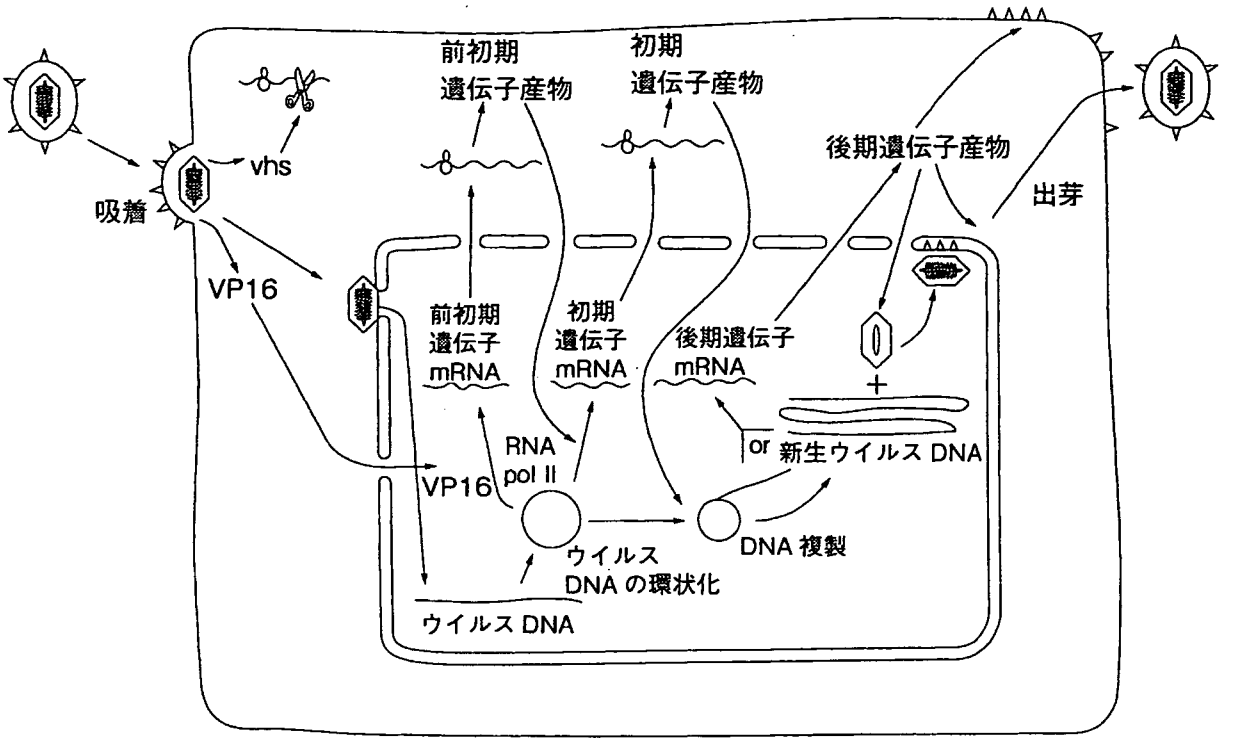

図 1　単純ヘルペスウイルスの増殖メカニズム

HSV はレセプターを介して宿主細胞に侵入．宿主細胞核に運ばれたウイルス DNA は環状化する．テグメント蛋白である VP16 により前初期遺伝子発現が亢進し，その遺伝子産物がつくられる．前初期遺伝子産物は初期遺伝子発現を亢進，それに伴い初期遺伝子産物がつくられる．初期遺伝子産物（DNA ポリメラーゼなど）は遺伝子複製と後期遺伝子発現に働き，ウイルス遺伝子複製とウイルス構成蛋白（後期遺伝子産物）の合成が進む．最終的に空のウイルスカプシドに遺伝子がパッケージングされ，さらにエンベロープ蛋白を獲得して新生ウイルス粒子として出芽する．テグメント蛋白である vhs は，宿主細胞の mRNA を分解し宿主細胞蛋白合成を阻害する．

(Roizman B, et al: Fields Virology. 4th ed[1])

ヘルペスウイルスの生物学

ヘルペスウイルスは前述のように初感染後宿主体内に潜伏感染し，宿主免疫能が低下した際に再活性化し，時に回帰発症するという生物学的特徴をもつ．初感染ならびに再活性化時にはウイルスの遺伝子複製や遺伝子の転写，蛋白合成が行われ，新生ウイルス粒子が産生される（**図 1**）[1]．しかし，潜伏感染時にはウイルスゲノムは存在するものの，基本的に遺伝子複製，遺伝子転写，蛋白合成は起こらず，宿主免疫能からエスケープし，長年宿主体内に潜むことができる．再活性化は各ウイルスの潜伏感染細胞になんらかのシグナルが入ることにより前初期遺伝子の転写が開始され，新生ウイルス粒子の産生が再開されることになる．しかしながら，ウイルスの再活性化は直ちに宿主免疫により感知され，新生ウイルス粒子産生の制御機構が働く．もし宿主免疫能が低下していると，このような再活性化を制御できず回帰発症（臨床症状の出現）につなが

