Molecular mechanisms of cell death in periventricular leukomalacia

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Abstract—*Objective:* To investigate the cytokine-related molecular cascade leading to neural cell death in periventricular leukomalacia (PVL). *Methods:* The authors explored potential tumor necrosis factor α (TNFα) signaling pathways in human brains with PVL and conducted in situ immunohistochemical investigations to search for possible expression of cytokine receptors in these brains. They also investigated likely links to molecules potentially involved in neurocytotoxicity, particularly pathways involving nitrosative-induced apoptosis. *Results:* TNFα overexpression was associated with immune reactivity for p75TNFαR2 and p55TNFαR1 receptors in affected PVL areas. p75TNFαR2 labeling was intense on cerebrovascular endothelial cells in PVL areas, whereas no vascular p55TNFαR1 immunoreactivity was detected therein. Immune labeling for both receptors was detected on many white matter parenchymal cells. In contrast, there was no immune reactivity for either receptor in tissues taken from non-PVL areas. Additionally, in situ overexpression of inducible nitric oxide synthase was found in PVL brain regions where apoptotic cell death was detected. *Conclusions:* Both p75TNFαR2 and p55TNFαR1 receptors and nitric oxide may be implicated in the pathogenesis of periventricular leukomalacia.

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Periventricular leukomalacia (PVL)[1,2] is multifactorial.[3-6] We previously reported cytokine overexpression in PVL brains[7-10] and suggested that activation of inflammatory cells and the associated cytokine up-regulation lead to the white matter damage (WMD) in this perinatal encephalopathy. Animal studies also showed the deleterious effects of proinflammatory cytokines, and, overexpression of tumor necrosis factor α (TNFα) in transgenic mice caused myelination defects and oligodendroglial apoptosis. Pretreatment with TNFα and interleukin 1β increased the WMD induced by injection of a glutamatergic agonist.[11] Exposure to TNFα or infection, additionally , induces nitric oxide (NO) formation.[12] NO, too, could cause neural damage.[13,14]

Physiopathologic actions of TNFα in various organs are mostly mediated by a receptor, namely, p55TNFαR1. However, some TNFα functions, including apoptosis, are attributed to a different receptor, namely, p75TNFαR2.[15,16] We hypothesized[17] that TNFα exerts neurotoxicity through a direct pathway involving activation of p55TNFαR1 or p75TNFαR2 receptors or both or by an indirect cascade involving the induction of inducible nitric oxide synthase (iNOS).

Methods. Twelve brains from early infantile deaths in which neuropathologic examination revealed periventricular leukomalacia (PVL) were explored in this study. The main clinicopathologic features for these infants are outlined in the table.

Neural tissues analyzed in this study have been processed within the legal frame of complete autopsy procedures involving adequate and full neuropathologic examinations. All such autopsy and postmortem procedures were conducted according to the legal and ethical rules applied in our institutions.

Neuropathology, immunohistochemical, and molecular techniques. The brains were fixed and processed according to standard pathology techniques.[7] From each of these brains, two distinct sets of paraffin-embedded tissue blocks, containing relevant areas of cerebral wall, were prepared. The first set of blocks contained neural tissues that were picked up from affected cerebral areas that showed on routine neuropathologic examination both macroscopic and microscopic evidence of typical PVL lesions. These were designated as "PVL areas." In brief, these PVL area samples comprised cerebral hemispheric tissues that contained diseased white matter zones at the histopathologic phase of "early-stage PVL" (namely, coagulative necrosis foci or lesions in the stage of "early resorption") or at the phase of "late-stage PVL" (comprising mainly the histopathologic stage of "late resorption" or the more advanced cystic or gliotic stages in these lesions).[7] The second set of tissue blocks were collected from nonaffected brain areas where there was no evidence for tissue disease according to classic neurohistopathologic examination. These latter blocks were characterized as "non-PVL areas" and similarly included central white matter but without being affected histopathologically.

