もみられた。さらに、RTT-ES細胞由来神経細胞の電位依存性ナトリウムチャネルとカリウムチャネルの未発達傾向が観察された。

D．考察

(1) 血中GRL濃度は食事と日内リズムに影響されることから、本研究では定点採血を行い、比較的安定した結果が得られた。GRLは自律神経調節を介して消化管運動を促進することが知られ、RTTの血中GRL濃度の低下が摂食行動異常や消化管運動障害に影響をおよぼしていると考えられる。

(2) 我々の確立したRTT-ES細胞は神経発生から分化過程の連続的実験を可能にした。今回の結果から、MeCP2は神経発生・分化よりも神経細胞の成熟過程で重要な役割を担うと考えられた。また、RTT-ES細胞の膜特性に有意差が認められた。これは、神経細胞がネットワークを形成する過程において、病的なシナプス形成が引き起こされている可能性が考えられる。

E．結論

RTTの血中GRL濃度の低下を明らかにした。GRLの機能低下がRTTの臨床症状の一部の発症病態に関与していると考えられた。血中GRL濃度はRTTの臨床像と一定の相関を示すことから、RTTの生物マーカーの可能性がある。また、MeCP2の神経細胞の成熟過程での重要性を示唆した。

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H．知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）

分担研究報告書

レット症候群の診断と治療・療育マニュアルの作成と生体試料の収集

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研究要旨

本研究ではこれまで試みられてきたレット症候群の診断基準をレビューし、我々が検討してきている本症の診断に必要な臨床的特徴を述べ、本症の診断について検討した。またこれらをもとに科学的根拠に基づいた治療と療育のマニュアルを検討した。生体試料の収集に関しても検討した。

A．研究目的

レット症候群（RTT）の診断は、本症の臨床的特徴に基づいて行われてきた。1999年にその病因遺伝子としてMethyl CpG binding protein2(*MECP2*)遺伝子の発見後、*MECP2*変異が診断に取り入れられてきている。しかし、臨床像の捉え方に共通の認識がなされておらず、これまで複数の診断基準が提唱されてきた。本研究はこれまで提唱されてきた診断基準を検討し、正確な診断法を確立する。また、現時点では根本的な薬物による治療はないため、個々の病態にあった理学療法、作業療法、音楽療法などの指導のためのマニュアル化を行なう。

B．研究方法

これまでに提唱されてきた診断基準をレビューし、それぞれの意味を検討し、独自の診断基準の策定と治療、療育法マニュアル化を行なう。

（倫理面への配慮）

本研究は瀬川小児神経学クリニック倫理規定を遵守して行った。患者本人、患者本人が未成年または知的障害のために判断が不可能な場合は保護者に説明し、同意を得られた場合のみ研究を行った。個人の情報、プライバシーの保護に十分配慮した。

C．研究結果

[診断基準について]　RTTは、1966年Andreas Rettにより、特異な症状を呈する大脳萎縮症として初めて報告された。以来、RTTの診断は臨床的特徴に基づいて行なわれてきた。これまでの主な診断基準は次のようなものがある。

(1) Hagberg B, Goutières, Hanefeld F, Rett A, Wilson J. *Rett syndrome: criteria for inclusion and exclusion. Brain Dev* 1985;7:372-3.

(2) The Rett Syndrome Diagnostic Criteria Work Group. *Diagnostic criteria for Rett syndrome. Ann Neurol* 1988;23:425-8.

(3) Hagberg B, Hanefeld F, Percy A, Skjeldal O. *An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett syndrome clinical criteria consensus panel.* Satellite to European Paediatric Neurology Society Meeting. Baden Baden, Germany, 11 September 2001.

(4) DSM-IV-TR

(5) ICD-10

我々は本症の重要な臨床的特徴である特異な精神運動発達遅滞と症状の年齢依存性に注目し、報告してきた。

発症は乳児期早期でおとなしく、筋緊張低下し、睡眠覚醒リズムの異常があり乳児期早期より日中の睡眠時間が多い。これらの症状は軽微で、見逃されることが多い。乳児期後半には這い這いの異常が特徴的で、四つ這い移動の不獲得や遅くれ、移動運動パターンの異常などがみられる。これら本症の歩行はロコモーション障害と考えられる。また、随意性の手の機能の獲得が遅れる。頭囲の拡大が減退する。乳児期後半から幼児期にかけて、精神運動発達の退行、周囲に対し反応を示さなくなる。こうした特徴のうち、乳児期早期の症状と這い這いの異常、周囲に対する反応の消失は自閉症の早期症状と共通である。本症に特異な、手の随意機能の消失と同

時に出現する手の常同運動は、しばしば上肢全体、口、舌、歯軋り、時に下肢におよぶことが少なくない。小児期初期より、ジストニア性筋緊張が下肢から出現し、経過と共に四肢、体幹のジストニア姿勢が進行する。これが後彎、側彎となって現れ、小児期から思春期に進行することが多い。頭囲の発達は減衰し、小頭を呈してくる。てんかんもしばしばみられる。睡眠覚醒障害に加えて、突然の笑いや泣きもみることがある。息つめ、過呼吸などの呼吸障害も特異で、呑気による異常な腹部膨満をきたすこともある。咀嚼障害、嚥下障害もしばしばみられ、便秘症もほぼ必発である。小児期には、冷足などの自律神経障害が出現する。身長、体重、手足の大きさの成長が停滞し、極度の痩せ、時に肥満をみることがある。

病態理解の上で重要な年齢依存性の変化はステージング（Habgerg and Witt-Engerström, Kerr）と名付け、症状を整理している（資料４）。また、*MECP2*の変異解析結果も診断には重要である。

[治療・療育のマニュアル]　治療・療育のマニュアルは、一般の小児神経医師、小児科医師、その他関連の医師、療育機関のパラメディカル担当者、患者家族、教師などを対象としている。その概要を記す。

(1) レット症候群の定義について
本症は特異な発達障害で、主として神経系を障害するが、他の内蔵機能の症状も見逃すことができない。代表的な症状について述べる。
(2) レット症候群の病態について
(3) レット症候群の病因について
(4) レット症候群の治療・療育について
現時点での主となる治療・療育の指針として次の如くまとめられる。いずれも対症療法である。
(4-1) サポーティブな治療・療育：理学療法、作業療法、言語療法、心理療法、音楽療法、その他。
(4-2) 併発症、合併症に対する治療：てんかん、側彎症、呼吸異常、心臓リズム障害、その他。

[生体試料の収集]　本症の正確な診断と今後の病態・病因の研究、それに基づいた治療法開発を目指した臨床および基礎研究は臨床医と基礎研究者の密接な協力と連携が欠かせない。患者とその家族の理解のもとに種々の生体試料を採取、保管を進める。現在、臨床データ、画像データ、血液・尿・髄液試料、筋・末梢神経・皮膚などの生検を収集している。現在、iPS細胞研究を目的とした線維芽細胞の収集を準備中である。

D．考察

本症の診断基準は、臨床的特徴と病態の関連性に基づいて作成する必要がある。RTTの病態理解が不十分なだけでなく、RTTの全例に*MECP2*変異はなく、他の疾患にも*MECP2*変異がみつかっているため、これまでの診断基準は実態を反映していなかった。したがって、今回作成した診断基準(案)に基づいて、新たな診断基準を作成し、標準化をはかる。

[治療・療育のマニュアル]　本症は特異な発達障害であり、主として神経系を障害するが、他の内蔵機能の症状も重要である。治療・療育のマニュアル作成は、本症の臨床的特徴を詳細に理解し、かつそれぞれの病態に基づいた対応のために必要である。

E．結論

RTTの診断は当初より臨床診断であり、病因遺伝子が解明された以後も基本的には臨床診断であることは変わりない。しかし、病因遺伝子の解明により病態の理解が深まり、診断基準もリファインされている。

治療は現時点では対症療法であるが、本症の認知が進むに従いより正しい治療が個々の患者になされてくることが必要である。

G．研究発表

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H．知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）

