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SELECTIVE DEGENERATION OF OLIGODENDROCYTES MEDIATED BY REACTIVE OXYGEN SPECIES

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The mechanism underlying demyelination in inflammatory canine distemper encephalitis is uncertain. Macrophages and their secretory products are thought to play an important effector role in this lesion. Recently, we have shown that anti-canine distemper virus antibodies, known to occur in chronic inflammatory lesions, stimulate macrophages leading to the secretion of reactive oxygen species (ROS). To investigate whether ROS could be involved in demyelination, dog glial cell cultures were exposed to xanthine/xanthine oxidase (X/XO), a system capable of generating O_2^-. This treatment resulted in a specific time-dependent degeneration and loss of oligodendrocytes, the myelin producing cells of the central nervous system. Initial degeneration was not associated with a decrease in viability of oligodendrocytes as judged by trypan blue and propidium iodide exclusion. Astrocytes and brain macrophages were not affected morphologically by this treatment. Further, an evaluation of the effect of several ROS scavengers, transition metal chelators and inhibitors of poly (ADP-ribose) polymerase suggests that a metal dependent formation of ·OH or a similar highly oxidizing species could be responsible for the observed selective damage to oligodendrocytes.

KEY WORDS: Cytotoxicity, demyelination, dog glial cell cultures, oligodendrocytes, oxygen radicals.

INTRODUCTION

The mechanisms underlying demyelination in the central nervous system of dogs suffering from canine distemper encephalitis, an animal model for human demyelinating diseases,[1] are still unclear. Macrophage-like cells, also referred to as microglial cells[2,3] and their secretory products have been suggested to be partly responsible for the severe necrosis observed in the white matter of animals with distemper that develop chronic inflammatory lesions.[4,5] Recently, we have shown that anti-canine distemper virus antibodies binding to canine distemper virus infected glial cells are able to stimulate brain macrophages *in vitro*, leading to the release of reactive oxygen species (ROS), potentially harmful products.[4,5] It is likely that such events also occur *in vivo* since canine distemper virus infected glial cells, macrophages and Ig-bearing cells, (known to produce antiviral antibody[6,7]) are present in close proximity in inflammatory lesions in distemper.[6] Our results *in vitro* therefore supported the concept of bystander demyelination in canine distemper encephalitis.[8] Superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are the primary reduction products generated by activated macrophages.[9] In the presence of available transition metals

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such as iron or copper, O_2^- and H_2O_2 can give rise to the highly reactive hydroxyl radical (·OH) and other ROS.[10] Hydroxyl radicals are known to cause considerable tissue damage through reaction with DNA, protein and membrane lipids.[10-13] The brain has a high oxygen consumption, is rich in iron[14-16] and contains large amounts of polyunsaturated fatty acids that are readily oxidized.[17] Myelin, the major component of the white matter of mammalian brain, contains 15–30% proteins and 70–85% lipids.[18] Exposure of isolated myelin to ROS *in vitro* leads to damage to its protein[19,20] and lipid.[21,22]

Since the above described tissue destruction associated with inflammation is predominantly seen in white matter lesions in canine distemper,[6] the question must be raised whether myelin, or oligodendrocytes (the myelin producing cells) are particularly susceptible to ROS. To address this question, we have exposed primary cultures of dog brain cells to an extracellular source of O_2^- and examined, based on morphological observations, the effect of this type of oxidative stress on oligodendrocytes, astrocytes and brain macrophages, the three main cell types present in these cultures.[4,5,23,24] The results obtained clearly show that oligodendrocytes, which — in differentiated cultures — produce all myelin products and large myelin membranes[25] are more susceptible to ROS than other glial cells *in vitro*. An evaluation of the effect of ROS scavengers, transition metal chelators and inhibitors of poly (ADP-ribose) polymerase, indicates that metal ion-dependent formation of ·OH, or a similar highly oxidizing species,[10] seems to be responsible for the observed damage to oligodendrocytes.

MATERIALS AND METHODS

Dog Brain Cell Cultures

Primary cell cultures from dog cerebella were prepared as described previously.[23] Briefly, cerebella from neonatal dogs were dissociated mechanically and seeded at 1–1.5 × 10^6 cells/ml in Petri-dishes (3003, Falcon) containing several glass-coverslips (18 × 18 mm, Assistent) that were mounted onto the Petri-dish with silicon grease. Cultures were maintained in Dulbecco's modified Eagle medium (DMEM, Gibco BRL) supplemented with 10% fetal calf serum, penicillin (250U/ml) and streptomycin (100 μg/ml) and incubated at 37°C in a water-saturated atmosphere of 5% CO_2 and 95% air. Medium was changed every other day. Cells were used at day 16.

Exposure of Brain Cells to O_2^-

After 16 days of culture, glial cells were rinsed twice with Ca^{++} — and Mg^{++} — free Hanks balanced salt solution (HBSS, Gibco BRL)[26] before DMEM (pH 7.4) containing xanthine (X, 160 μM, Sigma) was added. Production of O_2^- was initiated by the addition of xanthine oxidase (XO, 4 Units/ml, Sigma) to the medium.[27] Under these conditions 2.5 ± 0.5 nmol O_2^- are formed per minute as determined by the superoxide dismutase-inhibitory reduction of ferricytochrome C.[28] After an initial exposure of the cells to O_2^- for 1 hr, some coverslips were taken from 2 separate Petri-dishes and harvested while the remaining cells were washed once with DMEM before being re-exposed to O_2^- for an additional 1 or 5 hrs using fresh prepared X/XO containing DMEM. Cells incubated with either DMEM, DMEM plus X, or DMEM plus XO

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served as controls. Experiments were repeated 4 times. The harvested cells were fixed and stained for oligodendrocytes (using the monoclonal antibody (MAB) 1 6G1 described below), astrocytes and brain macrophages. The morphological appearance of oligodendrocytes, astrocytes and brain macrophages was evaluated light microscopical (using 40 × magnification) by examining 10 equally sized fields ($0.24\,mm^2$) that were chosen arbitrarily on each coverslip. Based on repeated observations,[23,24] oligodendrocytes with extensive branching and well-defined cytoplasm were considered as normal whereas cells with few short processes and ill-defined cytoplasm were regarded as degenerated. Selected coverslips were incubated in addition with propidium iodide[29](PI) and trypan blue, both accepted indicators for cell cytotoxicity.

