Cytokines Induce Nitric Oxide-mediated mtDNA Damage and Apoptosis in Oligodendrocytes

PROTECTIVE ROLE OF TARGETING 8-OXOGUANINE GLYCOSYLASE TO MITOCHONDRIA*

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Nitric oxide (NO) that is produced by inducible NO synthase (iNOS) in glial cells is thought to contribute significantly to the pathogenesis of multiple sclerosis. Oligodendrocytes can be stimulated to express iNOS by inflammatory cytokines, which are known to accumulate in the multiple sclerosis brain. The potentially pathological levels of NO produced under these circumstances can target a wide spectrum of intracellular components. We hypothesized that one of the critical targets for damage that leads to disease is mtDNA. In this study, we found that cytokines, in particular a combination of tumor necrosis factor-α (50 ng/ml) and IFNγ (25 ng/ml), cause elevated NO production in primary cultures of rat oligodendrocytes. Western blot analysis revealed a strong enhancement of iNOS expression 48 h after cytokine treatment. Within the same time period, NO-mediated mtDNA damage was shown by Southern blot analysis and by ligation-mediated PCR. Targeting the DNA repair enzyme human 8-oxoguanine DNA glycosylase (hOGG1) to the mitochondria of oligodendrocytes had a protective effect against this cytokine-mediated mtDNA damage. Moreover, it was shown that mitochondrial transport sequence hOGG1-transfected oligodendrocytes had fewer apoptotic cells compared with cells containing vector only following treatment with the cytokines. Subsequent experiments revealed that targeting hOGG1 to mitochondria reduces the activation of caspase-9, showing that this recombinant protein works to reduce apoptosis that is occurring through a mitochondria-based pathway.

Oligodendrocytes, one of the primary types of glial cells, produce the unique lipid-rich myelin membrane that forms multilamellar spirally wrapped sheaths around neuronal axons in the central nervous system (CNS). Alterations in oligodendrocyte function lead to disruption of myelogenesis and can cause severe neurological deficits, such as those found in multiple sclerosis (MS) and in the extensive white matter degeneration observed after an ischemic insult to the CNS (1). In demyelinating diseases, such as MS, oligodendrocytes are under immune attack, both cell-mediated and humoral (1, 2). Recent evidence suggests that oligodendrocytes, besides being targets, may also be sources of cytokines and nitric oxide (NO), under inflammatory conditions (3, 4). Cytokines are important mediators in the inflammatory demyelination observed in human MS, as well as in animal models of MS, such as experimental allergic encephalomyelitis or Thielir virus infection. In these pathological conditions, proinflammatory cytokines released by endogenous cells or infiltrated macrophages and CD4 + Th1 cells accumulate and exert pleiotropic effects on oligodendrocytes (5, 6). It has been shown that two proinflammatory cytokines, tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ), accumulate in the brain and cerebrospinal fluid of MS patients (7, 8). Separate studies using transgenic mice have revealed the development of a spontaneous inflammatory disease with experimental allergic encephalomyelitis-like symptoms and demyelination as a direct consequence of CNS-specific expression of TNFα (9) and IFNγ (10).

Among the cytotoxic effector molecules evoked by proinflammatory stimuli mentioned above, increasing evidence supports a role for NO in the tissue damage observed in demyelinating diseases. In agreement with this notion is the finding that there is significant inducible nitric-oxide synthase (iNOS) expression in these pathologies (11, 12). Additionally, several reports have shown that oligodendroglial cells could be induced to express iNOS by proinflammatory cytokines and bacterial lipopolysaccharide and release micromolar concentrations of NO (3, 14, 15).