分担研究報告書

遺伝子改変細胞及びマウスの作製と行動、機能解析

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研究要旨
レット症候群の臨床的臨界期の特定や神経発達におけるMeCP2発現の重要性を明らかにするため、MeCP2発現をコントロールできるノックインマウス作製を試みた。胎生期において安定な遺伝子発現が得られるROSA26領域に、テトラサイクリンで発現制御可能なMeCP2遺伝子を持つ発現ユニットを導入し、トランスジェニックマウス作製を行った。

A．研究目的

レット症候群（RTT）の研究は、原因遺伝子であるメチル化CpG結合タンパク質2（MeCP2）の基礎生物学的研究やMeCP2欠損マウスによる病態解析が行われ、IGFBP3のantagonistを用いた治療実験が進められている。しかし、発達障害特有の症状の時間的特異性や回復可能な臨界期の研究は未だ行われていない。

MeCP2発現を制御して発達段階の異なる時期にその発現を変化させ、その結果生じうる神経発達の異常を検索することは、RTTの病態解明の上で今後重要な取り組みとなると考えられる。また、その病態の形成時期を特定することは、今後のRTTの治療の取り組みに際して臨床的臨界期を特定することにつながり、極めて重要な情報となりうる。

我々は、胎生期における遺伝子発現が極めて保たれ、発現抑制を受けないとされるROSA26遺伝子座領域に注目し、この領域にジーンターゲティング・ノックイン手法を用いて遺伝子発現の制御を可能とさせるテトラサイクリン発現制御システムとMeCP2遺伝子を組み込む。組み込んだ制御系は、ROSA26領域の持つ特性から、遺伝子発現抑制から免れて安定したテトラサイクリン発現制御を行うtet遺伝子産物を産生し、さらにその産物がテトラサイクリンの濃度によってMeCP2遺伝子発現を制御するシステムが構築できる。

B．研究方法

ROSA26領域をノックインするプラスミドベクターは、大阪大学宮崎教授より供与されたものを使用した。このベクターの構造をより詳細に明らかにするため、ベクター上にある各遺伝子の既知配列を用いてシークエンスプライマーを作製し、特に各遺伝子間の接合部を中心にDNA配列を得て、プラスミドのほぼ全長の配列を確認する。その情報をもとに、MeCP2 cDNA配列を組み込み、MeCP2の発現制御用のジーンターゲッティング・ノックイン用ベクターを構築する。

その基本構造は、ROSA26遺伝子のゲノム配列に、MeCP2遺伝子およびPGK-neoカセットが挿入され、pBluescriptにサブクローニングされている。MeCP2遺伝子の発現コントロールは、Cre組み換えタンパク質によりneo遺伝子を取り除いた後、テトラサイクリンで制御されるtranscription activator(tTA)がROSA26プロモーターにより発現され、MeCP2とEGFP両遺伝子が発現コントロールされる。

得られたノックインベクターは、大量培養の後に精製し、マウスES細胞（129Sv/EvTac系統）にエレクトロポレーションにより導入する。PCRならびにサザンハイブリダイゼーションによりG418耐性ES細胞のコロニーをスクリーニングし、正しくターゲッティングされたES細胞クローンを選別する。

このES細胞をBlastcystにインジェクションすることにより、キメラマウスを作製する。高効率にES細胞由来の組織を有する雄マウスを、さらにC57BL/6J系統の雌マウスと交配し、F1マウスを得る。このマウスをさらに交配することにより、ROSA26遺伝子領域にMeCP2発現コントロールユニットを有するマウスを作製する。実際の発現コントロールは、さらにこのマウスをCreマウスと交配することにより、LoxPの組み換えを起こさせた後に得られる。

（倫理面への配慮）

本研究は環境中への拡散防止しつつ遺伝子組み換え生物の使用を行う「第二種使用等」に分類され、DNA組換え安全委員会の承認の元に行われる。

C．研究結果

オリジナルのノックインベクターの構造配列を解析した後、MeCP2遺伝子を組み込んだノックインベクターの作製に成功した。その後、研究方法に従って、目的のES細胞の20株を得た。そのうち正常核型を有する2株（A2、A20）を胚盤胞に注入し、偽妊娠ICR系統マウスの子宮に移植した。得られた産子のうち毛色判定により、129系統マウス由来のES細胞とホストマウス系統のキメラマウスを確認した。

その結果、2株のES細胞を注入した初期胚(計63個)から、合計3頭のキメラマウスを得ることが出来た。これらのうち、毛色判定で高キメラ個体と判定されたのは、A20由来のキメラマウス2頭であった。引き続き、これらの高キメラマウスをC57BL/6マウスと交配させ、F1マウスを得た。今後はさらに、Creマウスと交配することにより、MeCP2発現制御可能なマウスを作製していく。

D．考察

テトラサイクリン誘導系を用いて目的の遺伝子発現制御を行う実験は、過去多くの遺伝子座で試みられてきたが成功した例は少ない。これは、ノックインした遺伝子座の多くで、発現コントロールユニットの発現が抑制され、制御が働かなくなる可能性が指摘されている。今回我々は、すでにいくつもの成功例を持つROSA26領域をそのノックインの場所として採用している。その点で、我々の目的とする発現制御が達成される可能性は高いと考えている。

ノックインベクターは、そのサイズが極めて大きく、取り扱いが非常に困難であった。そのため、出来るだけその配列の詳細を明らかにして、MeCP2発現調節用に改変したことは、成功した一つの要因であると考えられた。完成したノックインベクターを使ってのROSA26領域への導入は極めて順調にに進行している。今後は、発現のコントロールが実際に可能かどうかを検証し、その後Rett症候群の表現型が得られるかなどの検討を行っていく予定である。

E．結論

ROSA26領域にMeCP2発現をコントロールするユニットを組み込むノックインマウスの作製を行っている。これまで、予定通りの進展が得られている。

G．研究発表

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H．知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）

分担研究報告書

インプリンティング遺伝子の細胞生物学的解析

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研究要旨
本研究では、レット症候群（RTT）の原因遺伝子であるメチル化CpG結合タンパク2（MeCP2）のヒト15番染色体のAngelman症候群(AS)領域の分子機構を解明する。MeCP2抗体によるクロマチン免疫沈降（ChIP）法でMeCP2結合領域を同定した。また、この領域における*UBE3A*とアンチセンスRNA（*UBE3A-AS*）の転写領域を明らかにした。今後、これらの領域のdsRNAの産生について解析し、分子生物学的アプローチからの新たな治療法の開発へ発展させる。

A．研究目的

レット症候群（RTT）の原因遺伝子であるメチル化CpG結合タンパク2（MECP2）はエピゲノム機構の中心的分子であり、ヒト15番染色体のAngelman症候群(AS)領域のインプリンティング機構をも制御している。このAS領域におけるMeCP2の分子機構を解明することで、RTTのみならずASやPrader-Willi症候群（PWS）などの類縁疾患の発症病態の解明へも発展させる。

B．研究方法

MeCP2 抗体を用いて、クロマチン免疫沈降(ChIP)法によるヒト 15 番染色体上の MeCP2 結合領域を同定する。さらに，MeCP2 によって *UBE3A* とアンチセンス RNA（*UBE3A-AS*）の発現がどのように制御されているかMeCP2ノックダウンSH-SY5Y細胞を作製し、機能および発現解析を行う。

（倫理面への配慮）
本研究では、確立された培養細胞を用いた実験であり、遺伝子組み換えにおいては金沢大学遺伝子組換えDNA安全委員会の承認を得ている。

C．研究結果

ChIP法に用いるMeCP2抗体の選択と条件検討が重要であり、ヒト15番染色体上のMeCP2結合領域を選択的に解析した。ヒト15q11-q13にある*UBE3A*遺伝子の3’UTR領域にMeCP2結合領域を同定した。また，*UBE3A*とアンチセンスRNA（*UBE3A-AS*）の転写領域をRT-PCRで詳細に検討した結果、このMeCP2結合領域内に複数のオーバーラップする領域を見出した。