Effect of Oxygen Radical Scavengers, Transition Metal Chelators and Inhibitors of Poly (ADP-ribose) Polymerase

The oxidant scavengers superoxide dismutase (SOD), catalase, mannitol, thiourea and the transition metal chelators diethylene triamine penta acetic acid (DETAPAC) and EDTA, as well as the inhibitors of poly (ADP-ribose) polymerase were obtained from Sigma. Poly (ADP-ribose) polymerase, an enzyme activated under certain conditions of DNA damage can be inhibited by theophylline, 3-aminobenzamide or nicotineamide.[30] Desferrioxamine (Desferal®) was supplied by Ciba-Geigy AG (Switzerland). Cells in 2 separate Petri-dishes were exposed to X/XO for two times 1 hr in the absence (control) or presence of the various scavenging compounds at concentrations indicated in Table I. After exposure, coverslips were harvested and oligodendrocytes (anti-MAG), astrocytes (anti-GFAP) and brain macrophages stained as described above. Morphology of MAG-positive oligodendrocytes was judged double-

TABLE I

Effect of oxygen radical scavengers, transition metal chelators and inhibitors of poly (ADP-ribose) polymerase on degeneration of oligodendrocytes in dog glial cell cultures. After treatment of dog glial cell cultures with xanthine/xanthine oxidase and oxygen scavengers, metal chelators and enzyme inhibitors, MAG-positive oligodendrocytes were judged double-blindly and classified in three groups according to their morphological appearance. (+) Cells that have lost their peripheral fine branching of processes, (++) cells with major loss of processes and cytoplasmic protrusions, (+++) cell-fragments without processes.

Scavenged species	Scavenger	Concentration	Damage
O_2^-	SOD	100 U/ml	++
H_2O_2	Catalase	100 U/ml	++
$H_2O_2 + O_2^-$	SOD/Catalase	100 U/ml	+
·OH	Mannitol	50 mM	+
O	Thiourea	10 mM	+
	Theophylline	2.5 mM	+++
Poly(ADP-ribose) polymerase	3-Aminobenzamide	2.5 mM	+++
	Nicotineamide	5 mM	++
	DETAPAC	50 μM	+
Fe^{++}	Desferal®	50 μM	+
	EDTA	50 μM	++
None			+++

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blindly and the cells classified in three groups defined as follows: (+) Cells that have lost their peripheral fine branching of processes, (++) cells with major loss of processes and cytoplasmic protrusions, (+++) cell-fragments or oligodendrocytes without processes.

Antiglial Antibodies

Oligodendrocytes were visualized using two different antibodies. The human antibody against myelin-associated glycoprotein (MAG), binds strongly and selectively to cultured canine oligodendrocytes.[23,24,31] The murine MAB 1 6G1 (kindly provided by Dr. M. Dumas, Inselspital, 3010 Bern) was shown previously to bind to galactocerebroside[23] (GalC), a well established marker for oligodendrocytes.[32] For the demonstration of astrocytes, a commercially available antiglial fibrillary acidic protein (GFAP) antibody (Dakopatts) was used. To visualize astrocytes *within* cell preparations that had been stained previously for oligodendrocytes with anti-MAG, coverslips were subjected to additional staining with hematoxylin. Brain macrophages were detected using their ability to form rosettes with IgG-coated erythrocytes.[33]

Immunocytochemistry

At the time points indicated, coverslips from treated and untreated cultures were harvested and the cells fixed with either 3.7% phosphate buffered formalin for 20 min (anti-GalC) or with ethanol:acetic acid (95:5, v:v) for 5 min at −20°C (anti-MAG and anti-GFAP). To visualize the above mentioned markers, both the unlabeled antibody peroxidase anti-peroxidase[34] (PAP) and the indirect immunofluorescence assay were applied as described previously.[5,24] MAB 1 6G1 was combined with rabbit anti-mouse IgG-fluorescein isothyocyanate (FITC, Cappel) whereas treatment with either anti-GFAP or anti-MAG was followed with the corresponding second antibody-layer and PAP using diaminobenzidine as chromogen.

Cytotoxicity Assay

Cell viability was assessed using trypan blue (47285, Serva) and propidium iodide (PI, 33671, Serva). Both substances are normally excluded by the intact cells. PI is a poorly fluorescent substance that upon permeabilization of cells can intercalate with DNA, resulting in the appearance of highly fluorescent nuclei.[29] PI was added to unfixed coverslips at a final concentration of 100 μmol. To visualize oligodendrocytes, some coverslips were subjected to additional anti-GalC-FITC staining. 200 GalC-positive cells per coverslip were counted, and the percentage showing PI uptake was calculated. Trypan blue (0.5%) was added for 5 min to unfixed coverslips which were evaluated similarly.