All formalin-fixed, paraffin-embedded blocks were serially cut in 8-μm sections and first studied with routine stains and then processed for 1) immunohistochemical (IHC) detection of TNFα, TNFα receptors (p55TNFαR1 and p75TNFαR2) and for detection of iNOS; and 2) exploration with apoptotic cell death detection techniques. Details of our IHC staining procedures for the detection of TNFα cytokine were previously described.[7] Detection of

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Table *Clinicopathologic features of the studied cases*

No./PVL stage/sex	GA, wk/ survival, d	TNFα (WM): intensity and labeling index	p55TNFαR1: PVL/non-PVL	p75TNFαR2: PVL/non-PVL	iNOS: PVL/non-PVL	Apoptosis: PVL/non-PVL	Clinicopathologic data
97-52;E/F	34/2	+ (20)	−/−	+ (11)/−	++ (42)/−	++ (70)/−	Hyaline membrane disease
1448;E/M	34/2	+ (21)	−/−	−/−	−/−	+ (6)/−	Heart failure (malformation)
1907;E/F	38/8	++ (68)	+ (40.5)/+ (5.7)	++ (51)/++ (3)	+ (34)/−	+ (48)/−	Heart failure (malformation)
98-18;E/M	37/21	+++ (61)	−/−	++ (39)/+ (8)	+++ (66)/−	+ (13)/−	Heart failure (malformation)
1566;E/M	30/3	+ (40)	−/−	+ (9.5)/−	+ (9)/−	+ (8)/−	Hyaline membrane disease, septicemia
1299;E/M	31/1	+ (23)	−/−	−/−	+ (2)/−	++ (32)/−	Hyaline membrane disease
97-102;E/M	32/2	++ (38)	−/−	+ (12)/+ (4)	++ (37)/−	−/−	Heart failure, chorioamnionitis
1637;L/M	27.5/28	+ (6)	−/−	+ (19)/−	++ (2.5)/−	+++ (66)/−	Hyaline membrane disease
1536;L/M	29/18	++ (14)	++ (45.5)/−	+ (41.5)/+ (6)	+++ (41)/−	++ (16)/−	Respiratory failure
1791;L/M	36/2	++ (65)	+ (33)/−	+ (15)/−	+ (22.5)/−	−/−	Asphyxia, peritonitis
1992;L/M	39/10	+ (1)	−/−	−/−	++ (8)/−	−/−	Heart malformation, pneumonia
2125;L/M	28.5/21	+ (31)	−/−	−/−	−/−	+ (20)/−	Asphyxia, chorioamnionitis

Labeling index = mean percentage of labeled cells. Intensity is defined as mild (+), moderate (++), intense (+++), or absent (−). Percentages are in parentheses.

PVL = periventricular leukomalacia; GA = gestational age; WM = white matter; iNOS = inducible nitric oxide synthase; E = early; L = late.

TNFα receptors and iNOS relied basically on the same IHC procedure. Both TNFα receptors were monoclonal (mouse) antibodies (Santa Cruz Biotechnology) and were used in a dilution of 1:40. iNOS monoclonal antibodies (Transduction Laboratories, Lexington, KY) was used in a dilution of 1:50.

Tissue preservation, handling, fixation, and sampling were carried out in identical ways and done by the same person. Immune detection of particular antigens in each of the study groups (PVL and controls) was done in identical experimental and environmental conditions. Thus, tissue sections from both groups (often located adjacently on the same slide) were subjected identically to the same technical conditions as all the immune-staining procedure for the detection of a given molecule was done by the same person, at the same time, and precisely in the same staining session and equipments (staining baths, racks, etc.).

For the detection of apoptotic cell death, we used a kit (Apop-DETEK Cell Death Assay System, Enzo Diagnostics, NY). Manufacturer instructions were applied using formalin-fixed, paraffin-embedded sections. The incorporated deoxynucleotides were detected with a horseradish peroxidase–diaminobenzadine in situ detection system using a kit (SimplySensitive) provided by the same manufacturer.

Serial section analysis. Serial sections from the same blocks in each of the two series were prepared. These were first processed with routine staining techniques to establish the state of neural tissue in each particular site. Then, serial sections were explored with the various IHC and apoptosis detection techniques for the evaluation of the different molecular markers used in this study on consecutive sections from the same areas.