D．考察

*UBE3A*とアンチセンスRNA（*UBE3A-AS*）の転写制御機構について、MeCP2結合領域内に複数の候補領域が得られたことは、MeCP2のエピゲノム機構の関与を改めて証明したことであり、RTTに限らずASやPSWなど広く発達障害の病態形成に関与していることを意味している。今後、これらの領域のdsRNAの産生について解析する。MeCP2ノックダウンの結果とあわせて、*UBE3A*とアンチセンスRNA（*UBE3A-AS*）の転写制御機構を解明することで治療法の開発へ発展させることが期待できる。

E．結論

MeCP2による下流遺伝子UBE3Aの転写制御機構を明らかにすることは、RTTの複雑な病態を明らかにする上で大変重要である。本研究では，UBE3Aの3’UTR領域に新規のMeCP2結合領域を見出した。このことはMeCP2がこの結合領域の*UBE3A*とアンチセンスRNA（*UBE3A-AS*）の転写制御に関与している可能性がある。

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H. 知的財産権の出願・登録状況

なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Nomura Y	Rett syndrome	Kompoliti K Verhagen L	Encyclopedia of Movement Disorders	Elsevier	New York	2010	(in press)

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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研究成果の刊行物・別刷

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Seminars in
Pediatric
Neurology

A History of Our Understanding of Cerebral Vascular Development and Pathogenesis of Perinatal Brain Damage Over the Past 30 Years

Sachio Takashima, MD,* Masayuki Itoh, MD,† and Akira Oka, MD‡

This article reviews our studies focusing on cerebral vascular development, the pathogenesis of subependymal/intraventricular hemorrhage (SEH/IVH), periventricular leukomalacia (PVL), and pontosubicular neuron necrosis (PSN). Their pathogenesis consists of predisposing developmental and causal factors. SEH/IVH may be caused by reperfusion or overperfusion following ischemia in the subependymal germinal matrix with characteristic vasculature. The cause of PVL is multifactorial (ie, ischemia and inflammation), predisposed by the maturational status of the vasculature and oligodendroglia in the white matter. Focal PVL is ischemic necrosis, and diffuse PVL or white matter injury may include cytotoxic damage. PSN has an apoptotic character, and may be induced by ischemic and oxidative stress on specific immature neurons. Further studies on preventive and therapeutic measures are necessary in clinical, pathologic, and experimental fields. The monitoring and control methods of brain hemodynamics and cellular stability should be more developed to prevent brain damages.
Semin Pediatr Neurol 16:226-236

Perinatal brain damage is caused by hemorrhagic, hypoxic–ischemic, infectious, metabolic, toxic, or traumatic events during the prenatal or neonatal period. Some of the perinatal brain diseases are caused by genetic abnormalities. These perinatal brain injuries lead to cerebral palsy, intellectual disabilities, and epilepsy. Recently, psychiatric disorders, attention deficit/hyperkinetic disorders, or learning disabilities in survivors of high-risk neonatal periods have received increasing attention in both the medical and educational fields. Understanding the pathogenesis and prevention of these events is of great importance. Immature structure and function of the developing cerebral vasculature may play a central role in these different etiologies. This article reviews our studies on cerebral vascular development, the pathogenesis of perinatal brain damage, and the areas of controversy over the past 30 years, focusing on subependymal/intraventricular hemorrhage (SEH/IVH), periventricular leukomalacia (PVL) and pontosubicular neuron necrosis (PSN).

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Neuropathology of Fetuses and Neonates

Neuropathology of stillbirths often provides insight into the pathogenetic mechanisms leading to neurologic disorders in liveborn infants. A variety of abnormalities are found in the brains of stillbirths, the most common including PVL, SEH/IVH, cerebral infarcts, PSN, and spinal cord or brainstem necrosis.

On the other hand, the neuropathology of neonates shows both greater range and extent of brain abnormalities than in the fetus, and all lesions described in the fetopsies may also be seen in neonatal autopsies. The prenatal insults may become a predisposing factor for postnatal severe brain damage. Recently, the incidence of SEH/IVH has been markedly reduced, but perinatal hypoxic–ischemic encephalopathy (HIE) remains the most important cause of cerebral palsy and intellectual disabilities. Brain lesions of perinatal HIE consist of cortical laminar necrosis, cerebral infarct, basal ganglia necrosis, PVL, Ammon's horn necrosis, PSN, and brainstem necrosis. The pathogenesis of each lesion type may be different. Despite advances in perinatal intensive care, these vari-

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ous lesion types continue to be identified in recent cases of cerebral palsy. Induced hypothermia in infants with severe HIE is a recently developed intervention aimed at decreasing the incidence and severity of neurological sequelae. Recent advances in neuroimaging, including diffusion tensor MRI, are promising for the early diagnosis and intervention of these lesions.

Cerebrovascular, Neuronal, and Glial Development in the Human Brain

Perinatal brain lesions occur age-dependently in specific sites, and their pathogenesis consists of a causal factor and predisposing factors, such as vascular, glial, or/and neuronal development.

The structural and functional development of the brain vasculature is closely associated with regional cerebral blood flow and metabolic demand, and in its underdeveloped state forms a very important predisposing factor for regional brain damage in hypoxia–ischemia. In particular, fetal and neonatal leukomalacia is still an important cause of cerebral palsy and intellectual impairment.

On the other hand, neuronal development is also related to the vulnerability of the neonatal brain. For example, PSN is often found in autopsied fetal and neonatal brains, and shows a characteristic apoptotic feature of neuronal karyorrhexis and distribution in the pontine basis and subiculum.[1]

Characteristic Angioarchitecture in the Cerebral Hemisphere

In the cerebral hemispheres, the perforating arteries branching from the leptomeningeal arteries supply the cortex to the deep white matter as cortical, subcortical, and medullary branches. Most of the perforating branches are short in the second trimester and develop with gestational age (Fig 1). The ventriculofugal arteries, which are found in some parts, are end branches of the lateral striatal, choroidal, or other perforating arteries,[2,3] and remain short in the premature fetus.[3] However, there is a controversy over the importance of ventriculofugal arteries in the brain injury in premature infants.[4] Nelson et al[5] have studied the vascular development of the fetal telencephalon and found perforating vascular channels freely anastomosing with each other at deeper levels of the cerebral hemisphere.

On the other hand, venous drainage in the cerebral mantle is divided into 2 pathways, in the meningeal direction from the cortex and superficial white matter (cortical and subcortical veins), and in the ventricular direction from the deep white matter (medullary veins).[6] The medullary veins in the deeper cerebral white matter are more developed than are the veins in the subcortical white matter. These characteristics of the developing vascular structure may be predisposing factors for perinatal brain damage.[7]

Additionally, there are the developmental characteristics of vessel density in the human brain. In the cerebral cortex and subcortical white matter, the vessel density is low at

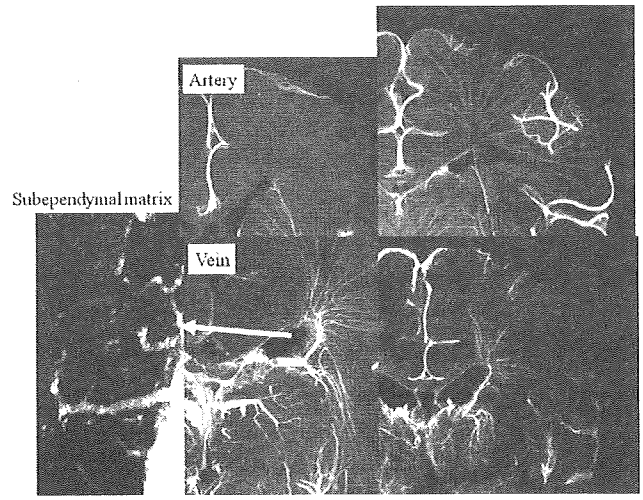

Figure 1 Development of the arterial and venous architecture in the human cerebrum. TV: Terminal vein. Numerous small venules are present with the appearance of a bamboo bush, on the ventricular side of the terminal vein. SEH originates in this bush-like venous structure, which drains the terminal branches of the thalamostriatal arteries. SEH may then progress to IVH by rupture through the ependyma. (Color version of figure is available online.)

