, a higher than expected (3/9 monozygotic; 3/12 dizygotic twins affected) level of concordance was demonstrated.5 Twin studies are not conclusive, even in these ideal circumstances, as subclinical abnormalities of the nigrostriatal dopamine system may not be expressed for several years. Thus, a PET study found abnormal 6-fluorodopa uptake in 40% of asymptomatic monozygotic twins of PD patients and in 28% of dizygotic twins. However, 3 of the 4 “asymptomatic” MZ twins had tremor at the time of the PET scan (and 1 went on to develop PD within 2 years).6 The largest twin study to date, based on 19,842 white male twins enrolled in the National Academy of Sciences/National Research Council World War II Veteran Twins Registry was recently reported. Concordance rates were similar in monozygotic and dizygotic twin pairs; however, the concordance risk was much higher when

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disease onset prior to the age of 50, suggesting that genetic factors may play a greater role in disease of earlier onset.6a

There is an increased incidence of family history of Parkinson’s in affected individuals (16% vs. 4% in controls; age-adjusted odds ratio 3.5),7 which may not entirely reflect genetic factors. Families with a dominantly inherited condition which is pathologically indistinguishable from PD and clinically similar have been identified, but most investigators consider these to be special cases. This includes the recently described association of familial parkinsonism with a mutation (Ala53Thr) of the gene encoding α-synuclein in the Contursi kindred, where the age of onset is younger than average, tremor is less frequent and progression is more rapid.8,9 Although this mutation was also found in other families, they were all of Greek or Sicilian origin, raising the possibility of a founder effect. A different (Ala30Pro) mutation in this gene has been described in a German family with parkinsonism.10 The α-synuclein mutation has now been excluded in numerous other families with dominantly inherited parkinsonism,11,12 as well as pathologically verified sporadic PD.13 Linkage in one family has recently been demonstrated to the short arm of the 2nd chromosome.14 Despite the recent interest in mitochondrial abnormalities (see below), there has been no convincing evidence of maternal transmission or mitochondrial DNA abnormalities in PD, although analysis of cytoplasmic hybrids (cybrids) suggests that the abnormality of Complex I is indeed derived from mitochondrial DNA.14a While juvenile parkinsonism is linked to a region of the long arm of the 6th chromosome, where mitochondrial (Mn) superoxide dismutase (SOD2) is encoded, SOD2 expression was high in a single patient with this disorder15 and the mutation has recently been identified in a gene called parkin. The N-terminal sequence of Parkin is similar to the ubiquitin family of proteins and it is therefore possible that juvenile parkinsonism results from impaired proteolytic processing.16 The importance of this mechanism is further supported by the recent identification of an Ile93Met mutation of the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) gene in a German pedigree with typical, adult-onset parkinsonism.16a Genetically inherited susceptibility to certain toxins has been suggested by diminished debrisoquine hydroxylation, sulfoxidation and S-methylation reactions. An increased incidence of mutant alleles of the CYP2D6 gene has been reported by multiple groups, but this does not seem to segregate with disease expression within families with multiple affected members. Similarly, reports that PD may be associated with increased frequency of an MAO-B allele17 have not been consistently reproduced. A number of other candidate genes (GPX1, TH, BDNF, CAT, APP and SOD1, as well as CYP2D6) have been excluded in at least some families.18,19

Nature vs. Nurture – Population Studies

Although PD is in general widespread, some populations seem to have a lower incidence. This would include South African and Nigerian blacks, although blacks living in Mississippi are affected to a comparable degree as the white population20 and a recent autopsy study suggests that black Africans have an equivalent prevalence of incidental Lewy body disease.21 Lower incidences have likewise been reported in Oriental populations (but not in Oriental Americans and possibly not in Taiwanese).22,23 While many investigators have reported an increased risk of having a close relative with PD in patients compared to controls, one interesting study of families in which members of multiple generations were affected suggested that disease onset clustered around the same calendar year rather than a comparable chronological age,24 again suggesting shared exposure (and susceptibility?) to some unidentified environmental factor.