We counted immune-labeled and apoptosis-positive cells using a high-power (×40) microscopic lens. Counting was performed in regions of the white matter with the highest numbers of labeled cells, in both PVL and non-PVL areas (both study groups). Cell counting was performed in each individual area in all samples from both study groups. For each area, counting was done in five high-power fields, and the mean value from these readings was calculated. The mean percentage of the number of labeled cells (i.e., labeled cells over all-cells counts) in each of these areas was termed the "labeling index" and was used for comparative analysis (table). We also determined the "labeling intensity" of immune-reactive or apoptosis-positive cells. "Labeling intensity" refers to how strong the immune label (or apoptosis signal) looked. Thus, the intensity of labeling with the various staining techniques was visually determined and portrayed as mild (+), moderate (++), intense (+++), or absent (−).

Data on the proportions of immune-reactive cells are presented as the means ± SEM. Comparisons were performed using Fisher exact test and unpaired t test (Welsh corrected). The significance level was set at $p < 0.05$.

Results. Apoptosis was detected in PVL lesions in 9 of the12 brains (table). Apoptotic cells were particularly abundant in the most affected foci of PVL lesions. The number of apoptotic cells gradually decreased centrifugally. The mean percentage of apoptotic cells in these white matter lesions was 31% (SEM = 8.2%). This was significantly higher than in non-PVL areas, where no apoptotic signal was detected in any of the cells. In affected areas, apoptosis was detected in different neural cell types, with morphologic characteristics of astrocytes, microglia, and oligodendrocytes (figure 1A).

TNFα was expressed in PVL lesions in all the brains (n = 12). This cytokine immunoreactivity was observed in the various inflammatory cells that were seen in affected sites, namely, microglia, astroglia, and monocytes/macrophages (figure 1B). Cytokine was not remarkable in non-PVL areas.

TNFα receptors. Both p75TNFαR2 and p55TNFαR1 receptors were expressed in PVL lesions (table). p75TNFαR2 expression was detected in 8 of the 12 brains and was pronounced on vascular endothelial cells (figure 1C). p75TNFαR2 immune staining was additionally noticed on white matter cells, particularly astrocytes, in these PVLs. The mean percentage of immune-positive cells for p75TNFαR2 in the white matter lesions in these PVL brains was 24.75%. This was significantly higher than in non-PVL areas, where less p75TNFαR2 immune reactivity (mean percentage: 2.6%, SEM = 1.1%) was detected in white matter cells. It is noteworthy that whereas endothelial/vascular cells in PVL heavily expressed p75TNFαR2 receptors, there was no immunoreactivity for p55TNFαR1 in this vascular compartment. On the contrary, p55TNFαR1 immune labeling in these PVL areas was detected on various white matter cells, namely, with morphologic characteristics of astroglia, microglia, and oligodendrocytes. p55TNFαR1 expression (figure 1D) was