16-28 GW, and then increased after 36 GW. In the deep white matter, the vessel density is high in the middle fetal period (16-24 GW), transiently decreases at 28-36 GW, and then increases after 39 GW. In the putamen, the vessel density is high at 20-21 GW, remains high throughout the fetal period, and then rapidly increases after birth. In the basis pontis, the number of vessels increases after 28 GW, and after 32 GW is greater than in the pontine tegmentum. These alterations in vessel density may play a role in the pathogenesis of perinatal hypoxic–ischemic brain injury.[8,9]

Glial Development in the Cerebral White Matter

In the cerebral white matter, microglial as well as astroglial activation with myelination glia precedes the myelination of normal development and becomes a predisposing factor for leukomalacia. The number of microglia increases slightly and transiently between 25 and 40 GW (Fig 2).[10]

Using immunoperoxidase methods in normal infants, glia that are positive for glial fibrillary acidic protein (GFAP) are found first in the deep zones of white matter, and with increasing age they become more prominent in the subcortical zone. This shifting, transient increase of GFAP-positive glia in cerebral white matter may be another predisposing factors leading to perinatal leukomalacia.[11,12]

Myelin transcription factor 1 (MyT1) is expressed in early progenitors of oligodendrocytes, and has been examined in the developing human brain. MyT1-positive glial cells are first detected at 19 GW and then gradually increase until 26-29 GWs. Then, they decrease and become very rare at 1 year of age. The expression of MyT1 immunoreactivity shifts from the nucleus to the cytoplasm of the glial cells in the developmental time course.[13]

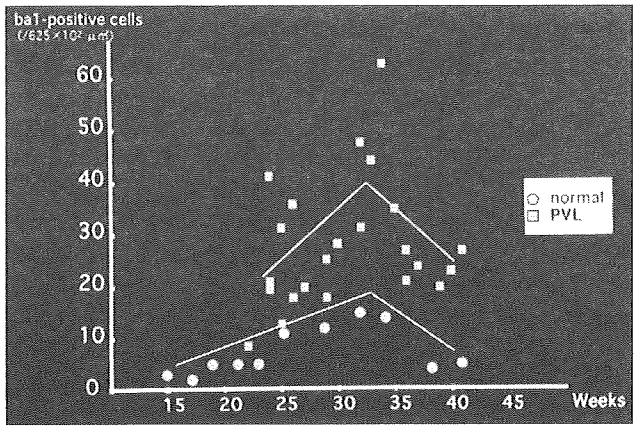

Figure 2 Development of Iba1-1 positive microglia in deep white matter in normal and PVL brains. Microglia are increased in PVL cases, releasing cytokines. (Color version of figure is available online.)

Pathogenesis and Complications of IVH

The incidence of intracranial hemorrhage is markedly reduced with recent advances in obstetric and neonatal intensive cares. However, SEH/IVH remains a significant problem in extremely low birth weight infants and fetuses.

Pathogenesis of SEH and IVH

In the pathogenesis of SEH/IVH, there are predisposing factors and causal factors. One of the predisposing factors, is that the microvascular architecture of the subependymal matrix in preterm infants is characteristic in the end zone of thalamostriatal arteries and in the bamboo bush-like venule structure zone on the ventricular side of terminal vein.[6] Hypotension or hypoxemia may induce focal hypoxic–ischemic changes in the characteristic arterial end zone within the subependymal germinal matrix, and with reperfusion or overperfusion following ischemia, venous hemorrhage may occur in the subependymal germinal matrix, with its paucity of supportive connective tissue.[7] Monitoring of intracranial microcirculation should be a goal for future management of the high-risk newborn.

Complications of IVH with Irreversible Brain Damage

Periventricular white matter hemorrhage (PWMH) occurs as hemorrhagic infarction in the territory of the medullary veins that are tributaries of the terminal vein. PWMH, with or without intraventricular rupture, is found at the deep arterial border zones of the frontal or occipital lobes. The ischemic tissue damage induced by hypoperfusion may be a predisposing factor for PWMH. Also, the high incidence of intravascular thrombi and the fan-shaped appearance of hemorrhage suggest venous thrombosis with coagulopathy, which may be another important factor for the pathogenesis of PWMH.[14]

Posthemorrhagic hydrocephalus is caused by stenosis at cerebral aqueduct or cerebellar foramina, because astrogliosis with ependymal cell deletion is induced by hemosiderin deposits. Subarachnoid hemorrhage (SAH) in the cerebrum induces absorption disturbance of cerebrospinal fluid, resulting in communicating hydrocephalus.[15]

Olivocerebellar transsynaptic degeneration is a unique phenomenon in the low birth weight infant. Cerebellar SAH or ischemia may lead to Purkinje cell and granular neuron loss, with gliosis in the inferior olivary nuclei.[16] Although Bergmann's glial cells have glutamate transporters that remove glutamate from the extracellular space surrounding Purkinje cells during the early stage of brain hypoxia–ischemia, the expression of the EAAT4 and GLAST glutamate transporters is decreased in the cerebellar cortex underlying SAH, and the immature glia are delayed in their clearance of glutamate (Fig 3). These characteristics of glutamate transporters in immature cells may predispose to cell death and olivocerebellar degeneration.[17,18] This transsynaptic degeneration is found in other perinatal brain injuries. These systemic brain damages through transneuronal connection and function may be important, although its clinical significance is not clear.

Pathogenesis and Prevention of PVL

White matter injury in fetuses as well as in preterm infants is related to cerebral palsy and intellectual impairment. Perinatal leukomalacia may be categorized, according to its distribution, severity, and age at onset. PVL occurs in the deep white matter of preterm infants, and subcortical leukomalacia in the superficial white matter of term or older infants.[19] The pathogenesis of these 2 types of white matter lesions may be different.[20] PVL is regarded as a major cause of motor sequelae in infants born prematurely, and the development of techniques for its prevention of major importance.

Pathology and Brain Imagings of PVL

Histologic examination of PVL in the acute stage in neonates reveals characteristic features of multiple foci with axonal

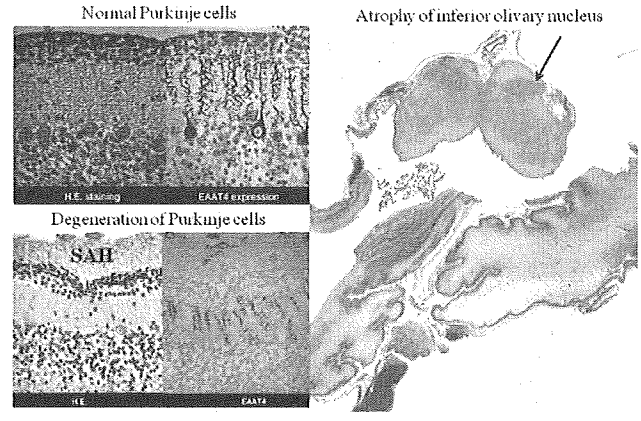

Figure 3 Purkinje cell death and olivocerebellar degeneration following subarachnoid hemorrhage. (Color version of figure is available online.)

swellings, microglial activation, and reactive astrocytosis. At the remote stage of PVL in preterm-born children, there are multiple foci of fibrillary gliotic or cystic changes in the centers of the lesions, which are surrounded by areas with astrogliosis and loss of myelination, resulting in focal atrophy of the affected white matter and leading to an irregular ventricular wall and ventricular dilatation.[19] In terms of reactive vascular changes with PVL, neovascularization appears around foci of necrosis. The expression of vascular endothelial growth factor in both astrocytes and endothelial cells in vessels plays an important role in embryonic angiogenesis as well as in neovascularization around foci of necrosis.[21]