Epidemiological studies have also led to more direct support for an environmental hypothesis. Young-onset parkinsonism has been associated in a number of studies with exposure to well water.25 No specific toxin has been identified and the well water link has not been found in all studies, although a more consistent factor seems to be rural upbringing. One compelling rural factor is pesticide/herbicide exposure, although this has also not been reproduced in all studies.26,27 The association may be strengthened by a recent post-mortem report of increased detection of the lipid-soluble pesticide (and mitochondrial toxin) dieldrin in the brains of Parkinson patients compared to Alzheimer and normal controls.28

While rural environments are associated with an increased risk of PD in the industrialized world, the opposite seems to be true in China, where exposure to industrial chemicals is less likely to occur in the countryside. In China, consumption of well water or high ethanol intake are associated with a reduced risk of Parkinson’s, whereas the risk is increased in those who consume river water or live in proximity to rubber plants.31 Data from the Canadian study on ageing recently suggested a relationship to plastic or epoxy resin exposure.32

Numerous investigators have suggested an inverse relationship between smoking and Parkinson’s disease, but this finding has remained controversial and most of the reports were based on case-control studies.33-36 Whereas a recent case-control study confirmed a lower prevalence of current smoking in Parkinsonians, but no difference in prior exposure (suggesting that there is no protective effect, but rather that PD itself leads to reduced smoking),34 another prospective study of more than 8,000 men enrolled in the Honolulu Heart Program did indeed support a reduced risk of Parkinson’s in smokers or ex-smokers (relative risk = 0.39), with an apparent dose-response effect.35 If the relationship is indeed a real one, there still exists the question of whether it reflects a “rigid” premorbid personality trait, as has been repeatedly described in PD, or a lower propensity to nicotine addiction, rather than a “protective” effect of smoking, perhaps mediated by stimulation of toxin-neutralizing enzymatic pathways.

Some retrospective studies have found an increased risk of PD following head injury,27-29 but this has been quite variable.38

Infectious and Immunologic Mechanisms

The pandemic of encephalitis lethargica in the early 20th century led to parkinsonism which was clinically and pathologically distinct from idiopathic disease, but there were nonetheless expectations that an infectious etiology might be found for the “idiopathic” disorder. Sporadic post-encephalitic parkinsonism does still occur, albeit very rarely, but repeated efforts to identify an infectious agent which is consistently associated with the development of PD have failed. Interest has been rekindled by the appearance of clinically typical parkinsonism in patients with encephalitis, with image evidence of damage confined to the
as well as the demonstration of relatively selective involvement of the substantia nigra by neuroviral influenza A.\textsuperscript{40} Parkinsonism is not transmissible to primates, effectively ruling out a “slow viral” or prion-related etiology.

Activated microglia are seen in the substantia nigra of patients dying with Parkinson’s disease.\textsuperscript{41} Whether or not these reflect a response to ongoing cell death as opposed to the primary mechanism of neuronal degeneration is, however, unresolved. Although there have been reports of disease-specific antineuronal antibodies in the CSF and of complement-dependent dopaminergic toxicity in PD serum,\textsuperscript{52} there has been no direct evidence to suggest a primary immunological abnormality in PD.

**Is Parkinson's a Response to Normal Ageing?**

Normal ageing is associated with clinical features which are somewhat reminiscent of PD, including the assumption of a stooped posture, slowing of body movements and a reduction of associated movements. Ageing is associated with a linear decline of pigmented neurons in the substantia nigra\textsuperscript{43} and with decreased levels of striatal dopamine, tyrosine hydroxylase and dopa decarboxylase. This has led to the suggestion that PD may result from the effects of ageing superimposed upon an insult to the nigrostriatal system earlier in life, which may go unrecognized, as symptoms of parkinsonism do not become apparent until dopamine depletion exceeds 50%. However, the pattern of age-related striatal dopamine depletion is different from PD\textsuperscript{44} and the presence of activated microglia at all stages of PD\textsuperscript{41} suggests that there is an ongoing active process. The original ageing hypothesis has been revised, based on an analysis of rates of disease progression estimated from repeated clinical examination.\textsuperscript{45} It is now proposed that the rate of PD progression is compatible with either an event which kills some nigral neurons and damages others, leading to reduced survival, or with an event which initiates a process that subsequently unfolds at a constant rate.\textsuperscript{46,47}