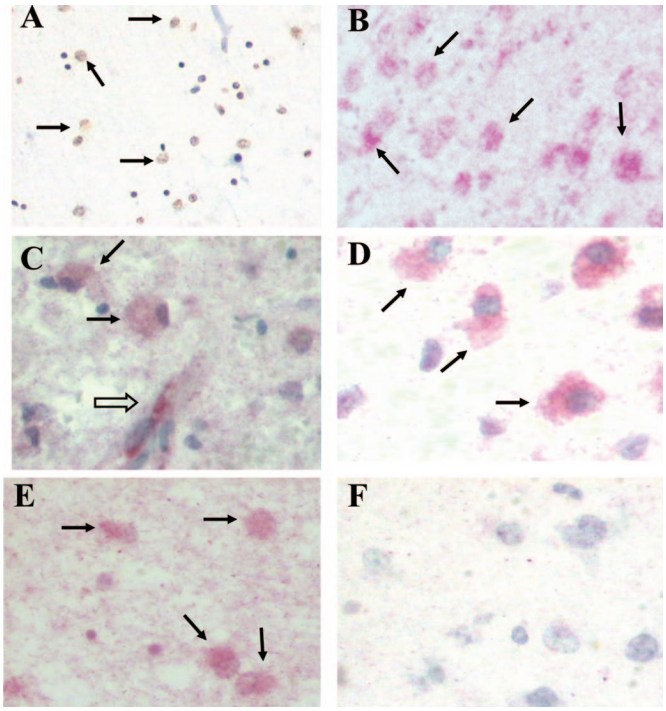

Figure 1. In situ immunohistochemical (IHC) detection of cytokine receptors and inducible nitric oxide synthase (iNOS) in periventricular leukomalacia (PVL) lesions in paraffin-embedded, formalin-fixed sections of cerebral white matter expressing apoptosis. (A) Apoptotic cells (brown; arrows), mainly seen in most affected zones of PVL brains. (B) Tumor necrosis factor α (TNFα)–labeled inflammatory cells (arrows) bordering a PVL area. (C, D) In situ IHC detection of p75TNFαR2 (C) and p55TNFαR1 (D) receptors, in affected white matter zones in PVL brains. Note the remarkable immune staining for p75TNFαR2 on vascular wall cells (open white arrow), besides its expression by inflammatory cells in the white matter (positive immune reactivity is expressed as red; arrows). (E) Marked immune staining (red; arrows) for iNOS in inflammatory cells in the white matter. The cytoplasmic expression (arrows) is quite intense in the two opposing gemistocytes (reactive astrocytes) near the lower margin of the figure. (F) White matter cells from a non-PVL (control) area showing no immune staining for cytokine receptor p75TNFαR2. (A through F) light microscopic photographs; original magnifications: ×250 (A), ×400 (B), ×500 (C through F); Mayer hematoxylin counterstaining.

observed in fewer brains (n = 3) when compared with p75TNFαR2 (n = 8) (table).

iNOS too was detected in PVL lesions. iNOS was expressed in 10 of the 12 brains. The mean percentage of immune-positive cells for iNOS in the white matter lesions in PVL brains was (26.4%). This was significantly higher than in non-PVL areas, where no iNOS signal was detected in any of the cells. iNOS immune reactivity was expressed particularly on astrocytes but also on oligodendrocytes, macrophages, endothelial cells, and to a lesser extent microglia (figure 1E). In affected PVL areas, combined expression of both iNOS and p75TNFαR2 was observed in 8 of the12 cases.

Finally, comparison between early and late PVL did not reveal noticeable differences in any of the various markers (table).

Co-expression of various markers. In brains where apoptosis was detected (n = 9), analysis of serial sections (see Methods) from tissue blocks from these affected sites showed that combined expression of TNFα, TNFα receptors p55TNFαR1 or p75TNFαR2, and iNOS in white matter lesions was observed in six cases (66%) (table).

Concerning gray matter neuronal centers, TNFα immune reactivity was observed in many neurons of cortex or deep gray nuclei adjacent to affected white matter areas in all PVL cases where these gray matter sites were available for neuropathologic examination (n = 9). No apoptosis was seen in any of these neuronal structures (whether cortex or deep gray neurons) from the nine brains. Appreciable expression of p75TNFαR2 on cortical or deep gray neurons was detected in only two cases. p55TNFαR1 neuronal expression was observed in one of these cases and in an additional one case. iNOS expression was also observed in gray matter neuronal structures but was present in fewer cases than with white matter expression; cortical or deep gray neuronal expression of iNOS was thus seen in 7 of 12 brains. Similarly to white matter, immune expression was detected on neural and vascular cells.

Discussion. Studies from our laboratory[3,7,8,10,17] and others'[18-20] suggested the implication of TNFα and the related immune–inflammatory cellular reactions in the causal mechanisms underlying brain damage in PVL. The steps leading from the immune-mediated inflammatory response down to WMD and cell death of myelin precursors, in PVL, however, remained unknown. Further, no studies so far have assigned the actions of TNFα to a specific protein or receptor in PVL, and the molecular pathways involved in neural cell injury and death in PVL/Cerebral Palsy remain unknown.

In this observational study, we revealed the expression of TNFα receptors in PVL brains, and we found predominance of p75TNFαR2 over p55TNFαR1 (in a distinct topography). Besides, we report an intracerebral (parenchymal) up-regulation of iNOS in these affected neonatal brains. These findings were associated with the presence of apoptosis-positive cells, exclusively detected in the affected white matter compartment. Cortical and deep gray neuronal structures, in contrast, were apoptosis-free.