RESULTS

Morphology of Untreated Dog Brain Cell Cultures

After 10 to 14 days of culture, dog brain cells became confluent. These cultures predominantly contained astrocytes, oligodendrocytes, brain macrophages and very

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few neurons.[4,5,23,24] Oligodendrocytes and brain macrophages were distributed regularly as a superimposed cell layer over the entire culture (Figure 1A). Throughout duration of the culture, 1-5% of the total cell population was identified as brain macrophages using the rosette assay with IgG-coated erythrocytes.[4] About 85% of the GalC-positive oligodendrocytes had a striking morphology, characterized by a sharply defined cytoplasm and cell processes that were extensively branched (Figure 1A). Also superimposed, but clearly in the minority (15%) were small, round GalC-positive cells with dense cytoplasm and fewer, unbranched processes (Figure 1A). These latter oligodendrocytes were considered as still immature. Oligodendrocytes stained with anti-MAG had a relatively small angular perikaryon and a limited number of long, extensively branched cell processes (Figure 1C).

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The Effect of Oxygen Radicals on Glial Cells

Formation of O_2^- was achieved by the application of the X/XO-system.[27,35] Using the standard concentrations of X and XO, O_2^- was produced continuously over a period of at least 60 min and at an initial rate of 2.5 ± 0.5 nmol/min, as determined by the reduction of ferricytochrome C.[36] For comparison, maximally stimulated human polymorphonuclear leucocytes at a concentration of 2×10^6/ml produce O_2^- at a rate of about 20 nmol/min.[36] The rate of O_2^- production with the X/XO system was independent of whether the reaction was carried out in phosphate buffered saline or DMEM, indicating that the latter medium does not contain compounds that react readily with O_2^- giving rise to secondary radicals.

Incubation of glial cells in tissue culture medium containing X/XO, resulted in a clear time-dependent loss of the total number of oligodendrocytes per coverslip. Thus from 27 GalC-positive oligodendrocytes present per field prior to exposure only 25, 20 and 12 GalC-positive oligodendrocytes remained after 1, 2 and 6 hrs of O_2^- treatment, respectively (Figure 2).

After a single 1 hr exposure of cells to O_2^-, 55% of GalC-positive oligodendrocytes were degenerated as judged by fluorescence microscopy (Figure 2). The remaining oligodendrocytes started to show first signs of alterations. Their cell processes became shortened and peripheral fine branching disappeared. After a second, additional 1 hr of exposure to O_2^-, 75% of the examined GalC-positive oligodendrocytes showed clear signs of pathological degeneration (Figure 1B, D). The cell processes were shortened or even lost. In addition, the cytoplasm appeared to contain small and ill-defined protrusions without sharp contours. In some focal areas oligodendrocytes had disappeared completely at this time. After 6 hrs of exposure, 85% of GalC-positive oligodendrocytes were considered as degenerated (Figure 2). In contrast, neither the morphological appearance of astrocytes nor brain macrophages were influenced by X/XO treatment for up to 6 hrs as judged by anti-GFAP (astrocytes) and erythrocyte rosette assay (brain macrophages) (Figure 3A, B). From 200 GalC-positive oligodendroglial cells, less than 1% were found to be PI-positive after 2 hrs treatment. Astrocytes did not exhibit a significant change of PI-uptake compared to the control. Comparable results were obtained when using trypan blue as an indicator for cell injury.

Injurious Species

It is well known that O_2^- produced by X/XO in aqueous solutions dismutates to O_2

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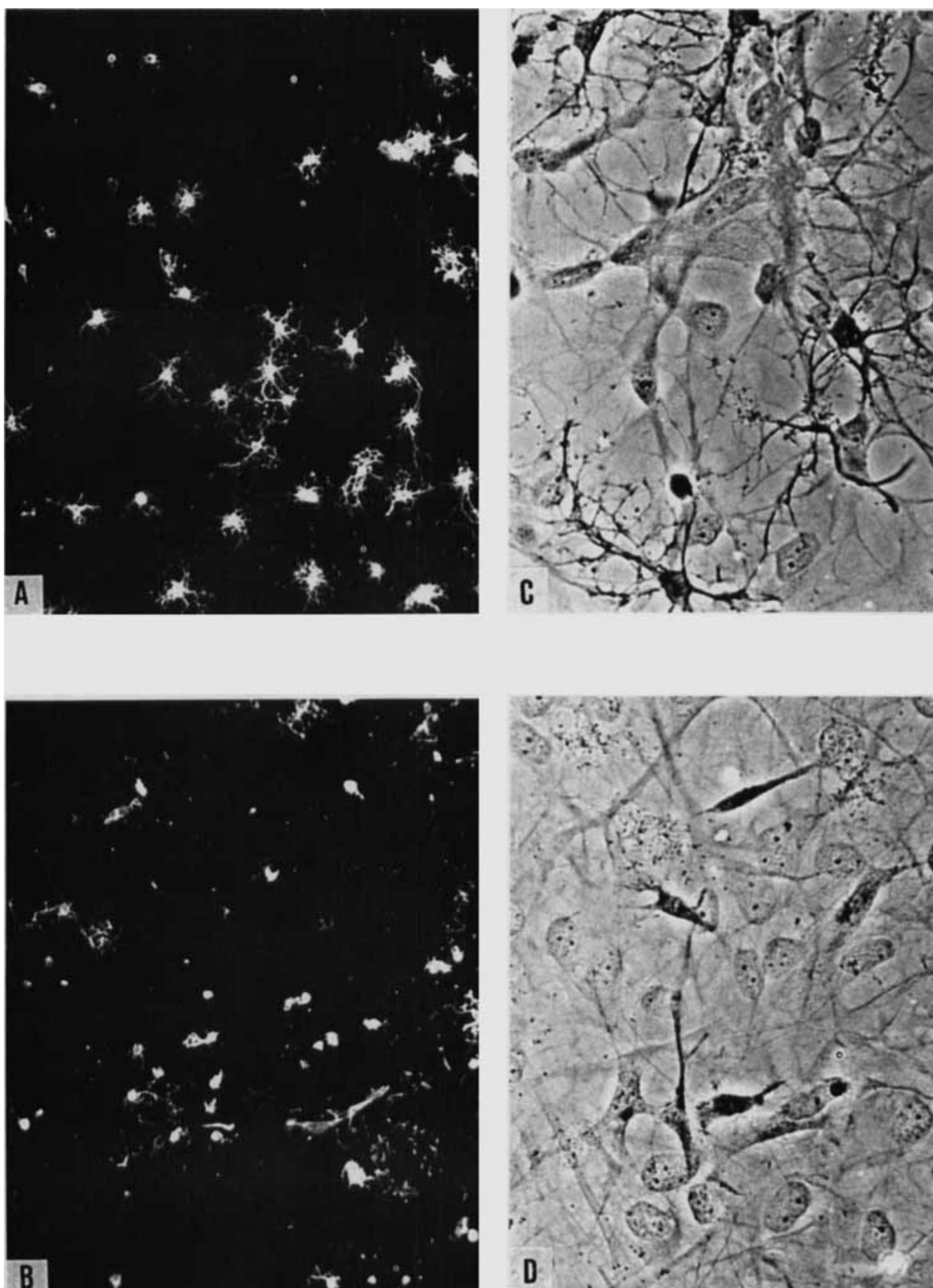