NO is a potentially toxic molecule that has been implicated in a wide range of diverse (patho)physiological processes in the CNS. It is known that the production of NO from L-arginine is important for intercellular signaling, nonspecific host defense, and in helping to kill tumors and invading pathogens. Massive NO formation, under certain conditions, can be cytotoxic and initiate apoptosis. In vitro studies have shown a direct toxicity of NO on oligodendrocytes, suggesting a role for NO in cell injury (16, 17). NO cytotoxicity may result in the production of peroxynitrite, a highly reactive product of the reaction between NO and superoxide. Nitrosylation of proteins, peroxidation of lipids, S-nitrosylation of thiol groups, and inhibition of mitochondrial activity are among the cytotoxic effects resulting from exposure to NO/peroxynitrite and other reactive nitrogen species. Because NO is a highly reactive molecule that can interact with a variety of cellular components, it appears likely that damage to several key cellular constituents may lead to the final demise of the cell. One likely critical site for injury is...
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DNA. NO can react with DNA via multiple pathways. Once produced, subsequent conversion of nitric oxide to nitrous anhydride can lead to the nitrosative deamination of DNA bases, such as guanine and cytosine. Complex oxidation chemistry can also occur causing DNA base and sugar-oxidative modifications (18). In a previous study from our laboratory (19), it was shown that, in pancreatic β-cell cultures, NO damages mtDNA to a greater extent than nuclear DNA. Additionally, Ballinger et al. (20) showed that, following exposure to NO and peroxynitrite in vascular endothelial and smooth muscle cells, mtDNA was preferentially damaged relative to the nuclear β-globin gene, resulting in decreased cellular ATP levels and mitochondrial redox function. Mutations and deletions in mtDNA, which could arise from unrepaired DNA damage, have been linked to a variety of diseases and aging. Moreover, alterations in the mitochondrial genome, which influence electron transport, could affect cells through the initiation of progressive cell death. Neurodegenerative diseases, including MS, are associated with a progressive loss of cells (21). Previously, we found a correlation between the induction of apoptosis by oxidative stress and repair of mtDNA in oligodendrocytes (22). In the present study, we investigated the mtDNA damage caused by reactive nitrogen species produced after iNOS induction by cytokines. Additionally, we targeted the DNA repair enzyme 8-oxoguanine DNA glycosylase (hOGG1) with intrinsic AP lyase activity, which may be crucial for the repair of damage to the DNA sugar-phosphate backbone, to mitochondria of oligodendrocytes. Overexpression of hOGG1 in mitochondria of oligodendrocytes had a protective effect against NO-mediated mtDNA damage and increased cellular survival following treatment with cytokines.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfection, and Treatment—Rat oligodendrocytes were harvested and cultured as previously described (23). Differentiation of oligodendrocytes was assessed by immunofluorescence microscopy, using antibodies to surface antigens (A2B5, O4, O1), in which sequential and partially overlapping expression defines early precursor, late precursor, and terminally differentiated oligodendrocyte stages, as described previously in detail (22). For several experiments, a vector containing a mitochondrial transport sequence (MTS) upstream of the sequence for hOGG1 was transfected into the cells. The construct was described previously (22). Cells were transfected with a combination of 2 μg of DNA/μl of FuGENE 6 (Roche Diagnostics) according to the specification of the manufacturer. The highest levels of recombinant protein expression were observed 24–72 h after transient transfection. The efficiency of transfection and the localization of the recombinant protein were described as previously (22). Transfected or untransfected cells were exposed to 50 ng/ml TNFα (R & D Systems, Minneapolis, MN) and 25 ng/ml IFNγ (eBioscience, San Diego, CA) individually or in combination for 24, 48, or 72 h.

NO Determination—The intracellular production of NO was determined as the accumulation of nitrite (a stable oxidation product of NO) in the culture medium using the Griess method (24). Aliquots of 100 μl of culture medium were mixed with an equal volume of the Griess reagent (0.1% (1-naphthyl)ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid). The absorbance at 550 nm was measured using a spectrophotometer.

Assay for mtDNA Damage Detection by Southern Blot—DNA extraction and LM-PCR conditions have been previously described in detail (28). Briefly, untreated and alkali-treated DNA from each sample was subjected to ligation-mediated PCR. Primer extension and ligation were all performed using the procedure initially described by Pfeifer et al. (26, 27) with certain modifications (28). After ligation, the reaction products were precipitated and then resuspended in H2O. PCR amplification was performed as previously described (26, 27), the only exception being the use of 5 pmol/μl primer pairs for the oligonucleotide probe sequences at the mtDNA light-strand and heavy-strand ends.