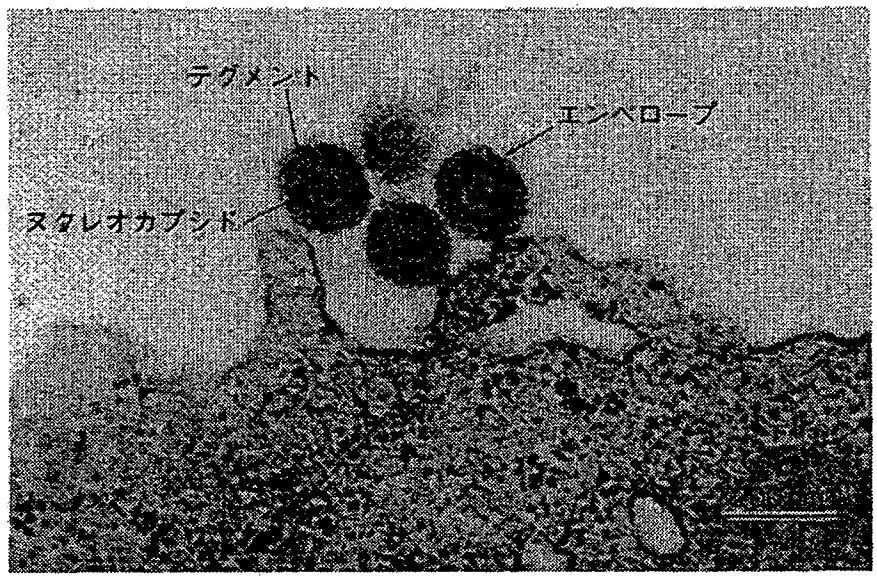

図2　HHV-6粒子の電子顕微鏡写真

表1　ヘルペスウイルス亜科の特徴

亜科	ウイルス	宿主域	培養細胞での増殖速度	潜伏感染部位	その他の特徴
α	HSV-1 HSV-2 VZV	広いもの（例：HSV）から狭いもの（例：VZV）までさまざま	速い	神経節	
β	CMV HHV-6 HHV-7	狭い	遅い	分泌腺，リンパ網様系細胞，腎臓など	感染細胞の肥大化（巨細胞形成）
γ	EBV HHV-8	狭い		リンパ組織	細胞の不死化

る．

ウイルス粒子を電子顕微鏡で観察すると，最外側を数種類の糖蛋白がスパイク状に突き刺さったエンベロープとよばれる外膜で覆われ，内側に遺伝子情報としての二本鎖DNAをもつヌクレオカプシドが存在し，ヌクレオカプシドとエンベロープのあいだにはテグメントとよばれる構造物が存在する（図2）．

8種類のヒトヘルペスウイルスは，それぞれの亜科ごとに特徴的な潜伏感染部位（細胞）やゲノム構造，増殖形態を示す．表1に示したように，HSV-1，HSV-2，水痘-帯状疱疹ウイルス（varicella-zoster virus：VZV）はそれぞれ神経節に潜伏感染し，αヘルペスウイルス亜科に属する．また，サイトメガロウイルス（cytomegalovirus：CMV）やHHV-6，HHV-7は単球マクロファージ系の細胞や唾液腺に潜伏あるいは持続感染し，βヘルペスウイルス亜科として

- 40 -

まとめられている．一方，Epstein-Barr ウイルス（EBV）と HHV−8（Kaposi 肉腫関連ヘルペスウイルス〈Kaposi sarcoma-associated herpesvirus：KSHV〉）はリンパ球などに潜伏感染し，トランスフォーメーション活性をもつという共通性があり，γヘルペスウイルス亜科として分類されている．

初感染臨床像

前述のように，日本においてはほとんどの人が HSV−2 と HHV−8 を除く 6 種類のヒトヘルペスウイルスに感染している．多くのヘルペスウイルスが小児期に初感染を起こすが，CMV や HSV に関しては生活水準の向上に伴い初感染年齢が上昇しつつある．これらヒトヘルペスウイルスのなかで，HHV−6 は初感染時期が生後 6 か月から 1 歳ごろに集中しているのが特徴的である．

一部のヘルペスウイルスにおいては，妊婦が初感染を受けることにより胎内感染を起こし臨床上問題になる．CMV の胎内感染が最も有名であり，先天性巨細胞封入体症を起こす．頭蓋内石灰化を伴う重度の中枢神経障害（**図 3**），難聴，肝脾腫，血小板減少などを合併する重篤な疾患である．また，まれではあるが，VZV の胎内感染に伴い皮膚にケロイド様の瘢痕形成を残すことがある．