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and H_2O_2 either spontaneously or catalyzed by SOD. Furthermore, in the presence of transition metals such as iron and copper, O_2^- and H_2O_2 can give rise to the highly toxic $\cdot OH$.[10,11,13,37] To address the question whether O_2^- itself or one of the ROS produced from it is the injurious species for oligodendrocytes, we tested the effects of some enzymatic and non-enzymatic ROS-scavengers as well as transition metal chelators on the X/XO-induced cell damage.

Exposure of certain cells to H_2O_2 can cause injury through a sequence of events initiated by DNA damage with subsequent activation of poly (ADP-ribose) polymerase.[30] As this type of cell damage can be prevented by blocking poly (ADP-ribose) polymerase,[38] we also tested the effect of the known inhibitors 3-aminobenzamide, nicotineamide and theophylline on X/XO-mediated damage to oligodendrocytes. No inhibitory effect was seen when glial cells were exposed to O_2^- in the presence of either theophylline or 3-aminobenzamide while nicotineamide provided some protection (Table I). A partial protective effect was observed when either SOD, catalase or EDTA alone was present (Figure 4A). In these cases, oligodendrocytes mainly showed a loss of cell processes and some cells had also ill-defined cytoplasm contours. Best protection was achieved when a combination of SOD and catalase or either mannitol, thiourea, DETAPAC or desferrioxamine alone were present (Table I). Under these conditions oligodendrocytes showed only minor signs of degeneration characterized by disappearance of fine branching processes only (Figure 4B). Astrocytes and brain macrophages were *not* affected morphologically by these treatments.

DISCUSSION

To date, only a few substances are known to exhibit toxic effects on oligodendrocytes *in vivo* or *in vitro* such as tumor necrosis factor,[39,40] cuprizone,[41] astrocytic cytotoxic factor,[42] complement[29] and anti-myelin antibodies.[43] In this study, we demonstrate that oligodendrocytes, present in large numbers in glial cell cultures prepared from dogs, are obviously the main target for oxygen free radicals produced by the X/XO-system. In contrast, treatment with X/XO had no effect on the *morphological appearance* and *number* of other glial cells (such as astrocytes and brain macrophages) also present in the cell culture. The PI and the trypan blue studies also showed that the treatment with X/XO had no general cytotoxic effect within the time span of the experiment. While oligodendrocytes were damaged primarily in their periphery (processes), few cells actually appeared to be non-viable, when examined by staining. Cell loss was probably due to detachment of damaged oligodendrocytes as these cells are superimposed on the other cell types present in these cultures.[23]

FIGURE 1 Morphological appearance of oligodendrocytes in normal and X/XO treated dog glial cell cultures. A: Oligodendrocytes immunostained with murine monoclonal antibody 1 6G1 (anti-GalC)-rabbit anti-mouse IgG-fluorescein isothyocyanate in *untreated* normal dog glial cell cultures. Many labeled GalC-positive oligodendrocytes can be seen with profusely branching cell processes covering large areas of the culture, × 100. B: Oligodendrocytes after 2 hrs incubation in X/XO-containing medium. Immunostaining is performed as in A. Most of the GalC-positive cells show severe signs of degeneration, × 100. C:MAG-positive oligodendrocytes in *untreated* control culture with sharply defined long slender branching processes. The underlaying cell layer, consisting mainly of astrocytes is faintly stained with hematoxylin, PAP, phase contrast, × 250. D: MAG-3-positive oligodendrocytes in X/XO treated culture. Immunostaining is performed as in C. Advanced oligodendroglial damage with small cytoplasmic protrusions and loss of cell processes. Note that the morphological appearance of the underlaying cell layer is unaltered, phase contrast, × 250.

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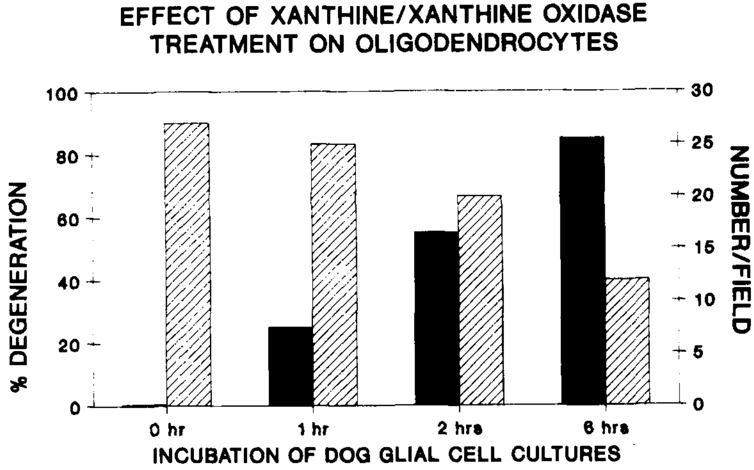

FIGURE 2 Dog glial cell cultures were incubated up to 6 hrs in the presence of xanthine/xanthine oxidase containing tisue culture medium. At the time points indicated, GalC-positive oligodendrocytes were counted (■). GalC-positive oligodendroglial cells were classified depending on their morphogical appearance. The percentage of degenerated cells (few short processes and ill-defined cytoplasm) is shown (■). Results expressed are the means of individual determination of 10 fields on 2 coverslips derived from different Petri-dishes.