**Toxins and Parkinson’s Disease**

A variety of toxins have been associated with the development of parkinsonism and neuronal death, including cyanide, manganese, carbon disulfide, methanol and organic solvents. In general, however, the pattern of neuronal death is different from that seen in idiopathic PD and clinical features are variable. As noted above, PD may be associated with exposure to well water, pesticides, epoxy resins or other chemicals, but to date, no specific toxic constituent has been identified. Thus, the identification in the early 1980s of a group of drug addicts who developed typical clinical features following exposure to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and the concurrent demonstration that MPTP is a selective nigral toxin in primates was a significant breakthrough. In non-human primates, MPTP leads to selective depletion of dopamine and preferential destruction of the A9 dopamine neurons, but lesser involvement of the A10 neurons and locus coeruleus are also seen. Furthermore, aged animals exposed to MPTP may develop structures resembling Lewy bodies. Some strains of mice develop dopamine depletion, but nigral cell death is not universally observed,\textsuperscript{48,49} with strain-related differences. Rats are resistant to peripherally administered MPTP, in part related to catabolism in capillary walls,\textsuperscript{50} but also reflecting reduced sensitivity to MPP+,\textsuperscript{51} although aged rats are partially susceptible and MPP+ is partially effective following direct intracerebral administration.\textsuperscript{52,53}

MPTP undergoes MAO-B-dependent conversion to MPP+ in glial cells. MPP+ is then in turn released into the extracellular space and selectively taken up by DA neuronal terminals (dependent upon the membrane DA transporter), where it then inhibits mitochondrial Complex I activity.\textsuperscript{54} A variety of naturally occurring isoquinolines which are structurally analogous to MPTP, inhibit mitochondrial Complex I and can enhance apoptotic death in dopaminergic neurons\textsuperscript{55} have been implicated as endogenous neurotoxins in PD.

There is a considerable amount of controversial literature on the relationship between MPTP toxicity and excitatory amino acid transmission. Excitatory amino acid inhibitors might be expected to reduce the symptoms and signs of PD by blocking excessive output from the subthalamic nucleus, but the potential role of excitatory amino acids as endogenous toxins in PD is less clear. Some investigators have reported that MPTP or MPP+ toxicity can be prevented by the non-competitive NMDA antagonist MK-801\textsuperscript{56} and MPP+ enhances glutamate toxicity to dopaminergic neurons in culture.\textsuperscript{57} It has been suggested that the acute phase of MPTP/MPP+ toxicity may be associated with excessive depolarization-induced stimulation of glutamate receptors and that neurons are more susceptible to the resultant influx of calcium ions because of impaired mitochondrial function (the “weak excitotoxic” hypothesis).\textsuperscript{58} There is indeed an excitatory amino acid input to the substantia nigra from the pedunculopontine and subthalamic nuclei, but many labs have failed to reproduce the protective effects of MK-801 in this model and the proposed mechanism remains speculative. However, protection against 6-hydroxydopamine-induced nigral degeneration following subthalamic nucleus lesions in the rat has also been described.\textsuperscript{59} Given that amantadine has properties as an excitatory amino acid antagonist, a novel rationale for this medication in Parkinson’s disease is suggested and indeed its use has been associated with prolonged survival.\textsuperscript{60}

**Endogenous Toxins – the Free Radical Hypothesis**

A converging amount of evidence suggests that Parkinson patients may suffer the combined effects of multiple factors culminating in free radical-induced damage. MAO-catalyzed oxidative deamination of dopamine results in the formation of hydrogen peroxide. Dopamine additionally undergoes autooxidation reactions which result in the formation of reactive quinones and semiquinones (also the mechanism of 6-hydroxydopamine toxicity) as well as the superoxide anion radical, the latter subsequently cleared by superoxide dismutase to form more hydrogen peroxide. Under usual conditions, hydrogen peroxide is cleared by reduced glutathione (via glutathione peroxidase) or catalase, but in Parkinson patients, there is a deficiency of reduced glutathione (GSH) which appears to be relatively disease specific and restricted to the substantia nigra.\textsuperscript{62} A partial deficiency is also seen in the brains of people with incidental Lewy body disease.\textsuperscript{63} This deficiency may in part be a consequence of increased oxidative stress (via conversion to oxidized glutathione), but may also reflect degradation secondary to increased activity of γ-glutamyltranspeptidase.\textsuperscript{64} This depletion of GSH may lead to impaired clearance of hydrogen peroxide, which is now available for neuromelanin to bind oxygen free radicals (referred to as the “weak excitotoxic” hypothesis). There is indeed an excitatory amino acid input to the substantia nigra from the pedunculopontine and subthalamic nuclei, but many labs have failed to reproduce the protective effects of MK-801 in this model and the proposed mechanism remains speculative. However, protection against 6-hydroxydopamine-induced nigral degeneration following subthalamic nucleus lesions in the rat has also been described. Given that amantadine has properties as an excitatory amino acid antagonist, a novel rationale for this medication in Parkinson’s disease is suggested and indeed its use has been associated with prolonged survival.\textsuperscript{61}
to undergo non-enzymatic (Fenton) reactions with ferrous ions, resulting in the formation of the highly toxic hydroxyl radical. The resultant ferric ions can also react with the superoxide anion radical to form hydroxyl radicals (Haber-Weiss reaction). Hydroxyl radicals are highly reactive and will in particular result in the formation of lipid peroxides which damage cell membranes.