These results provide new insights into the TNFα signaling pathways and characterize key elements apparently involved in triggering molecular cascades leading to neural cell death and injury in PVL brains.

TNFα is believed to mediate most of its biologic activities via two distinct cell surface receptors, namely, p55TNFαR1 and p75TNFαR2.[15,21] Many of the cytotoxic and pro-inflammatory actions of TNFα are mediated by p55TNFαR1, which has an intracellular "death domain" capable of triggering apoptosis following ligand binding by this receptor. p75TNFαR2 does not possess a death domain, and p75TNFαR2 signaling is reportedly operational in

fewer cell systems. The role of p75TNFαR2 in disease has thus been less clear and has awaited further explorations.[15,21,22] In vitro and animal studies showed that microglia could express both p55TNFαR1 and p75TNFαR2 and that astrocytes and oligodendrocytes predominantly express p55TNFαR1. p75TNFαR2 was also shown recently to have a pro-inflammatory role, mainly at the level of vascular endothelia in the CNS of transgenic mice, resulting in ischemic brain damage,[21] whereas p75TNFαR2, under basal condition, was found to be barely detectable in the rat brain.[23] These and other animal studies thus revealed that both TNFα receptors (p55R1 and p75R2) could be expressed in neural tissues under experimental conditions and that expression of TNFα receptors could be implicated in the pathogenesis of immune–inflammatory conditions (such as experimental cerebral malaria and sepsis) and ischemic brain damage.[15,21,22] The studies in transgenic mice suggested that p55TNFαR1 signaling could specifically trigger WMD with oligodendrocyte apoptosis, parenchymal inflammation, and primary demyelination, whereas p75TNFαR2 mediated CNS vascular inflammatory ischemia.[21] In light of these data (relating the expression of TNFα receptors to severe pathologic conditions), on the one hand, and given the absence of these receptors in normal "non-PVL" central white matter areas from our material (Results), on the other, it becomes inferable that our detection of a high expression of these receptors in PVL areas (with WMD) suggests a role for these receptors in PVL immunopathogenesis triggered by TNFα and could imply increased sensitization of these brains to the effects of this cytokine.

Our results further suggest that TNFα in human PVL brains is likely to mediate its neurotoxic effects including apoptotic cell death via p55TNFαR1 or p75TNFαR2 or both. This conclusion is based on our detection of both of these protein receptors (together with their ligand, namely TNFα) in PVL brains and on the growing knowledge of the molecular interactions in which these receptors could be involved, triggering distinct chains of interactions leading to inflammatory reactions, neural cell injury, and apoptosis.[15,21,22] Apoptosis is a form of programmed cell death executed by caspase enzymes activated along signaling pathways initiated by ligation of cell surface receptors to intracellular death domains (extrinsic pathway) or by perturbation of the mitochondrial membrane promoted by physical or chemical stress agents (intrinsic pathway).[24,25] The implication of p55TNFαR1 in apoptotic cell death in PVL would thus become easy to understand, as this receptor is known to possess an intracellular death domain specifically linked to it. TNFα can thus trigger the molecular chain down to apoptosis via p55TNFαR1, thereby activating the "extrinsic pathway." But the lower prevalence of p55TNFαR1 in our PVL brains (in comparison with p75TNFαR2) would suggest that apoptosis detected in the damaged white matter in these brains is apparently not mediated principally by p55TNFαR1. We then argued whether p75TNFαR2, too, could have been involved in cell injury and death in PVL. p75TNFαR2 does not have a death domain. However, animal studies showed that it could as well be involved in inflammatory and apoptotic events, and it is known to be highly induced in pathologic conditions.[22] p75TNFαR2 is thus thought to cooperate with p55TNFαR1 by forming heterocomplexes with the latter or by potentiating the pro-apoptotic effects of TNFαR1 activation.[26-29] Experimental studies also revealed that p75TNFαR2 could participate in TNFα-mediated inflammation and injury including apoptosis and necrosis in renal epithelial cells.[15,16] Besides, animal studies showed that TNFα signaling by the p75TNFαR2 (independently of p55TNFαR1) triggers inflammatory ischemia at the level of vascular endothelium in the CNS of transgenic mice.