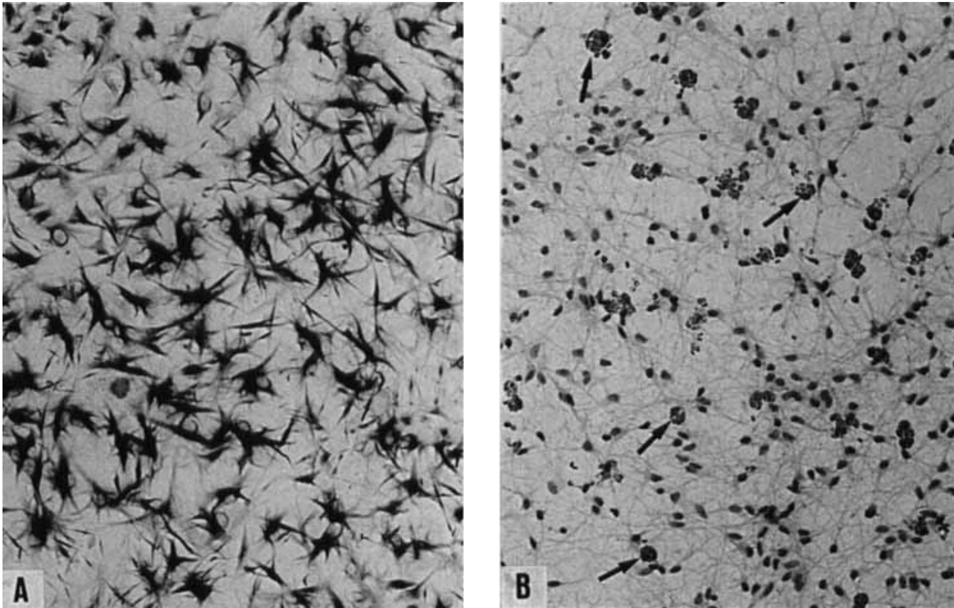

FIGURE 3 Morphological apperance of astrocytes and brain macrophages in X/XO treated dog glial cell cultures. Dog glial cell cultures after treatment with X/XO for 2 hrs. Neither GFAP-positive asrocytes (A) nor erythrocyte-positive macrophages (B, marked by arrows) are morphogically affected by this treatment, × 100.

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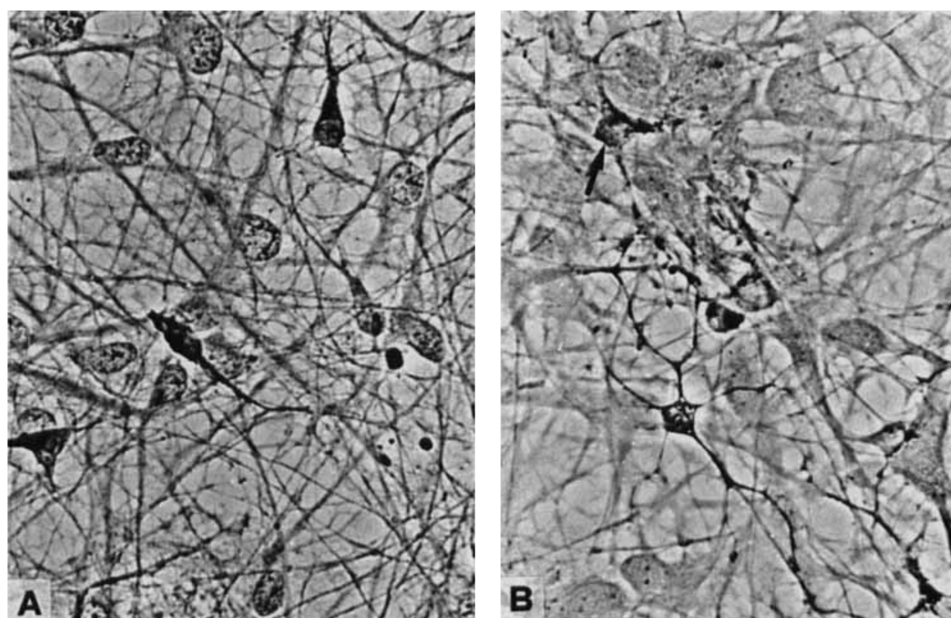

FIGURE 4 Effect of oxygen radical scavengers on the X/XO-induced morphological alteration of cultured dog glial cells. MAG-positive oligodendrocytes in X/XO-treated cultures supplemented with (A) SOD (100 U/ml) only, or (B) SOD (100 U/ml) and catalase (100 U/ml). Immunostaining is performed as in figure 1C. While oligodendrocytes have lost in (A) their fine branching processes, those in (B) show nearly normal appearance. Some MAG-positive cells show degeneration (marked by arrow), phase contrast, ×250.

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It is well known that the primary product of the X/XO-system is O_2^- and that the superoxide anion dismutates to H_2O_2 either spontaneously, or catalyzed by SOD.[10] However, neither O_2^- nor H_2O_2 alone seems to be the injurious species in our system since the presence of either SOD or catalase only slightly ameliorated the X/XO-induced degeneration of oligodendrocytes. The much better protection observed when SOD was present *together* with catalase indicates that both O_2^- and H_2O_2 are required for oligodendrocyte degeneration.