Interest in oxyradical mechanisms of neurodegenerative disease increased with the identification of SOD1 mutations in some cases of familial ALS. However, motor neuron degeneration is associated with a gain of mutant SOD1 function and degeneration may in part be mediated by peroxynitrite formation; SOD mutations have not been associated with idiopathic PD. In juvenile autosomal recessive parkinsonism secondary to mutations of the parkin gene on chromosome 6q, there may be a gain of SOD2 function.

What evidence, direct or indirect, suggests that this cascade of events might occur in PD? Nigral iron content is increased in PD substantia nigra and it may furthermore be insufficiently "trapped" by ferritin, which is (variably) deficient. Neuromelanin can bind iron and facilitate Fenton and Haber-Weiss reactions. However, 6-hydroxydopamine-induced degeneration of the substantia nigra also results in elevated iron levels, as do multiple other degenerative conditions, so this finding may be secondary. Deficiency of reduced glutathione has been demonstrated, as have changes in SOD, which are less consistent, but would nonetheless be compatible with oxidant stress. Depletion of GSH alone fails to result in nigral cell loss, but potentiates MPTP or MPP+ toxicity. In cortical neurons, GSH deficiency results in cell death mediated by 12-lipoxygenase. A number of studies have suggested abnormal lipid peroxidation in PD and further evidence of free radical toxicity is derived from the demonstration of increased 8-hydroxy-2-deoxyguanosine, a marker of oxyradical-mediated DNA damage, in PD brain. This increase was seen in all brain regions studied (except the cerebellum), however, and may be secondary to L-DOPA therapy, so the pathogenetic significance of this finding is as yet unresolved.

Mitochondrial Dysfunction

A number of studies now suggest that there is an abnormality of nigral mitochondrial Complex I in PD. This appears to be relatively region specific within brain, but abnormalities in other tissues (platelets and muscle) and of other components of the electron transport chain have been less consistent. Some degree of disease specificity has also been demonstrated, suggesting that this does not simply reflect cell loss. It is of interest that MPP+ also exerts its toxicity by inhibition of Complex I, suggesting a close link to idiopathic PD. Complex I abnormalities may lead to increased formation of oxyradicals which are normally tightly bound to the electron transport chain. Indeed, transgenic mice with high Cu/Zn (cytosolic)-SOD activity are resistant to the toxic effects of MPTP and MPP+. Mitochondrial abnormalities may also result in impaired formation of reduced glutathione. Conversely, oxyradicals are toxic to mitochondria and the Complex I abnormalities may be secondary. The brains of people with incidental Lewy body disease demonstrate a deficiency of reduced glutathione which is intermediate between normal controls and Parkinson subjects, but the reduction of Complex I activity was not statistically significant.

Furthermore, systemic administration of the specific complex I inhibitor rotenone induced striatal and pallidal, but not nigral cell loss. Although 7 of the approximately 40 subunits of Complex I are encoded by mitochondrial DNA, there has been no convincing evidence of altered mitochondrial DNA in PD.

The indirect evidence to support the free radical/mitochondrial hypothesis is compelling, but caution is warranted before it is accepted as the primary abnormality as opposed to either a series of secondary abnormalities or a contributor to ongoing cell death in the presence of another as yet unidentified process. These issues have been reviewed in detail in a number of excellent reviews and critiques.

Implications of the Free Radical Hypothesis

If the events outlined above are indeed important for the pathogenesis of PD, then it is potentially possible to intervene in the disease process. MAO-B inhibitors might theoretically help by preventing enzymatic oxidation of dopamine, while other antioxidants (Vitamins C and E) may quench free radicals. To date, there is no convincing evidence that any of these compounds affect disease progression, although Vitamin E deficiency may lead to (asymptomatic) impairment of nigrostriatal function. Although abnormalities of iron are likely to be largely secondary, chelation might be of theoretical interest to slow down further disease progression. While protective effects have been demonstrated in animal models, this approach has not been tested in humans.