These animal studies thus reveal that p75TNFαR2 is also implicated in cell injury including in the CNS and provide further evidence in support of our hypothesis that TNFα receptors (including p75TNFαR2) could play a role in WMD of human PVL brains. Human studies also provide additional evidence in support of this hypothesis, whereas pathologic studies suggested the possible implication of both TNFα receptors in certain CNS disorders like multiple sclerosis wherein both receptors were detected at the rim of active multiple sclerosis lesions.[30]

P75TNFαR2 immunoreactivity detected in our PVL brains was remarkably intense and particularly expressed on vascular linings (see Results; figure 1). The preferentially high expression of this receptor on vascular cells in these brains could be related to its physiopathologic mechanisms of actions. These results might thus identify a pro-inflammatory role for p75TNFαR2 at the level of the CNS vascular endothelium, which correlates with the expression pattern of this receptor in the brain in this perinatal encephalopathy. Evidence in support of this hypothesis comes from similar observations in animal studies, whereas transgenic mice generated to express human TNFα and p75TNFαR2 (but lacking p55TNFαR1) were found to develop a CNS vasculitic pathology characterized by endothelial cell activation and inflammation that could lead to multifocal CNS ischemic injury.[21] These PVL findings and animal explorations would therefore strongly suggest that TNFα-related neuro-immune interactions in these pathologic conditions largely involve p75TNFαR2-mediated immune inflammatory processes at the vascular level, leading to possible ischemic/anoxic brain damage.

Based on these personal and other reported data, it would seem that TNFα-induced WMD and apoptosis in PVL could be largely mediated by p75TNFαR2 and that such p75TNFαR2-mediated neural cell injury in this perinatal encephalopathy could come about as a result of both a "direct" pathway (through the p75TNFαR2 reported pro-inflammatory role or