Axonal injury is a major feature of PVL pathology in the prenatal or neonatal brains. Amyloid precursor protein (APP) immunohistochemistry conspicuously demonstrates focal lesions with swollen axons in the periventricular white matter, and is useful to detect the distribution of the lesions, which is predominant in the posterior regions of the cerebral hemispheres.[22-24] Of note, the lesions in near-term neonates tend to be well localized, often as multiple foci, whereas they are typically widespread in very preterm neonates (Fig 4). Based on the evaluation in autopsied cases, we classified the pattern of PVL distribution into 3 types, ie, focal, widespread, or diffuse types. Focal PVL often occurs frequently in near-term neonates, and widespread PVL more frequently in preterm infants.[25]

Beta-APP immunoreactive axons are found in the acute stage of prenatal PVL, coinciding with other early features, such as coagulation necrosis, microglial activation, axonal swelling, or astrogliosis. In addition, beta-APP immunoreactivity is also observed in pyramidal neurons in the fifth layer of the cerebral cortex, corresponding to the beta-APP positive axons in PVL lesions. This immunoreactivity becomes undetectable in the later stage of prenatal PVL, indicating that beta-APP accumulation is an early phenomenon. Because beta-APP is a substrate of the axonal transport system, the early induction of beta-APP immunoreactivity in neurons in the cerebral cortex as well as in axons may indicate the disturbance of axonal flow, ie, axonal dysfunction.[26]

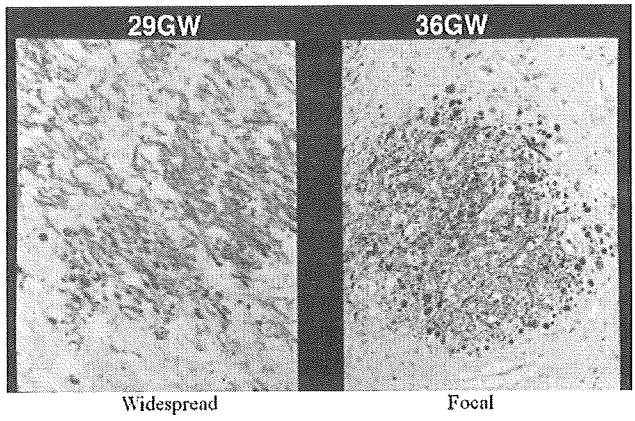

Figure 4 APP-positive axons in PVL. (Color version of figure is available online.)

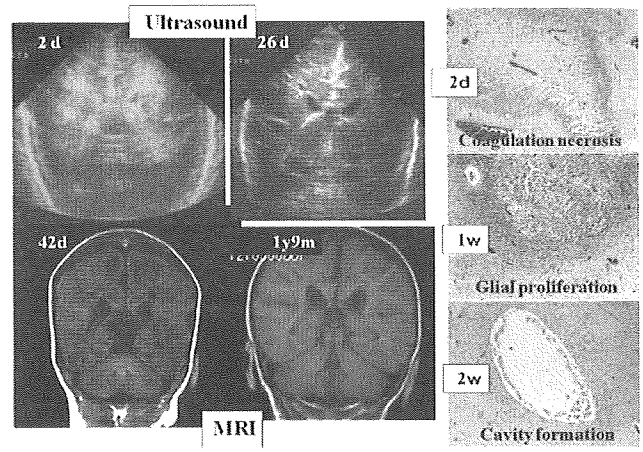

Figure 5 Brain imaging and histologic findings in PVL. (Color version of figure is available online.)

In terms of neuroimaging, ultrasonography has been the most widely used bedside technique for the detection of white matter lesions. Persistent high echodensity defined as higher density than the choroid plexus (grade 3), or cystic changes on ultrasonography, leads to the diagnosis of PVL. These ultrasonographic findings are well correlated with leukomalacia on brain pathology, particularly in the more severe cases with multicystic encephalomalacia. With echodensity similar to the choroid plexus (grade 2), there is a less but still fair correlation (about 40%) with the neuropathology of PVL (Fig 5).

Before the MRI era, brain CT showed periventricular low-density areas, irregular ventricular wall, and reduced white matter volume in children with PVL. Currently, MRI is central to the evaluation of PVL, and periventricular hyperintensity on T2-weighted and FLAIR sequences is closely related to PVL. MRI can significantly predict neurological outcome.[27,28] Furthermore, earlier stage PVL lesions have been observed routinely by early MRI, which delineates the precise site and extent of PVL.[29] Recent developments in MRI, such as diffusion tractography or fractional anisotrophy maps, have advanced the assessment of the cerebral white matter, allowing enhanced evaluation of axonal damage in infants with PVL.

Pathogenesis of PVL

Immature Cerebrovascular Development as a Predisposing Factor to PVL

The developmental status of the cerebral vasculature is a critical predisposing factor in the pathogenesis on PVL. The topographic predilection of white matter injury is distinct between immature and mature brains, because regional cerebral blood flow and metabolic demand are rapidly changing during the perinatal period. In the immature brain, the end-zones of perforating arteries through the cerebral cortex are located in the periventricular white matter, which is a watershed area vulnerable to PVL.

The ontogeny of the developing cerebral vasculature as studied by collagen type 6 immunoreactivity, indicates a discrepancy between arterial and venous development in the

deep white matter.[30] Collagen type 6 immunoreactive vessels, which are mainly medullary veins, terminal branches of internal cerebral veins, already appear from 21 to 29 GW in the deep white matter, whereas cortical and subcortical veins as well as perforating arteries lack the immunoreactivity. The positive vessel number increases with maturation. Therefore, the developmental discrepancy between the later maturation of arteries and the earlier maturation of veins in the deep white matter may be an important predisposing factor for PVL and PWMH.

Furthermore, in white matter with PVL lesions, collagen type 6-positive vessels with abnormally thick walls are found predominantly in the deep white matter. The immunoreactivity is markedly increased in remote PVL lesions, exhibiting abnormal vessels with thick collagen 6-positive walls. The distribution of the lesions with abnormal vessel changes, depends on the gestational age, ie, it is very widespread in the cerebral white matter of extremely preterm infants less than 24 GW, relatively widespread in very preterm infants of 26-29 GW, and focal in preterm or term infants of more than 34 GW.[31] These results are consistent with the notion that the distribution of white matter injury in PVL is well correlated with the development of perforating arteries in the cerebral white matter.

Glial Development and Activation in PVL Pathology

GFAP immunoreactive glia, including myelination glia in normal brains, are temporarily increased in deep white matter during the late fetal period, and in subcortical white matter during the postnatal period. PVL and subcortical leukomalacia occur in the white matter with developmentally upregulated glia during these periods, and the number of these glia is markedly increased in the lesions of leukomalacia.[11,32]

Therefore, the increase of GFAP-positive glia shifts from deep white matter to subcortical white matter in the normal developing brains, and the shift of glial upregulation, as well as the shift of the arterial border zones, is an important predisposing factor for leukomalacia.

Microglia and reactive astrocytes are identified by immunohistochemistry with Iba-1 and GFAP, respectively. Iba1-positive microglia transiently increase from 25 to 36 GW in the cerebral white matter.[33] In comparison with the developmental pattern in normal cerebral white matter, Iba-1-positive microglia and GFAP-positive astrocytes are upregulated in the early and later stages of PVL lesions, both focal and widespread.[34]

The expression of cytokines in PVL has been investigated extensively, and the immunohistochemistry of TNF-alpha demonstrates the reactivity of microglia in or around PVL lesions.[10,35,36] The TNF-alpha expression is surrounded by GFAP-positive astrocytes. In addition, IL-6-positive glia are found in PVL lesions. Therefore, the expression of cytokines by these glia in the lesions as an early phenomenon may be related to the formation of PVL.

Osteopontin immunoreactivity is detected in axons and macrophages in the subacute and chronic stages of PVL, in contrast to its absence in either normal white matter or acute PVL lesions. The marked reactivity in swollen and calcified axons bordering the ischemic zone suggests osteopontin is closely associated with death of swollen axons at the periphery of the ischemic zone, and potentially plays a role in regulating the calcification seen in some chronic lesions.[37]

Cerebral Hypoperfusion as a Cause of PVL

In addition to predisposing factors of vascular and glial maturation, cerebral hypoperfusion is a causal factor of PVL, triggered by extracranial conditions, such as systemic hypotension, shock, hypocarbia, bradycardia, or cardiac failure, and intracranial conditions, such as brain edema or meningitis/encephalitis. From a clinical viewpoint, the role of cerebral blood flow regulation is critical for the prevention of PVL, although this issue remains debated.