DNA extraction and LM-PCR conditions have been previously described (25), using the Poisson expression primer, which recognizes the 5′-ATACATCCAAGGCACGT- TAGATCTACCC-3′ from the sense strand and 5′-CACGGTATAT- TCTCTCAGTCCTCTCAAGTC-3′ from the antisense strand. The 516-bp LM-PCR product recognizes the 10.8-kb restriction fragment when hybridized to rat DNA digested with BamHI. Autoradiograms were densitometrically scanned. The break frequency was determined, as previously described (25), using the Poisson expression s = −lnPb, where s is the number of breaks/fragment and Pb is the frequency of fragments, free of breaks.

 Assay for mtDNA Damage at the Nucleotide Level by Ligation-mediated PCR—DNA extraction and LM-PCR conditions have been previously described in detail (28). Briefly, untreated and alkali-treated DNA from each sample was subjected to ligation-mediated PCR. Primer extension and ligation were all performed using the procedure initially described by Pfeifer et al. (26, 27) with certain modifications (28). After ligation, the reaction products were precipitated and then resuspended in H2O. PCR amplification was performed as previously described (26, 27), the only exception being the use of 5 pmol/μl primer pairs for the oligonucleotide probe sequences at the mtDNA light-strand and heavy-strand ends.

western blot analysis—Whole cell protein lysates, nuclear, mitochondrial, or cytosolic protein fractions, obtained as described previously (22), were used for Western blot assays. Protein concentration was determined using the Bio-Rad protein dye microassay according to the manufacturer’s recommendation. SDS-polyacrylamide gel electrophoresis and transfer of separated proteins to polyvinylidene difluoride membrane were performed by standard procedures. Blocking and antibody immunoblotting were performed in 6% nonfat dry milk, Tris-buffered saline (TBS)/0.1% Tween 20 (TBS-T). TBS-T and TBS were used for washing. The monoclonal anti-INOS/NOS type II antibodies were from BD Biosciences; the polyclonal anti-hOGG1 antibodies were from Novus Biologicals (Littleton, CO); anti-cytochrome c monoclonal antibody was purchased from Pharmingen; the anti-lamin B1 polyclonal antibodies were from Santa Cruz Biotechnology; the antisemur to caspase-9 was from Cell Signaling Technology (Beverly, MA); and the anti-actin antibody was from Sigma. The immune complexes formed by these antibodies were detected with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG antibodies (Promega, Madison, WI) using chemiluminescent reagents (SuperSignal; Pierce, Rockford, IL).

TUNEL Assay—The presence of DNA breaks were evaluated by a TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling) procedure as described previously (29). For detection and quantitation of apoptotic cells, the DeadEnd fluorometric TUNEL system from Promega was used. It measures the fragmented DNA of apoptotic cells by catalytically incorporating fluorescein-12-dUTP at 3′-OH DNA ends using the enzyme terminal deoxynucleotidyltransferase. The assay was performed according to the manufacturer’s procedure. Briefly, cells were grown on slides, fixed in 4% methanol-free formaldehyde, and permeabilized with 0.2% Triton X-100. Thereafter, DNA strand breaks were labeled with fluorescein-12-dUTP by the terminal deoxynucleotidyltransferase enzyme, and the nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). The number of nuclei-bound fluorescein (fluorescein-12-dUTP)/100 blue DAPI-stained nuclei was calculated by fluorescence microscopy.

Caspase-8 and -9 Activity Assay—After cytokine treatment, cells were rinsed with 1× phosphate-buffered saline and collected via centrifugation at 200 × g for 10 min. Caspase-8 and -9 activities were measured using colorimetric assay kits (R & D Systems, Minneapolis, MN) and a Bio-Rad microplate reader. Cells were incubated in 10,000 × g for 1 min, and 200 μg of supernatant proteins were incubated with caspase-8-specific or caspase-9-specific substrates. The assay was read on a microplate reader using a 405-nm wavelength of light.