In the presence of transition metals such as iron or copper, O_2^- and H_2O_2 can give rise to the highly reactive $\cdot$OH.[10] Indeed, such a process or one similar to it, is very likely to be involved in our experiments, since metal chelators and scavengers of $\cdot$OH provided good protection to oligodendrocytes. It is not surprising that EDTA had no protective effect on oligodendrocytes, as glial cells can not survive for an extended period of time without Ca^{++} (C. Griot, unpublished observation).

H_2O_2-mediated injury to cells that derive a great part of their metabolic energy anaerobically through glycolysis is characterized by cellular depletion of NAD and ATP which can ultimately lead to cell death. These metabolic alterations are preceded by an activation of poly (ADP-ribose) polymerase, a nuclear enzyme using NAD as substrate. Inhibition of this enzyme prevents H_2O_2-induced injury in these cells.[12,38] However, such mechanism is unlikely the basis of X/XO-induced damage to oligodendrocytes observed in our studies, as neither of the poly (ADP-ribose) polymerase inhibitors had a significant effect.

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An important question is the source and form of transition metal, most likely iron, involved in the cytotoxic action observed in our system. We can not exclude the possibility that iron is derived from contamination of the X/XO-containing medium. While such source of iron would explain the inhibitory effects observed with hydroxyl radical scavengers and iron-chelators (Table I), it can not easily explain how X/XO-derived oxidants selectively damage oligodendrocytes within the mixed cell culture system. Selective damage to oligodendrocytes is unlikely to be only the result of differences in cellular contents of oxidizable targets and/or antioxidant defences as, in contrast to the X/XO-system, exposure of dog glial cell cultures to either aqueous peroxyl radicals produced at constant rate,[44] hydrogen peroxide or cumene hydroperoxide, results in general damage to *all* cell types present (data not shown). These observations make it more likely that oligodendrocytes themselves represent the source of transition metal.

Oligodendrocytes seem to play a central role in iron metabolism in the brain as iron,[14–17] transferrin and ferritin[16,45–48] have been shown to be present in oligodendrocytes of several different species. Interestingly, in rat and monkey brains, iron has been localized cytologically in the inner loop of the myelin sheets.[15] Unfortunately, the biological form of this iron is not known. While ferritin-bound iron can function as an *in vivo* source of iron to promote $\cdot OH$ formation from O_2^- and H_2O_2,[45,50] transferrin-bound iron seems to be less able to catalyze deleterious biological oxidation such as lipid peroxidation.[51] However, the degree of iron saturation of the various iron binding proteins is an important additional factor determining their efficiency to promote lipid peroxidation. Furthermore, in addition to transferrin, oligodendrocytes specifically contain carbonic anhydrase C,[48,52] an enzyme that facilitates binding of iron to transferrin.[53] It seems possible that under the conditions of high iron turnover, the concentration of iron available to catalyze formation of $\cdot OH$ is also increased. Thus, oligodendrocytes that act as target cells in our system may themselves provide a critical factor necessary for their own destruction, much in analogy to the situation proposed by Ward and co-workers for the oxygen radical-mediated killing of endothelial cells by activated neutrophils.[54] These authors further proposed that O_2^- and H_2O_2 may enter target cells passively, and that in subsequent events iron could be released from intracellular iron stores to become "available" to form hydroxyl radical. It is also possible that such "available" iron is accessible to iron chelators present outside the cells, a process that may be aided by close association of chelators with the target cells, as discussed in the case of Desferal® and endothelial cells.[54] These hypothetical events would explain the protective effects observed with DETAPAC and Desferal® (Table I). Therefore, iron available for the production of $\cdot OH$ together with large amounts of easily oxidizable myelin sheets (which are produced by oligodendrocytes *in vitro*[25]) may render these cells particularly vulnerable to oxidative damage. In contrast, astrocytes have the capacity to release pyruvate,[55] a non-enzymatic scavenger of H_2O_2, suggesting some protection of these cells against oxidants. However, such protection seems to be of limited duration as long term incubation with X/XO (more than 3 days) leads to destruction of the entire cell culture, including astrocytes (data not shown).

Our observations are compatible with the hypothesis that macrophages are important effector cells in demyelination.[4,5,7] We have shown recently that in cultured dog glial cells infected with canine distemper virus, antiviral antibodies can activate macrophage-like cells to produce luminol-dependent chemiluminescence,[4,5] a process likely to result mainly from the production of O_2^- by these cells.[56,57] Thus, production

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of reactive oxygen species by activated macrophages together with high sensitivity of oligodendrocytes towards these oxidants may contribute significantly to the destruction of myelin in dogs suffering from chronic inflammatory canine distemper encephalitis and probably in other inflammatory demyelinating diseases. The exact biochemical mechanism of the observed selective oligodendroglial damage needs further investigation which will perhaps ultimately provide data for therapeutic intervention of brain damage in encephalitis caused by ROS.