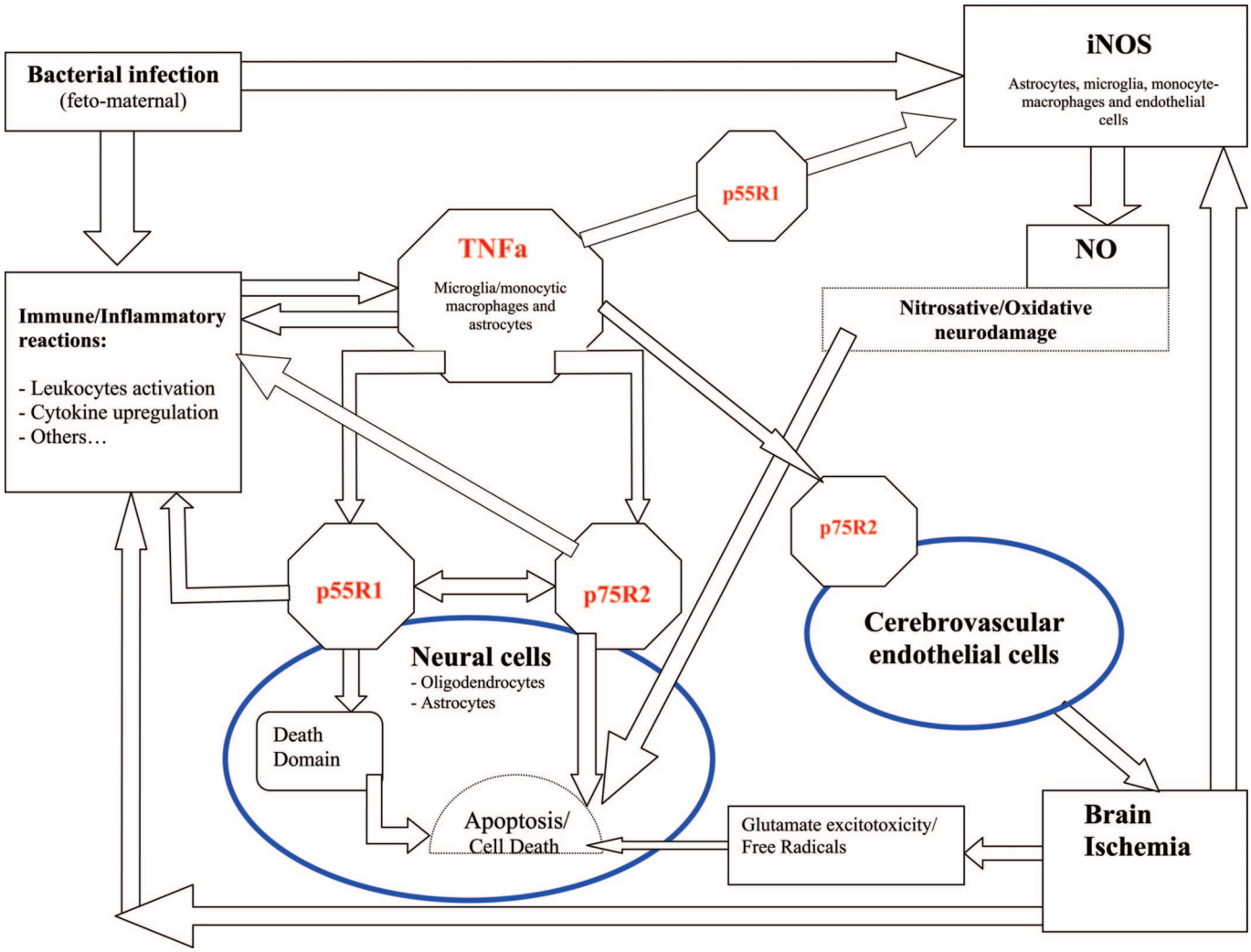

Figure 2. Schematic drawing of a hypothesis highlighting the central role of tumor necrosis factor α (TNFα) receptors in a complex molecular network implicated in neural cell injury and death in periventricular leukomalacia (PVL) brains. This figure shows that whereas TNFα could trigger apoptosis directly through p55TNFαR1 and activation of the death domain (extrinsic pathway; see text for details and references), TNFα in the brain seemingly uses mainly p75TNFαR2 to mediate its actions in PVL by triggering a multipons offensive: p75TNFαR2 thus seems to play a pivotal role in mediating most of the effects of TNFα leading to neural cell damage. Pathways involving p75TNFαR2 include the triggering of vascular cerebral ischemia and the consequent induction of inducible nitric oxide synthase leading to nitrosative/oxidative cell damage through activation of intrinsic pathways of apoptosis. p75TNFαR2 could also induce non-receptor-dependent apoptosis directly or via inflammation promotion. It can, additionally, enhance the action(s) of p55TNFαR1, with which it forms complexes. This figure, besides, highlights the intricate link(s) between the ischemic/hypoxic and the immune/infectious bases often debated in the literature as possible etiopathogenic factors underlying PVL. It clearly shows that these two "apparently" different mechanisms in origin are not "opposing" and actually could be closely related to each other. Both factors might interact, and either mechanism might potentiate the other as some of the final molecules and pathways are seemingly shared.

the currently defined neuro-vascular involvement or both) and via an "indirect" pathway (through the enhancement of p55TNFαR1 by p75TNFαR2 and their possible interactions and formation of hetero-complexes, as defined above) (figure 2).

Our finding that p75TNFαR2 could mediate WMD in this infantile pathology thus expands a small but growing list of neurologic disorders in which this protein has been shown to play an important role in CNS pathologies.

Besides the overexpression of TNFα and p75TNFαR2 observed in PVL brains, there was also a parallel up-regulation of iNOS in these affected cerebral sites. iNOS is an inducible isoform of the enzyme that produces NO and has been detected in microglia, astrocytes, and endothelial cells.[31] It is a major source of excessive NO in pathologic conditions.[32] It is noteworthy that iNOS mRNA is reportedly undetectable in normal brain tissue but becomes observable following experimental pathologic conditions. It was also found that iNOS activity increases following exposures to cytokines, namely, TNFα and microbial products (lipopolysaccharide).[12] p55TNFαR1 but not p75TNFαR2 was reported to

mediate iNOS induction particularly in astrocytes.[33] When its concentration increases, NO is known to exert neurotoxic effect through oxidative/nitrosative stress, and NO is believed to cause damage to myelin and its precursors.[14] Further, iNOS is thought to contribute to the pathogenesis of human diseases with cerebral WMD like multiple sclerosis.[34,35] On the other hand, TNFα signaling involving exclusively p75TNFαR2 was reportedly involved in triggering inflammatory ischemia in the CNS of transgenic mice lacking p55TNFαR1.[21] Other experimental models showed that ischemia was associated with excessive NO formation,[36] whereas inhibitors of iNOS during reperfusion reduced cell damage.[37]