Data Analysis—All statistical analyses were performed using the Student’s t test to compare individual means with significant differences at a confidence level of p < 0.05.
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RESULTS

Several studies have reported that rat oligodendrocytes produce nitric oxide as a result of the induction of the type II iNOS gene (5, 13). In agreement with these reports, our experiments utilizing the combination of TNFα (50 ng/ml) and IFNγ (25 ng/ml) stimulated NO production in oligodendrocytes. As shown at Fig. 1A, NO production was determined as nitrite released into the cell medium using a colorimetric method (see “Experimental Procedures”). To evaluate the expression of iNOS in cytokine-treated cultures, we carried out immunoblot analyses using antibodies specific for iNOS. The membrane was cut, and the lower part was blotted with anti-actin antibody for equal loading. A representative blot is shown at Fig. 1B.

To identify the specific pattern of cytokine-mediated mtDNA damage at the nucleotide level, LM-PCR was performed on a 200-bp sequence from the heavy strand of mtDNA from treated oligodendrocytes. This sequence contained one of the break points for the 5-kb “common deletion” that accumulates in mtDNA with aging (30, 31). Previously, we have shown that NO can modify DNA by causing the deamination of purines, whereas reactive oxygen species generated by the enzymatic reaction of xanthine oxidase and substrate hypoxanthine cause modifications in both purines and pyrimidines (28). The pattern of cytokine-induced mtDNA damage was compared with the pattern of damage produced by PAPA/NO, xanthine oxidase/hypoxanthine, and peroxynitrite (Fig. 4). Using a Maxim-Gilbert sequencing ladder to identify bases, it appeared that most of the damage caused by PAPA/NO involved guanines, whereas reactive oxygen species generated by the enzymatic reaction of xanthine oxidase and substrate hypoxanthine cause modifications in both purines and pyrimidines (28). The pattern of cytokine-induced mtDNA damage was compared with the pattern of damage produced by PAPA/NO, xanthine oxidase/hypoxanthine, and peroxynitrite (Fig. 4).

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Recently, we reported (22) that targeting of hOGG1 to mitochondria in oligodendrocytes enhanced the repair of oxidative lesions in mtDNA. Oligodendrocytes were transiently transfected with a vector containing a MTS upstream of the sequence for human OGG1 or empty vector for control. Previously, localization of recombinant protein was confirmed by fluorescence microscopy (22). Fig. 6 shows that expression of the OGG protein is increased in the mitochondrial (but not nuclear) protein fraction of MTS-hOGG1-transfected cells as opposed to control transfectants.
compared with vector-only-transfected cells. Anti-lamin B1 and anti-cytochrome c antibodies were used as loading controls as well as a control for the purity of fractions.

Quantitative Southern blot analysis showed a significant decrease in cytokine-induced mtDNA damage in MTS-hOGG1-transfected oligodendrocytes compared with cells containing only vector (Fig. 7A). The break frequency/10.8-kb fragment is shown on Fig. 7B.

Next, we investigated whether the observed increase in mtDNA repair influences viability of the cells following TNFα and IFNγ treatment. The DeadEnd fluorometric TUNEL system used for the specific detection and quantitation of apoptotic cells revealed that MTS-hOGG1-transfected oligodendrocyte cultures had fewer apoptotic cells (17% ± 5.2), compared with cultures containing vector-only cells (31% ± 3.6) after 48 h of cytokine treatment. These results show that targeting the repair enzyme hOGG1 to the mitochondria of oligodendrocytes significantly protects against the induction of apoptosis by 48 h after TNFα and IFNγ treatment.

To determine the pathway of apoptosis affected by enhanced mtDNA repair, we examined the activation of both caspase-8 and -9 in MTS-hOGG1- and vector-transfected oligodendrocytes following cytokine treatment. The induction of apoptosis...
through the extrinsic death receptor mechanism resulted in the activation of caspase-8, whereas the intrinsic mitochondrial death signal led to the activation of caspase-9. Previously, it had been reported (32) that TNFα and IFNγ induce the expression of the Fas (death-receptor) in primary oligodendrocyte cultures, and cytokine-induced apoptosis mainly goes through the extrinsic pathway. In our study, colorimetric activity assays based on cleavage of caspase-8 (33) or caspase-9 (34) specific substrate were performed. For a positive control, Jurkat cells were treated with camptothecin. Fig. 8 shows that 48 h after TNFα and IFNγ treatment, both caspase-8 and -9 were activated in MTS-hOGG1 and vector cells. But intriguingly, following cytokine induction, caspase-9 activity in vector-only-transfected oligodendrocytes was significantly elevated compared with MTS-hOGG1 transfectants.