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References

1. M.C. Dal Canto and S.G. Rabinowitz (1982) Experimental models of virus-induced demyelination of the central nervous system. *Annals of Neurology*, **11**, 109–115.
2. K. Frei, C. Siepl, P. Groscurth, C. Bodmer, C. Schwerdel and A. Fontana (1987) Antigen presentation and tumor cytotoxicity by interferon-γ-treated microglial cells. *European Journal of Immunology*, **17**, 1271–1278.
3. F.L. Jordan and W.E. Thomas (1987) Identification of microglia in primary cultures of mixed cerebral cortical cells. *Brain Research Bulletin*, **19**, 153–159.
4. T. Bürge, C. Griot, M. Vandevelde and E. Peterhans (1989) Antiviral antibodies stimulate production of reactive oxygen species in cultured canine brain cells infected with canine distemper virus. *Journal of Virology*, **63**, 2790–2797.
5. C. Griot, T. Bürge, M. Vandevelde and E. Peterhans (1989) Antibody-induced generation of reactive oxygen radicals by brain macrophages in canine distemper encephalitis — a mechanism for bystander demyelination. *Acta Neuropathologica (Berlin)*, **78**, 396–403.
6. M. Vandevelde, R.J. Higgins, B. Kristensen, F. Kristensen, A.J. Steck and U. Kihm (1982) Demyelination in experimental canine distemper virus infection: immunological, pathologic, and immunohistological studies. *Acta Neuropathologica (Berlin)*, **56**, 285–293.
7. M. Vandevelde, A. Zurbriggen, A. Steck and P. Bichsel (1986) Studies on the intrathecal humoral immune response in canine distemper encephalitis. *Journal of Neuroimmunology*, **11**, 41–51.
8. H. Wisniewski, C.S. Raine and W.J. Kay (1972) Observations on viral demyelinating encephalomyelitis. Canine distemper. *Laboratory Investigation*, **26**, 589–599.
9. C.F. Nathan and Z. Cohn (1980) Role of oxygen-dependent mechanisms in antibody-induced lysis of tumor cells by activated macrophages. *Journal of Experimental Medicine*, **152**, 198–208.
10. H. Sies (1985) Oxidative stress. Academic Press, London.
11. J.A. Imlay and S. Linn (1988) DNA damage and oxygen radical toxicity. *Science*, **240**, 1302–1309.
12. I.U. Schraufstätter, P.A. Hystop, J.H. Jackson and C.G. Cochrane (1988) Oxidant-induced DNA damage of target cells. *Journal of Clinical Investigation*, **82**, 1040–1050.
13. T.F. Slater (1984) Free-radical mechanisms in tissue injury. *Biochemical Journal*, **222**, 1–15.
14. A.J. Dwork, E.A. Schon and J. Herbert (1988) Nonidentical distribution of transferrin and ferric iron in human brain. *Neuroscience*, **27**, 333–345.
15. C. Francois, J. Nguyen-Legros and G. Percheron (1981) Topographical and cytological localization of iron in rat and monkey brains. *Brain Research*, **215**, 317–322.
16. J.M. Hill and R.C. Switzer (1984) The regional distribution and cellular localization of iron in the rat brain. *Neuroscience*, **11**, 595–603.
17. B. Halliwell and J.M.C. Gutteridge (1985) Oxygen radicals and the nervous system. *Trends in Neuroscience*, **7**, 22–26.
18. W.T. Norton and W. Cammer (1984) Isolation and characterizationof myelin. In Myelin (ed. P. Morell), Plenum Press, New York and London, pp. 147–180.
19. L.S. Chia, J.E. Thompson and M.A. Moscarello (1983) Disorder in human myelin induced by superoxide radical: An *in vitro* investigation. *Biochemical and Biophysical Research Communications*, **117**, 141–147.