Based on these observations, and given the remarkable up-regulation of iNOS detected in our PVL brains in association with overexpression of TNFα and p75TNFαR2 in the distinctive (vascular) pattern we described, it might be inferred that TNFα-triggered neurotoxicity in human PVL brains[38,39] could also involve p75TNFαR2-mediated inflammatory ischemia leading to excessive NO formation. We thus believe that NO is involved in a TNFα-triggered molecular chain implicated in the pathogenesis of PVL by exerting a toxic effect on the developing white matter. Evidence in support of this hypothesis also came from experimental (cell culture)[14] and autopsy studies[13] that reported the vulnerability of developing oligodendrocytes (white matter precursors) to NO and suggested the enormity of nitrosative damage to white matter in PVL.

Studies in cell culture[14] also suggested that the vulnerability of developing oligodendrocytes to NO-induced cell death involves mitochondrial dysfunction and translocation of apoptosis-inducing factor from mitochondria to cell nuclei. Hence, up-regulation of iNOS in PVL brains could therefore be noxious via this reported mitochondrial toxicity, implying the activation of the "intrinsic pathway of apoptosis" (see above). It would thus seem that both the "extrinsic" and "intrinsic" pathways of apoptosis are involved in this perinatal encephalopathy, whereas both p55TNFαR1 and p75TNFαR2–ischemia–NO cascades are respectively activated.

The various molecular chains likely activated during the cascade of events leading to cell injury and death in PVL brains are schematically outlined in figure 2. These findings shed light on mechanisms involved in the pathogenesis of WMD in PVL brains and could pave the way for new strategies for neuroprotection in perinatal encephalopathy. Targeted molecules like NO could thus be stimulated or inhibited by pharmacologic or gene therapy approaches. Forthcoming neuromodulatory interventions might also consider inhibiting/reducing inflammatory cytokines, preventing transmigration of inflammatory cells to the CNS, or the immunogenic neutralization of offending molecules (such as TNFα or its receptors) by anti-cytokines or monoclonal antibodies.

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Corrections

Practice Parameter: Neuroprotective strategies and alternative therapies for Parkinson disease (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology

In the AAN Special Article "Practice Parameter: Neuroprotective strategies and alternative therapies for Parkinson disease (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology" by O. Suchowersky, G. Gronseth, J. Perlmutter, S. Reich, T. Zesiewicz, and W. J. Weiner (*Neurology* 2006;66:976–982), there is an error in the data presented in the description of the co-enzyme Q study (page 978). The correct information should read as follows: "Subjects treated with CoQ10 had less disability as shown by a change in UPDRS from baseline (11.99 in controls and 6.69 in the 1,200 mg group)." This does not change the conclusions or recommendations contained in the article, and the data are presented correctly in the accompanying tables that are on the Web site. The authors regret the error.

Phrenic neuropathy due to neuralgic amyotrophy

In the Brief Communication "Phrenic neuropathy due to neuralgic amyotrophy" by B.E. Tsao, D.A. Ostrovskiy, A.J. Wilbourn, and R.W. Shields, Jr. (*Neurology* 2006;66:1582–1584), under Methods, the second sentence is incorrect. It should read as follows: "Inclusion criteria were age older than 18 years." The authors regret the error.

Scale for the assessment and rating of ataxia: Development of a new clinical scale

In the article "Scale for the assessment and rating of ataxia: Development of a new clinical scale" (*Neurology* 2006;66:1717–1720) by T. Schmitz-Hübsch, S. Tezenas du Montcel, L. Baliko, et al., one of the authors, Roberto Fancellu (Department of Biochemistry and Genetics, Istituto Nazionale Neurologico C. Besta, Milan, Italy), was omitted from the list of authors. The publisher regrets the error.

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