Further, to confirm that overexpression of hOGG1 in mitochondria of oligodendrocytes led to a substantial inhibition of the activation of caspase-9 following exposure to cytokines, we performed Western blot analysis using an antibody against cleaved caspase-9. Cytosolic protein fractions of control and TNFα + IFNγ-treated samples from MTS-hOGG1 and vector-only-transfected oligodendrocytes were subjected to immunoblotting. A specific antibody that detects cleaved (activated) 40-kDa and 38-kDa subunits of caspase-9 was employed. As shown in Fig. 9, the bands corresponding to the cleaved subunits of caspase-9 were markedly increased in vector-only samples as compared with MTS-hOGG1 transfectants following cytokine induction. To ensure equal loading, the membranes were reblotted with an anti-actin antibody.

**DISCUSSION**

The results of the present investigation show that endogenous NO production following cytokine-induced iNOS expression can cause mtDNA damage in oligodendrocytes. Furthermore, targeting of the specific DNA repair protein (hOGG1) to mitochondria of oligodendrocytes decreases the sensitivity of mtDNA to NO-mediated damage and protects these cells against apoptosis.

In the CNS, astrocytes, microglia, and immune-derived cells all release inflammatory cytokines and cytotoxic mediators that may damage and destroy myelinating oligodendrocytes and their progenitors. A substantial body of evidence supports the involvement of cytokine-induced iNOS-mediated NO production in CNS-demyelinating diseases (35, 36, 11, 12). It is now apparent that two proinflammatory cytokines, TNFα and IFNγ, known to accumulate in the brain and cerebrospinal fluid of MS patients, play prominent roles in the immunopathogenesis of the disease (7, 8). In the present study, we observed that treatment of oligodendrocytes with the combination of TNFα and IFNγ resulted in the expression of iNOS and the concomitant production of NO. This observation is in agreement with previous reports, which show that cultured rat oligodendrocytes express iNOS and release NO following activation with lipopolysaccharide/IFNγ (5, 14) and that the central

**Fig. 6.** Expression of the recombinant protein in mitochondria of the transfected cells. 10 μg of mitochondrial (Mito) and nuclear proteins from the MTS-OGG-transfected (OGG) and vector-only-transfected (V) oligodendrocytes were subjected to SDS-PAGE, and the separated proteins were transferred to a polyvinylidene difluoride filter. The upper section of the membrane was incubated with antibody against Lamin B (70-kDa marker for nuclear equal loading). Middle section, human OGG1 (39 kDa); lower section of the membrane, with anti-cytochrome c antibody (15-kDa mitochondrial marker). The results are representative of three independent transfection experiments.

**Fig. 7.** Overexpression of the DNA repair enzyme hOGG1 in mitochondria of oligodendrocytes prevents cytokine-induced mtDNA damage. Oligodendrocytes were transfected with a vector containing a MTS upstream of the sequence for human OGG1 (OGG) and empty pcDNA3.0neo (V) for control. Both cultures were treated with TNFα (50 ng/ml) and IFNγ (25 ng/ml) for 24, 48, and 72 h. High molecular weight DNA was isolated and digested to completion with BamHI. Samples were exposed to 0.1N NaOH prior to Southern blot analysis and hybridization with a PCR-generated mtDNA probe. A, representative autoradiograph from cells treated 48 h (c, untreated control; tr, cytokine treated). B, break frequency/10.8-kb mtDNA restriction fragment. Autoradiographic bands representing a 10.8-kb mtDNA fragment were densitometrically scanned. The break frequency was determined using the Poisson expression \( s = -\ln P_b \), where \( s \) is the number of breaks/fragment and \( P_b \) is the fraction of fragments, free of breaks. Data are means ± S.E. of three separate experiments. *, \( p < 0.05 \) when the data from MTS-OGG transfecteds was compared with vector-only cells using Student’s t test.