One of the most disturbing issues is the question of whether levodopa itself may contribute to ongoing nigral cell death in PD, by increasing dopamine turnover and promoting oxyradical formation. While high concentrations of levodopa and dopamine are indeed toxic in cell culture systems (in the absence of glial cells; levodopa actually has trophic effects in neuronal-astrocyte co-cultures) and may also reduce nigral graft survival in animal transplant models, there has been no convincing evidence that levodopa hastens disease progression in PD. If anything, levodopa use has been associated with enhanced survival of remaining dopamine neurons in rats with partial 6-hydroxydopamine lesions and with decreased mortality in PD, which of course probably reflects symptomatic benefit. Nonetheless, the issue remains controversial and the oxyradical hypothesis has led some authors to suggest that symptomatic therapy should be initiated with dopamine agonists (which will actually reduce dopamine turnover) rather than levodopa. Dopamine agonists may lead to decreased free radical production, seem to retard the normal age-related loss of nigral dopamine neurons in rodents and their use may be associated with decreased mortality in PD. Whether or not their use as de novo therapy confers any advantage over initiating treatment with levodopa is the subject of a number of ongoing clinical trials.

Trophic Factors

Target-derived trophic factors are substances which are produced and released in limited quantities in the projection areas of neurons which respond to them. Within the nervous system, the primary effect is on survival and differentiation of neurons during embryonic development, as well the maintenance of neuron-specific function during adult life. Recent advances in molecular
biology have led to an explosion of interest in this area, with the identification of novel factors and better understanding of receptor structure and transduction mechanisms. These compounds may prove to be beneficial in a variety of neurodegenerative disorders, including Parkinson’s disease, and may also substantially promote the survival and function of neuronal grafts used for the treatment of these conditions.

The trophic factors attracting the greatest attention in PD are brain derived neurotrophic factor (BDNF), a member of the neurotrophin (nerve growth factor-related) family, which interacts with the trkB receptor, and glial cell-derived neurotrophic factor (GDNF), which belongs to the TGF (transforming growth factor-β) superfamily. BDNF promotes the survival of mesencephalic dopaminergic neurons in culture and protects against MPP+ toxicity. Although initially thought to be highly selective for dopaminergic neurons, GDNF is now recognized to have trophic influences on other neuronal populations, particularly motor neurons. GDNF selectively promotes dopamine uptake, dopamine neuronal survival and morphological differentiation in rostral mesencephalic tegmental cultures. GDNF is expressed in dopamine target areas during development and is transported retrogradely in the nigrostriatal pathway. Although its message is not upregulated by 6-hydroxydopamine lesions, GDNF markedly enhances survival of fetal dopaminergic grafts and recent studies have shown beneficial effects in MPTP, 6-hydroxydopamine and axotomy models of parkinsonism. Recent animal studies indicate that GDNF and other neurotrophic factors can be expressed in microencapsulated cells or by modified viral vectors, thus offering a novel approach to either enhance the survival of mesencephalic grafts used for the treatment of PD, or possibly even to delay disease progression.

Cyclosporin and the immunosuppressant FK506 both bind to proteins which interact with calcineurin. The recently described FK506 analogue GPl 1046 has been shown to have neuroprotective effects in 6-hydroxydopamine and MPTP-induced parkinsonism in rodents. This effect seems to be independent of action on calcineurin and GPl 1046 has no immunosuppressant properties. Other proteins of interest, which seem to preferentially promote the survival and differentiation of dopaminergic nigral neurons include sonic hedgehog, the GDNF analogue neurturin, and the orphan nuclear receptor family member Nurr-1.

While all these factors may be of interest in treating PD, there is no evidence to date to suggest that PD is associated with a specific deficiency of trophic factors, with the possible exception of basic fibroblast growth factor (bFGF), whose expression is reduced in nigral neurons of PD patients. Many of the neurotrophic factors are known to be expressed by glial cells. Thus, future studies on the mechanisms underlying neurodegeneration may need to pay closer attention to the possibility of abnormal glial function. This also highlights importance of providing an adequate glial support base when studying the effects of various manipulations on dopaminergic neurons in vitro (e.g., as noted above, levodopa is toxic to dopamine cell cultures in isolation, but has a trophic effect when studied in mixed neuronal-glial cultures).