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20. G.W. Konat and R.C. Wiggins (1985) Effect of reactive oxygen species on myelin membrane proteins. *Journal of Neurochemistry*, **45**, 1113–1118.
21. P.H. Chan, M. Yurko and B.A. Fishman (1982) Phospholipid degradation and cellular edema induced by free radicals in brain cortical slices. *Journal of Neurochemistry*, **38**, 525–531.
22. L.S. Chia, J. E. Thompson and M.A. Moscarello (1983) Changes in lipid phase behavior in human myelin during maturation and aging. *FEBS Letters*, **157**, 155–158.
23. A. Zurbriggen, M. Vandevelde, C.F. Beranek and A.J. Steck (1984) Morphological and immunocytochemical characterization of mixed glial cell cultures derived from neontal canine brain. *Research in Veterinary Science*, **36**, 270–275.
24. A. Zurbriggen, M. Vandevelde, M. Dumas, C. Griot and E. Bollo (1987) Oligodendroglial pathology in canine distemper virus infection *in vitro*. *Acta Neuropathological (Berlin)*, **74**, 366–373.
25. S. Szuchet, P. Polak and S.H. Yim (1986) Mature oligodendrocytes cultured in the absence of neurons recapitulate the ontogenic development of myelin membranes. *Developmental Neuroscience*, **8**, 208–221.
26. C.T. Bishop, Z. Mirza, J.D. Crapo and B.A. Freeman (1985) Free radical damage to cultured porcine aortic endothelial cells and lung fibroblasts: Modulation by culture conditions. *In vitro Cellular Development Biology*, **21**, 229–236.
27. J.M. McCord and I. Fridovich (1968) The reduction of cytochrome C by milk xanthine oxidase. *Journal of Biological Chemistry*, **243**, 5753–5600.
28. R. Stocker and E. Peterhans (1989) Antioxidant properties of conjugated bilirubin and biliverdin: Biological relevant scavenging of hypochlorous acid. *Free Radical Research Communications*, **6**, 57–66.
29. N.J. Scolding, A.J. Houston, B.P. Morgan, A.K. Campbell and D.A.S. Compston (1989) Reversible injury of cultured rat oligodendrocytes by complement. *Immunology*, **67**, 441–445.
30. I.U. Schraufstätter, D.B. Hinshaw, P.A. Hyslop, R.G. Spragg and C.G. Cochrane (1986) Oxidant injury of cells. DNA strand-breaks active polyadenosine diphosphate-ribose polymerase and lead to depletion of nicotineamide dinucleotide. *Journal of Clinical Investigation*, **77**, 1312–1320.
31. A.J. Steck, N. Murray, M. Vandevelde and A. Zurbriggen (1983) Human monoclonal antibodies to myelin-associated glycoprotein. *Journal of Neuroimmunology*, **5**, 145–156.
32. M.C. Raff, R. Mirsky, K.L. Fields, R.P. Lisak, S.H. Dorfman, D.H. Silberberg, N.A. Gregson, S. Leibowitz and M.C. Kennedy (1978) Galactocerebroside is a specific cell-surface antigenic marker for oligodendrocytes in culture. *Nature*, **274**, 813–814.
33. S.M. Taffet and S.W. Russel (1981) Identification of mononuclear phagocytes by ingestion of particulate materials such as erythrocytes, carbon, zymosan, or latex. In Methods for studying mononuclear phagocytes (ed. O.O. Adams, P.J. Edelson, A.S. Koren), Academic Press, London and New York, pp 283–293.
34. L.A. Sternberger (1979) Immunocytochemistry. Wiley and Sons, New York and Chichester.
35. E.W. III, Kellog and I. Fridovich (1975) Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. *Journal of Biological Chemistry*, **250**, 8812–8817.
36. R. Stocker and C. Richter (1982) Involvement of calcium, calmodulin and phospholipase A in the alteration for membrane dynamics and superoxide production of human neutrophils stimulated by phorbol myristate acetate. *FEBS Letters*, **147**, 243–249.
37. J.Z. Byczkowski and T. Gessner (1988) Biological role of superoxide ion-radical. *International Journal of Biochemistry*, **20**, 569–580.
38. I.U. Schraufstätter, P.A. Hyslop, D.B. Hinshaw, R.G. Spragg and L.A. Sklar (1986) Hydrogen peroxide-induced injury of cells and its prevention by inhibitors of poly (ADP-ribose) polymerase. *Proceedings of the National Academy of Science of the USA*, **83**, 4908–4912.
39. C.F. Brosnan, K. Selmaj and C.S. Raine (1988) Hypothesis: A role for tumor necrosis factor in immune-mediated demyelination and its relevance to multiple sclerosis. *Journal of Neuroimmunology*, **18**, 87–94.
40. K.W. Selmaj and C.S. Raine (1988) Tumor necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. *Annals of Neurology*, **23**, 339–346.
41. S.K. Ludwin (1979) Central nervous system demyelination and remyelination in the mouse: An ultrastructural study of cuprizone toxicity. *Laboratory Investigation*, **39**, 597–603.
42. D.S. Robbins, Y. Shirazi, B.E. Drysdale, A. Lieberman, H.S. Shin and M.L. Shin (1987) Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. *Journal of Immunology*, **139**, 2593–2597.
43. M.E. Smith and J.A. Benjamins (1984) Studies of disruption of myelin metabolism in tissue culture. In Myelin (ed. P. Morell), Plenum Press, New York and London, pp 457–458.

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44. R. Stocker, A.N. Glazer and B.N. Ames (1987) Antioxidant activity of albumin-bound bilirubin. *Proceedings of the National Academy of Science of the USA*, **84**, 5918–5922.
45. J.R. Connor and R.E. Fine (1987) Development of transferrin-positive oligodendrocytes in the rat central nervous system. *Journal of Neuroscience Research*, **17**, 51–59.
46. B. Bloch, T. Popovici, M.J. Levin, D. Tuil and A. Kahn (1985) Transferrin gene expression visualized in oligodendrocytes of the rat brain by using in situ hybridization and immunohistochemistry. *Proceedings of the National Academy of Science of the USA*, **82**, 6706–6710.
47. A Espinosa de los Monteros and B. Foucaud (1987) Effect of iron and transferrin on pure oligodendrocytes in culture; characterization of a high-affinity transferrin receptor at different ages. *Developmental Brain Research*, **35**, 123–130.
48. C. Griot and M. Vandevelde (1988) Transferrin, carbonic anhydrase C and ferritin in dissociated murine brain cell cultures. *Journal of Neuroimmunology*, **18**, 333–340.
49. P. Biemond, A.J.G. Swaak, H.G. Van Eijk and J.F. Koster (1988) Superoxide dependent iron release from ferritin in inflammatory diseases. *Free Radical Biology and Medicine*, **4**, 185–198.
50. C.E.T. Thomas, L.A. Morehouse and S.D. Aust (1985) Ferritin and superoxide-dependent lipid peroxidation. *Journal of Biological Chemistry*, **260**, 3275–3280.
51. M. Saito, L.A. Morehouse and S.D. Aust (1986) Transferrin-dependent lipid oxidation. *Free Radical Biology and Medicine*, **2**, 99–105.
52. W. Cammer (1984) Carbonic anhydrase in oligodendrocytes and myelin in the central nervous system. *Annals of the New York Academy of Science*, **429**, 494–497.
53. I. Schlabach and G.W. Bates (1975) The synergistic binding of anions and Fe^{3+} by transferrin. *Journal of Biological Chemistry*, **250**, 2182–2188.
54. D.E. Gannon, J. Varani, S.H. Phan, J.H. Ward, J. Kaplan, G.O. Till, R.H. Simon, U.S. Ryan and P.A. Ward (1987) Source of iron in neutrophil-mediated killing of endothelial cells. *Laboratory Investigation*, **57**, 37–43.
55. J. O'Donell-Tormey, C.F. Nathan, K. Lanks, C.J. Deboer and J. De La Harpe (1987) Secretion of pyruvate. An antioxidant defense of mammalian cells. *Journal of Experimental Medicine*, **165**, 500–514.
56. B. Sonderer, P. Wild, R. Wyler, A. Fontana, E. Peterhans and M. Schwyzer (1987) Murine glia cells in culture can be stimulated to generate reactive oxygen. *Journal of Leukocyte Biology*, **42**, 463–473.
57. C.A. Colton and D.L. Gilbert (1987) Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Letters*, **223**, 284–288.

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