**Fig. 8.** Activation of caspase-8 and -9 after cytokine treatment. MTS-hOGG1 (OGG)- and vector-only (V)-transfected oligodendrocytes were treated with TNFα (50 ng/ml) and IFNγ (25 ng/ml) for 48 h. The cells were rinsed with 1 × phosphate-buffered saline, collected via centrifugation, and lysed on ice for 10 min. The lysate was then centrifuged at 10,000 × g for 1 min and 200 μg of supernatant proteins incubated with caspase-9- or caspase-8-specific substrate. The assay was read on a microplate reader using 405-nm wavelength light. Data are means ± S.E. of three separate experiments. *, \( p < 0.05 \) when the data from MTS-OGG transfectants was compared with vector-only cells using Student’s t test. For a positive control, Jurkat cells (Jur) treated with 10 μM camptothecin for 5 h were used.
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NO is a highly reactive, potentially toxic molecule that can interact with a variety of cellular components. One likely critical site for injury is DNA. In the present work, we found that exposure of oligodendrocytes to the combination of TNFα and IFNγ induced mtDNA damage, which correlated with cytokine-induced NO production and the expression of iNOS. That this damage was due to the generation of NO was supported by the observation that it was attenuated by treatment with the inhibitor of iNOS, aminoguanidine. We were not able to detect appreciable DNA damage in similarly sized fragments of nuclear DNA. It has been reported that iNOS-mediated endogenous generation of NO in mammalian cells does not appreciably increase the steady-state level of nuclear DNA damage (37). However, studies from our laboratory and those of others have shown that mtDNA is vulnerable to NO-mediated damage (19, 20, 27, 38). Although in this study we cannot rule out the contribution of the nucleus to the damage to the intact or isolated nuclei, the results are representative of functional and protein changes in mtDNA.

DNA to DNA can occur through reactions with nitrous anhydride (N₂O₃), formed by the reaction of NO with molecular oxygen (O₂), or reactions with peroxynitrite (ONOO⁻), formed by the combination of NO with superoxide (39). Peroxynitrite can oxidize and nitrate DNA and may cause single-strand breaks through attack on the sugar-phosphate backbone (18). N₂O₃ can nitrosate amines to form N-nitrosamines, which, after metabolic activation, can alkylate purines. Additionally, N₂O₃ can cause nitrosation of primary amines in DNA bases, which leads to the formation of diazonium ions and subsequent deamination (18). Deamination of cytosine, adenine, and guanine may cause mutations in cells (40). Furthermore, xanthine (produced by the deamination of guanine) and hypoxanthine (produced by the deamination of adenine) are unstable and can form abasic sites that are both toxic and mutagenic (40, 41).

Previously, analysis of a 202-bp sequence of mtDNA by the technique of LM-PCR showed that exogenous NO generated by PAPANO damaged specific guanines and adenines (28). NO damage was detected to any pyrimidines in the sequence evaluated. For comparison, studies with the reactive oxygen species generator xanthine oxidase/hypoxanthine showed that we were able to detect a reproducible pattern of oxidative lesions that encompassed both purines and pyrimidines (28). In this study, we compared the pattern of nucleotide damage caused by the cytokines with the patterns produced by NO (PAPANO), reactive oxygen species (xanthine oxidase/hypoxanthine), and peroxynitrite.

The pattern of damage to mtDNA nucleotides produced by TNFα and IFNγ is virtually identical to that seen with peroxynitrite, suggesting that this molecule is the predominant damaging species. The spectrum of peroxynitrite DNA damage tends to be much more complex than that caused by N₂O₃. The two main types of base modification attributed to ONOO⁻ are oxidations and nitrations (42). Also, it has been shown that peroxynitrite can cause DNA strand breaks (43). Mitochondria constitute a primary site for the intracellular reaction of peroxynitrite with DNA. Although these organelles possess scavenging and repair systems for peroxynitrite-dependent oxidative modifications, our data reveal that they can be overwhelmed under enhanced cellular formation of NO, as well as under conditions that lead to augmented superoxide formation, such as through defects in electron transport chains (44).