Experimental models of PVL have used cerebral hypoperfusion as an insult. Yoshioka et al[38] have reported that ligation of the internal carotid arteries causes multiple necrotic foci in the deep white matter of puppies. The pathology of this experimental model is quite similar to that of focal PVL in human neonates.

As an experimental model of PVL in fetuses, repeated umbilical cord compression in fetal sheep has resulted in white matter lesions.[39,40] In this model, bradycardia during the compression, and hypotension following the compensatory hypertension, have been demonstrated, suggesting that PVL may be caused by fetal CNS hypoxia–ischemia due to systemic hypotension.

Fetal or Perinatal Infection and the Role of Inflammatory Substances

Infection in human fetuses and neonates is frequently accompanied by leukomalacia. Gilles et al[41] have reported perinatal telencephalic leukoencephalopathy (PTL) as a kind of leukomalacia, which consists of hypertrophic astrocytes, amphophilic globules, necrotic foci, and acutely damaged glia, widely spread in the cerebral white matter of newborns. PTL is epidemiologically related to bacterial infection. In experimental studies, small doses of endotoxin administered in kittens and puppies result in necrotic foci, astrogliosis, and calcification predominantly in the forebrain white matter, resembling PTL.

In earlier experiments,[42,43] neonatal puppies and rabbits were administered endotoxin, with monitoring of regional and serial cerebral blood flow changes. In addition, brain amino acid contents were at 24 hours and brain histology examined at 24 or 72 hours after endotoxin injection. Cerebral blood flow is slightly decreased in the cerebral cortex as well as white matter within 45 minutes, and is further downregulated in the cerebral white matter at 60 minutes or later. Several amino acids in brain tissue are increased, compared with controls, whereas they decrease in brain hypoxia. Threonine, valine, methionine, and histidine are increased in all areas, and glutamine is increased only in the cerebral white matter, diencephalon, and brain stem. However, GABA is decreased only in the cerebral white matter. The brain histology shows an increased vascular permeability and multifocal necrosis in the cerebral hemispheres.[44]

These results in endotoxin encephalopathy suggest that

there is different susceptibility to endotoxin among regions of the brain, and vascular damage, cerebral hypoperfusion, and metabolic activation of glial cells may be important pathogenetic factors leading to necrosis.

Clinical studies have documented that intrauterine infection is frequently found in cases of cystic PVL,[45] and inflammatory cytokines are increased in umbilical cord blood in patients with PVL.[46]

Experimental models of white matter injury by intrauterine infection have been developed in sheep and rabbits, and selective white matter lesions have been demonstrated.[47,48] In these experiments, chronic administration of endotoxin results in white matter lesions in the absence of hypoxemia and hypotension. However, further intracranial hemodynamic studies may be necessary. The white matter injury may be more related to PTL or to diffuse type PVL.

Oligodendroglial Death, Hypomyelination and Plasticity in PVL

Following cerebral hypoperfusion, axonal damage, glutamate increase and oligodendroglial damage appear, while ischemia induces microglial activation, cytokine release, and astroglial activation, with an increase of nerve growth factors.[49] Both the reparative factors as well as the factors of tissue destruction determine the resulting PVL lesions (Fig 6).

In the progression of PVL pathology, coagulation necrosis appears at the onset, followed by an astroglial reaction at the subacute stage and neovascularization and cavity formation at the chronic stage. In this process, tissue repair is involved after initial damage, and plasticity may play a role.

Myelination is mainly impaired in the necrotic or gliotic periventricular white matter of the brain with widespread PVL. The expression of lipid components is poorer than that of myelin basic protein, and the number of ferritin-containing oligodendrocytes is decreased in the necrotic or diffuse gliotic region of the widespread PVL brains compared with controls.[50] There is a significant relationship between the number of ferritin-containing oligodendrocytes and the degree of myelination. The impaired myelination in the PVL brains occurs in the necrotic regions as well as in gliotic

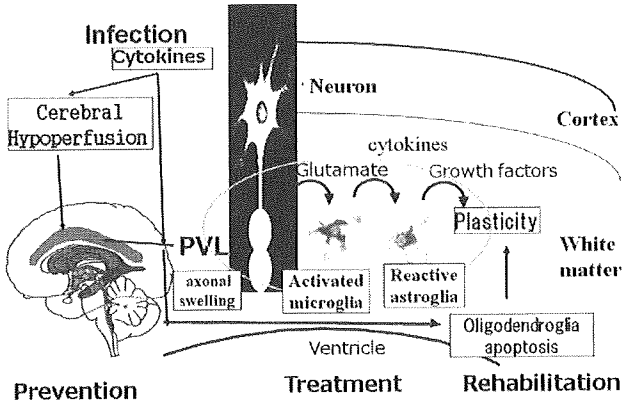

Figure 6 Pathogenetic factors related to PVL formation and repair. (Color version of figure is available online.)

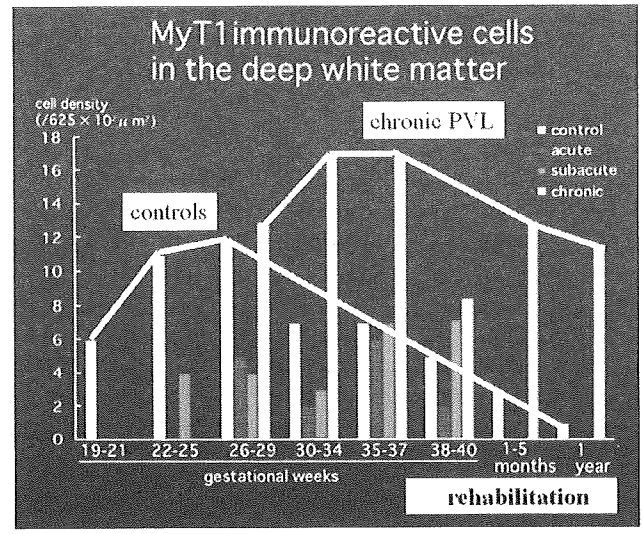

Figure 7 Development of MyT1 immunoreactive oligodendroglia in the deep white matter, and regeneration of immature oligodendroglia in PVL. (Color version of figure is available online.)

regions in the cerebral white matter, related to the decrease of normal oligodendrocytes.[50]

MyT1 is a zinc-dependent, DNA-binding protein and is expressed in the early progenitors of oligodendrocytes. MyT1-positive glial cells are detected from 19 GW and gradually increased until 26-29 GW in controls. In the chronic stage of PVL, MyT1-positive cells are significantly increased around necrotic foci, in comparison with age-matched controls, and some of the regions with increased MyT1-positive cells show an increased level of myelination (Fig 7). This result may indicate excessive myelination in response to oligodendroglial damage, indicating repair or plasticity around the PVL lesion.[51]

Nestin is a protein expressed during the fetal period, and its immunoreactivity is a marker of multipotential stem cells. Nestin immunoreactivity is found strongly in the molecular and superficial layers of cerebral cortices from 12 to 22 GW, gradually decreasing after 27 GW. The reactivity finally disappears after 2 months postnatal age. In the white matter, it is seen in glia, axons, and neurons until 26 GW. Nestin immunoreactivity may be correlated with the formation and differentiation of neurons and glial cells.

Nestin is reactivated in cells surrounding PVL lesions in subacute and chronic lesions.[52] In the white matter of infants with PVL who survived for several months, nestin-positive astrocytes and axons are found in proximity to PVL lesions, whereas they are not found in the white matter remote from the lesions. The reactivity of astrocytes and axons reappears in the chronic stage, following the gradual attenuation of the reactivity during acute and subacute stages of PVL. This re-expression of nestin in astrocytes and axons near PVL lesions in the chronic stage suggests the occurrence of tissue repair and synaptic plasticity in the white matter around PVL. These processes may contribute to a reduction in the size of PVL lesions, as well as an increase in neural plasticity around PVL

lesions, possibly minimizing the disabilities of cerebral palsy and intellectual deficits.