**Apoptosis**

While a variety of insults may lead to cell necrosis, often accompanied by an inflammatory response, normal cells may undergo death followed by rapid phagocytosis, particularly if deprived of trophic factors. This programmed cell death appears to be important for the regulation of cell number, the prevention of tumours and the appropriate mix of cell types (e.g., neurons only survive in the presence of the appropriate target tissue). Programmed cell death is an active, gene-regulated process which is morphologically distinct from necrosis. In addition to the absence of an inflammatory response, characteristic features include cell shrinkage, clumping of nuclear chromatin and cytoplasmic and nuclear fragmentation (apoptosis). This phenomenon has been shown most extensively in the worm Caenorhabditis elegans, where the genes ced-3 and ced-4 promote cell death and their inactivation leads to prolonged survival. The gene ced-9 opposes this function and a mammalian analogue, bcl-2 has been identified, which can prevent apoptosis when expressed in C. elegans. The fate of mammalian cells may be determined by the balance between anti-apoptotic proteins such as Bcl-2 or Bcl-x and opposing proteins such as Bax. The mammalian analogue of CED-3 is interleukin-1β converting enzyme (ICE), whose death-promoting properties can be prevented by the product of the crmA (cowpox virus cytotoxic response modifier) gene and the baculovirus antiapoptotic protein p35. ICE inhibitors arrest motoneuron death resulting from deprivation of trophic factors in vitro. ICE itself is not usually involved in apoptosis, but the related family of caspases are, in a self-activating cascade that may be triggered by extracellular (e.g., activation of the cell surface receptor Fas) or intracellular (e.g., release of mitochondrial cytochrome c) factors.

The role of apoptosis in Parkinson’s is as yet unresolved. One report described apoptosis in the substantia nigra of 8 of 11 parkinsonian patients studied post-mortem using the TUNEL (Terminal deoxynucleotidyl transferase dUTP-biotin Nick-End Labelling) method; however, glial cells were equally affected. This has been confirmed by other investigators, based on in situ labelling or electron microscopy. Other investigators have suggested that TUNEL labelling is seen in glia, but not neurons, within the SN. Expression of bcl-2 protein in the nigrostriatal path of parkinsonians has been demonstrated. Downregulation of SOD1 inhibition of mitochondrial Complex I or L-DOPA can induce apoptosis in PC12 cells and physiological concentrations of dopamine induce similar changes in cultured chick sympathetic neurons. MPTP induces apoptotic nigral cell death in the mouse in vivo. While high concentrations of NMDA or superoxide/peroxynitrite induce necrosis, lower concentrations induce apoptosis, which can be differentially affected by specific trophic factors. The recent demonstration of increased nuclear translocation of NF-κB in nigral dopamine neurons of Parkinsonian patients has been interpreted as evidence of apoptosis triggered by oxidative stress; however, the role of NF-κB itself (promotion of cell death vs. protection) is unclear.

**Other Factors of Potential Relevance**

The role of other factors which determine dopaminergic cell fate during development in the pathogenesis of Parkinson’s disease is not understood. One such factor which has attracted considerable interest is sonic hedgehog, a protein which is expressed in notochord and floor plate cells and induces a ventral cell phenotype. The weaver mutation is a spontaneously arising defect...
in mice, characterized by degeneration of cerebellar granule and midbrain dopaminergic neurons. It has been linked to a mutation of G protein-gated inward rectifying potassium (GIRK) channels. Further details on the mechanisms of nigral cell death are not yet understood, but it appears to be non-apoptotic.

**Summary**

This seemingly confusing array of etiological possibilities does not represent a series of mutually exclusive events. It seems clear that Parkinson’s is associated with excess generation of free radicals and impaired function of mitochondrial Complex I, although whether one of these may lead to the other and whether either is of primary pathogenetic importance is still unresolved. Genetic and environmental factors probably both influence these processes, to varying degrees from one individual to another. Whether a deficiency of trophic or other protective “anti-apoptotic” factors contributes to the development of Parkinson’s is also unclear. Recently discovered mutations of α-synuclein and parkin suggest that increased attention should be paid to protein handling. Improved understanding of these issues will allow the development of more rational treatment strategies for Parkinson’s as well as other neurodegenerative disorders.

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