Recently, we and others have reported (22, 45, 46, 47) that targeting of bifunctional glycosylase/AP lyases to mitochondria of various cells enhances the repair of oxidative lesions in mtDNA. In the present study, we observed that oligodendrocytes transfected with hOGG1, containing a MTS, showed a significant decrease in mtDNA damage following cytokine exposure compared with the cells transfected with vector only. The attenuation of cytokine-induced mtDNA damage by overexpression of hOGG1 in mitochondria may be explained by the AP lyase activity of OGG1. This activity may be crucial for the repair of damage to the sugar-phosphate backbone, which comprises a significant proportion of the total lesions in DNA following oxidative stress (48). The importance of the protective role of AP lyase activity against oxidative damage is suggested by the results that the bacterial enzymes endonuclease III (Endo III) and endonuclease VIII (Endo VIII), which have AP lyase activity but glycosylase activity for lesions other than 8-oxoguanine, protected mtDNA in HeLa cells from oxidative damage caused by exposure to menadione (46).

It has been reported that oligodendrocyte progenitors are particularly vulnerable to the activation of the cell death program following exposure to the combination of TNFα and IFNγ (32). Our results using TUNEL labeling showed that apoptosis was attenuated in MTS-hOGG1-transfected oligodendrocytes as compared with vector-only-containing cells 48 h following cytokine treatment. The finding that targeting hOGG1 to mitochondria of oligodendrocytes reduced peroxynitrite-induced apoptosis supports our previous report in which we found that overexpression of hOGG1 in oligodendrocytes protected cells against apoptosis caused by an oxidative insult (22). These findings allowed us to suggest that damage to mtDNA may be an early step in the induction of apoptosis. Our data employing a colorimetric activity assay revealed that 48 h after TNFα and IFNγ treatment, both caspase-8 and -9 were activated in both MTS-hOGG1- and vector-containing cells. However, caspase-9 activity in MTS-hOGG1 transfectants was significantly diminished compared with the vector-only-transfected oligodendrocytes. The majority of oligodendrocytes express TNF receptors, which recognize their specific ligands, resulting in the activation of the respective death receptors, followed by activation of caspase-8 (49). An increased expression of the death receptor Fas in cytokine-treated cultures of oligodendrocytes has been shown by others (31). It has recently become apparent that apoptotic signals coming from activated receptors can be amplified by mitochondria-dependent apoptotic pathways. For instance, caspase-8 can cleave the Bcl-2 family member Bid, and the truncated Bid is able to translocate from the cytosol to mitochondria and induce cytochrome c release followed by caspase-9 activation and thus amplify the apoptotic signal (50).
Our data indicate that cytokine-mediated mtDNA damage may be an additional stimulus that can activate caspase-9 to augment the apoptotic signal from caspase-8. The exact mechanism by which mtDNA damage caused by reactive nitrogen species may enhance cytokine-induced apoptosis in oligodendrocytes remains to be fully elucidated. However, it appears reasonable to speculate that, because mitochondria have a central role in energy metabolism and redox regulation, the accumulation of unreppaired lesions in mtDNA would cause an alteration in the transcription of mtDNA and modify the flow of electrons by changing key electron transport complexes (51) leading to defects in oxidative phosphorylation and impaired ATP/ADP exchange. This mitochondrial dysfunction would ultimately lead to the activation of the mitochondrial portion of the programmed cell death pathway. Overexpression of the DNA repair enzyme hOGG1 in mitochondria decreased the ratio of unrepaircd mtDNA damage and thus prevented apoptotic signaling by limiting the amount of mitochondrial dysfunction. Clearly, further exploration is required to fully understand the precise role of unrepaircd mtDNA damage in induction of the apoptosis.

Inflammatory and demyelinating diseases of the CNS, such as MS, are associated with a progressive loss of cells. Cytokines, which are major effector molecules in many disease processes, stimulate iNOS-mediated excessive production of NO in oligodendrocytes. This can cause NO-dependent mtDNA damage, which can contribute to the induction of apoptotic cell death. Targeting of a specific DNA repair enzyme to mitochondria decreases the ATP/ADP exchange. This mitochondrial dysfunction would ultimately lead to the activation of the mitochondrial portion of the programmed cell death pathway. Overexpression of the DNA repair enzyme hOGG1 in mitochondria decreased the ratio of unrepaircd mtDNA damage and thus prevented apoptotic signaling by limiting the amount of mitochondrial dysfunction. Clearly, further exploration is required to fully understand the precise role of unrepaircd mtDNA damage in induction of the apoptosis.

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