The developmental changes and patterns of the expression of parvalbumin (PA), a marker of GABAergic interneurons, during early development correspond to the establishment of thalamocortical connections, and to the functional maturation of cerebral cortices. In preterm cases with PVL, the expression of PA is increased in the cerebral cortices with focal type PVL, whereas it diminishes in the cerebral cortices corresponding to widespread or diffuse type PVL. These results suggest that increased expression of PA indicates neuronal plasticity in the cerebral cortex and that a reduction of PA reflects the impairment of thalamocortical neurons.[53]

Further Perspective

PVL is a major cause of long-term neurological sequelae, particularly in prematurely born infants, and interventions for its prevention and treatment are urgently sought. The neuropathological, experimental, and clinical studies discussed above, indicate that the pathogenesis of PVL is multifactorial, with 2 major factors, being ischemia and infection-related events. In terms of the latter, the detailed mechanisms remain to be elucidated. So far, no clinically effective therapeutic agents have been identified, and the most logical preventive measures currently available, including avoidance of systemic hypotension, hypocarbia, and severe chorioaminionitis. Improved monitoring and management strategies will be necessary to improve systemic and cerebral hemodynamics, and to ensure cellular stability before prevention of these brain lesions becomes a reality.

Recent epidemiologic studies suggest the possibility of a genetic predisposition to PVL, although these findings remain preliminary.[54] To date, several genetic polymorphisms have been implicated in the cerebrovascular and cellular susceptibility to PVL. Greater understanding of these predisposing genetic factors as well as of the regenerative tissue reaction to injury and the plasticity response will be essential for progress in this area.

Pathogenesis and Prevention of PSN

PSN is typically and frequently found in the pontine nuclei and hippocampal subiculum of fetal and neonatal brains. The morphologic study of PSN suggests neuronal apoptosis; however, its longer term clinical significance in survivors of prematurity remains unknown. The hippocampus and its connections, including the subiculum are important for the consolidation of new and short-term memories. On the other hand, children born preterm have an increased incidence of attention deficit/hyperactivity disorder and learning disability, but little is known about the specific nature of their cognitive deficits or the underlying neuropathology. PSN in the perinatal period may be connected to neurological outcome in preterm children.

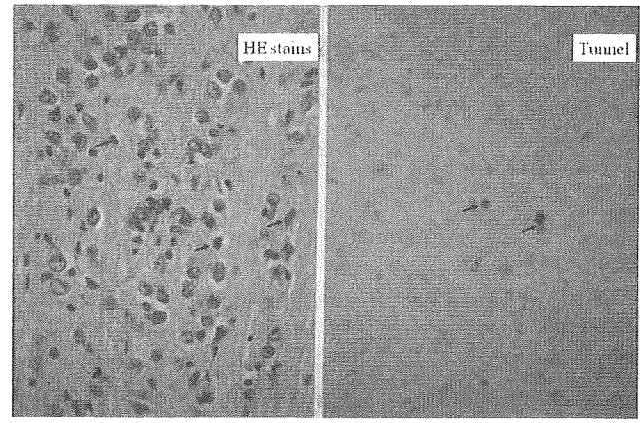

Figure 8 PSN: Karyorrhexis and apoptosis of small neurons in the pontine nuclei and subiculum. (Color version of figure is available online.)

Neuropathology of PSN

PSN is characterized by selective neuronal karyorrhexis in the pontine nuclei and in the hippocampal subiculum of the perinatal brains between 28 GW to 2 months after birth.[55] PSN is not visible in gross brain examination. Microscopically, PSN in the acute stage consists of dying neurons with karyorrhexis and perikaryal shrinkage. Karyorrhexis is characterized by loss of definition of the nuclear membrane and disintegration of the nucleus into basophilic granules, including complete loss of basophilic material. Thus, PSN has the characteristics of apoptosis,[56] with little astroglial reaction (Fig 8). In the chronic stage of severe cases, there is a progressive drop-out of neurons together with astrocytic proliferation, migrating microglia, and aggregations of macrophages scattered throughout the tissue.

The timing of karyorrhectic neurons is characteristic in infants delivered at more than 29 GW, but in very low birth weight infants delivered at younger than 28 GW there is an extended distribution of neuronal karyorrhexis to the inferior olivary nucleus, basal ganglia, thalamus, and cerebral cortex.[57]

Neuronal karyorrhexis in the subiculum as a part of PSN is a more frequent finding in the perinatal brain at autopsies than is Ammon's horn neuronal necrosis, another hippocampal lesion, seen after perinatal asphyxia.

Pathogenesis of PSN

Neuronal karyorrhexis in PSN has some characteristics of apoptosis on developing neurons. The pathogenesis of PSN consists of predisposing factors and causal factors. The predisposing factors exist in developing neurons, glial cells, and vasculature. Although the causal factors include ischemia, hyperoxemia, hypocarbia, and hypoglycemia, the pathogenesis of PSN remains unclear.

Slow Neuron Development in the Pontine Nuclei and Subiculum: A Predisposing Factor

In developmental studies of the pontine nuclei and subiculum, the neurons are small and round. Neuron specific enolase-positive cytoplasm is scanty in fetuses of less than 27

GW, and thereafter gradually enlarges with increasing age until 1 month, while the cell density is decreased after 24 GW. Compared with neurons in the facial nucleus and reticular formation of the pontine tegmentum, as well as the pyramidal cell layer of the hippocampus, neurons of the pontine nuclei and subiculum show delayed maturation, as indicated by gradual enlargement of the initially small cytoplasm.[58,59] The maturational course of these neurons and the timing of PSN are consistent.

The developing neuron is very susceptible to toxicity of NMDA during a restricted developmental period. Mitani et al[60] have demonstrated that the susceptibility to NMDA neurotoxicity in pontine nuclei peaks near postnatal day 15 in rats, and is mediated by NMDA receptor channels that are relatively insensitive to the normal magnesium blockade of NMDA channels. Therefore, maturational changes in NMDA-receptor channels may play a crucial role in the developmentally specific neuronal injury seen in PSN.

Relatively Mature Cerebrovascular Development in Pontosubicular Regions

There is relatively mature cerebrovascular development in pontosubicular regions of fetal and neonatal brains. The pons is supplied by pontine branches of the basilar artery. The basis pontis is perfused by proximal portions of penetrating arteries from the pontine branches in the leptomeninges. Venous angiography shows similar architecture to the distribution of the arterial vasculature. The arteries and veins are well developed in the pons of preterm and term infants. The hippocampal subiculum is supplied by proximal branches of the posterior cerebral arteries. These arteries are abundant and well developed in this area, compared with those of the temporal lobe. Thus, the developmental discrepancy between neurons exhibiting late maturation and arteries showing an early maturation in the basis pontis and subiculum may contribute to the occurrence of of PSN in fetuses and neonates.[58]

Ischemia, Hypocarbia, Hyperoxygenemia and Hypoglycemia as a Causal Factor

A stereotypical neuronal response to hypoxia, acute ischemia, hypoglycemia, hypocapnia, or hyperoxygenemia in a critical developmental time frame has been reported.

Immature developing neurons are more sensitive to ischemia than to hypoxia. The frequency of neonates with PSN who also show karyorrhexis or eosinophilic neurons in other regions of the brainstem, basal ganglia, and thalamus is higher than in controls, and acute ischemia may be an important underlying pathogenic factor.[59] Several factors may contribute to the resistance of immature neurons to hypoxia, such as smaller demands for energy-consuming processes, capacity for maintaining intracellular energy resources via anaerobic glycolysis, and capacity for downregulating the density of sodium channels after prolonged channel activation, which prevent irreversible functional deficits. On the other hand, ischemia is a form of multicomponent injury, including substrate deprivation (starvation), anoxia, and failure to remove toxic end-products. During starvation, the immature neurons produce excessive amounts of oxygen radicals, leading to membrane lipid peroxidation and cell death. Furthermore, oxygen radicals are produced during reoxygenation and reperfusion. The well-developed vascularity in the pontine nuclei and subiculum may contribute to the greater supply of oxygen-derived free radicals and predispose to the production of PSN.

Ischemia, hypocarbia, and hyperoxemia have been reported to be important factors in the pathogenesis of PSN. Karyorrhectic neurons are frequently found in neonates who show hyperoxemia more than 150 mm Hg,[61] and hypocarbia less than 20 mm Hg (Fig 9).[62] Vasoconstriction due to hyperoxemia or hypocarbia is speculated to be one of the pathogenetic mechanisms of PSN. PSN following hypoglycemia shows a large number of apoptotic cells and a higher expression of activated caspase 3.[63]

Experimental Animal Studies

In experimental models of acute hypoxic–ischemic injury, neuronal apoptosis and necrosis have been detected in the brains of newborn piglets[64,65] and immature rats.[66] Our experimental study of newborn rabbits has demonstrated selective neuron death with karyorrhexis in the subiculum and CA1 regions of the hippocampus by hyperventilation and ischemia associated with hypocarbia and decreased intracranial oxyhemoglobin and total Hb concentrations.[67] The damaged neurons are positive on DNA nick end labeling. A DNA ladder is detected on electrophoresis with a DNA sample extracted from hippocampal tissue in the hyperventilation and ischemia group, but not in the other groups. On electron microscopic examination, not only condensation of the nucleus but also disruption of mitochondria and the cell membrane are detected. These results suggest that hypocapnia in the setting of hypotension may cause neuronal cell death in the hippocampus of the neonatal rabbit. Ischemia with a metabolic change induced by hypocapnia may contribute to this apoptotic neuronal cell damage.[67]

Oxidative Stress and Apoptosis

During acute stage of PSN, an astroglial reaction is uncommon; during the later chronic stage, microglial activation and astrocytic reaction become evident.[68] Ferritin-positive glial cells are not increased in cases of selective karyorrhexis, but

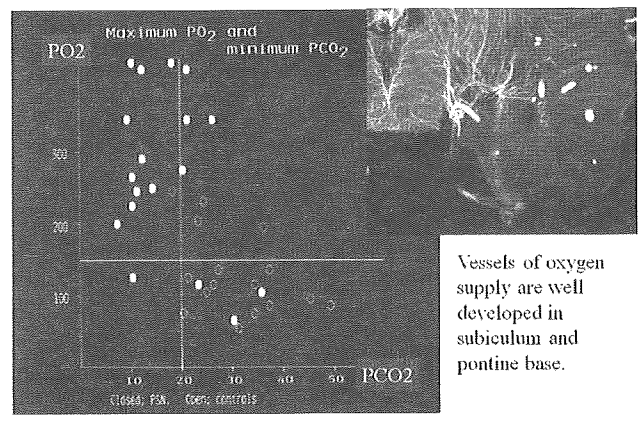

Figure 9 PSN is often found in neonates with hypocarbia and hyperoxygenemia just after birth. (Color version of figure is available online.)

are increased in cases of karyorrhexis with spongy changes and gliosis. Iron may be released to the damaged pontine tissue as a catalyst, and microglia may play an important role in the repair of the tissue.[69]

The immunoreactivities of apolipoprotein E and copper/zinc superoxide dismutase are increased in preserved neurons and glia of neonates with PSN.[70,71] Four-hydroxynonenal (an important marker of radical-induced lipid peroxidation) immunohistochemical study reveals that karyorrhectic cells in the pontine nuclei with PSN are positively stained, and that oxidative stress is present in these neurons. This oxidative stress may contribute to karyorrhectic neuronal death.[72]

The vulnerable neurons undergo karyorrhectic condensation, and exhibit *in situ* end labeling for DNA fragmentation leading to apoptosis in PSN. Neuronal apoptosis in PSN is accompanied by a pronounced activation of caspase-3.[73] Caspase activation plays a central role in apoptosis, and caspase-3 appears to be an especially important effector enzyme in neuronal apoptosis. Numerous CM1 (a specific marker of caspase-3 activation)- and fractin (a marker of caspase-like proteolytic activity) immunoreactive neurons are seen in the pontine nuclei and subiculum.[74]

Neurons of both PSN and control brains show expression of the Fas receptor, and the expression is significantly increased in degenerating neurons of PSN cases. In the developing human brain, cells expressing Fas receptor may be susceptible to undergoing apoptosis.[75] These results also suggest a potential role for caspase-inhibitors as an important element in the development of more effective neuroprotective strategies for asphyxiated infants (Fig 10).

Evolution of PSN and Plasticity

In the acute stage of PSN there is neuronal degeneration with little glial reaction, but microglial and astrocytic activation appears in spongy tissues with marked neuronal loss.

The expression of neuron-type glutamate transporters (EAAC-1), AMPA glutamate receptor subunits (GluR1 and GluR2/3), polyadenosine (5′ diphosphate-ribose) polymerase (PARP), and transforming growth factor-beta 1 (TGFb1) has been investigated in cases of PSN and age-matched controls. Developmental immunoreactivities of EAAC-1, GluR1, and GluR2/3 appear in the neurons of the pontine nuclei at 29-30 GW in controls, and then gradually increase with age. However, these activities are decreased in the pontine nuclei of patients with PSN, suggesting early degeneration of neurons. Although PARP and TGFb1 immunoreactivities are insignificant or very weak in the pontine nuclei at any ages in controls, PARP is markedly expressed in karyorrhectic neurons of the pontine nuclei in patients with PSN more than in controls. TGFb1 is expressed in remained cells and may play a role in the protection and repair of damaged neurons.[76]

Nestin is an intermediate filament protein, expressed in neuroepithelial stem cells of the developing central nervous system. The reexpression of this protein has been observed following pathologic situations in the central nervous system, including ischemia. This reexpression has been considered for the repair of neuronal processes and the synaptic plasticity. In our data, normal hippocampi express mild nestin immunoreactivity at neurons only in preterm infants of less than 27 GW. Nestin reactivity is increased in the brains of infants with PSN compared with control preterm brains. Furthermore, these increases are not only seen in the damaged regions, but also in those regions with otherwise preserved microanatomy. Additionally, nestin reactivity is more increased in subiculum and Ammon's horn that is more widely damaged. Nestin reactivity of neurons is increased only in the preterm infant, in contrast to the finding that only glial cells are increased in the term infants. Nestin, abundant in multipotential stem cells, is reactivated in surrounding cells of subacute and chronic lesions. Thus, local plasticity may be activated both in necrotic and apoptotic cell death, and the period of regeneration may be important for future treatment and rehabilitation strategies.

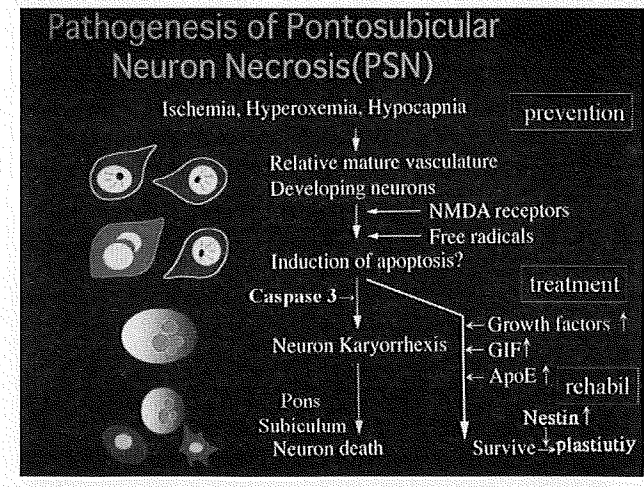

Figure 10 Pathogenesis and plasticity of PSN. (Color version of figure is available online.)

Acknowledgments

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