胎内感染に引き続き周産期感染の原因となるのは HSV と VZV である．神経

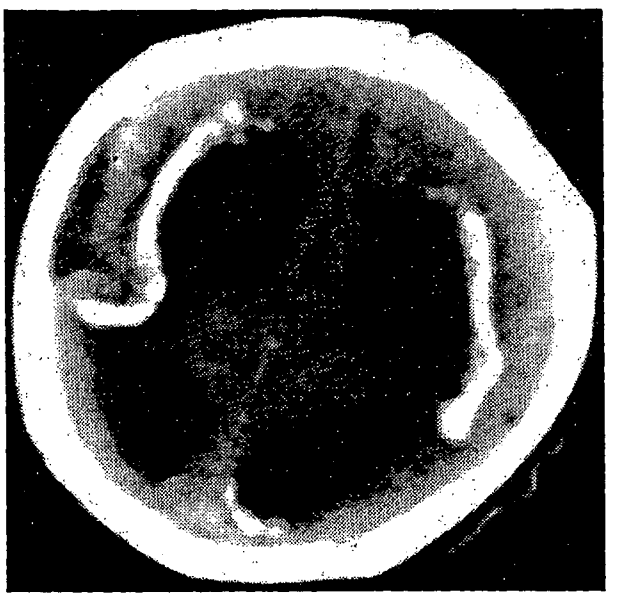

図 3　先天性巨細胞封入体症症例に認められた脳室周囲石灰化
（名古屋掖済会病院小児科，西川先生症例）

親和性のあるHSVは，主に出生時の産道感染により新生児ヘルペスとよばれる予後不良な疾患を起こす．臨床的には表在型，中枢神経型，全身型に分けられるが，中枢神経型と全身型の予後はとくに不良である（表2）[2]．また，出産前後に母体が水痘に罹患すると，新生児が重症水痘になる危険性が高い．前述のように，HSVとCMVの初感染年齢が次第に上昇してきており，未感染の妊

表2　新生児ヘルペスの予後（抗ウイルス薬投与なし）

臨床病型	頻度（%）	死亡率（%）	神経学的後遺症残存率（%）
表在型	18	0	38
中枢神経型	34	50	67
全身型	48	85	50

（Kimberlin D: Herpes 2004[2]）

表3　ヒトヘルペスウイルスの初感染ならびに再活性化時の臨床像

ヘルペスウイルス	ウイルス名	亜科	初感染時臨床像	再活性化時臨床像（健康人）	免疫不全宿主での再活性化時臨床像	その他関連疾患
HHV-1	herpes simplex virus 1	α	歯肉口内炎，角膜炎	口唇ヘルペス		脳炎，新生児ヘルペス
HHV-2	herpes simplex virus 2	α	性器ヘルペス	再発性性器ヘルペス		脳炎，新生児ヘルペス
HHV-3	varicella-zoster virus	α	水痘	帯状疱疹		
HHV-4	Epstein-Barr virus	γ	伝染性単核球症		日和見リンパ腫	Burkitt リンパ腫，上咽頭癌，胃癌
HHV-5	cytomegalovirus	β	多くが不顕性感染		発熱，肝炎，肺炎，骨髄抑制，網膜炎，腸炎など	
HHV-6	human herpesvirus 6 A human herpesvirus 6 B	β β	不明 突発性発疹症		発熱，発疹，肝炎，肺炎，骨髄抑制，脳炎など	
HHV-7	human herpesvirus 7	β	突発性発疹症		脳炎	
HHV-8	Kaposi sarcoma-associated herpesvirus	γ	不明		Kaposi 肉腫，body cavity based lymphoma	

娠可能女性が増すことにより先天性巨細胞封入体症や新生児ヘルペス症例の増加が懸念されている.

8種類のヒトヘルペスウイルスの初感染臨床像を表3にまとめた. HSVは口腔あるいは性器の粘膜病変やKaposi水痘様発疹症などの皮膚粘膜病変に加え, 角膜ヘルペスや先の新生児ヘルペスや単純ヘルペス脳炎（ヘルペス脳炎）といった侵襲性の強い病態も形成する. 周知のようにVZV初感染像は水痘で, その際まれに神経学的合併症をきたす. 水痘脳炎は小脳炎の形態をとる場合が多く, 水痘罹患後時間をおいて発症する二次性脳炎である.

HHV-6の初感染は突発性発疹症（突発疹）である. 突発疹はほかの熱性疾患に比べ熱性痙攣の合併頻度が高く, かつ複雑型の熱性痙攣を起こす頻度が高い[3]. また, HHV-6初感染時に脳症を起こすことも明らかにされており, 最近筆者らが実施した全国調査の結果から, 日本では年間約60例のHHV-6脳症が発生しているものと推定される[4]. このアンケート調査結果から, HHV-6脳症症例の約半数が重篤な神経学的後遺症を残すことも明らかになり(図4), 今後, 診断, 治療指針の整備が望まれる.

HHV-7初感染はHHV-6同様, 突発疹を起こすことが知られているが, 正確な顕性感染率は明らかではない. HHV-6よりは低いと考えられており, かつ初感染年齢が遅れるため2度目の突発疹として経験される場合が多い. 水痘は基本的に顕性感染であり, HHV-6もほぼ80%が顕性感染と考えられている.

一方, CMVとEBVの初感染は不顕性感染であることが多く, 気がつかないうちに抗体陽性となっている場合が多い. 一部の症例, とくにEBV初感染例

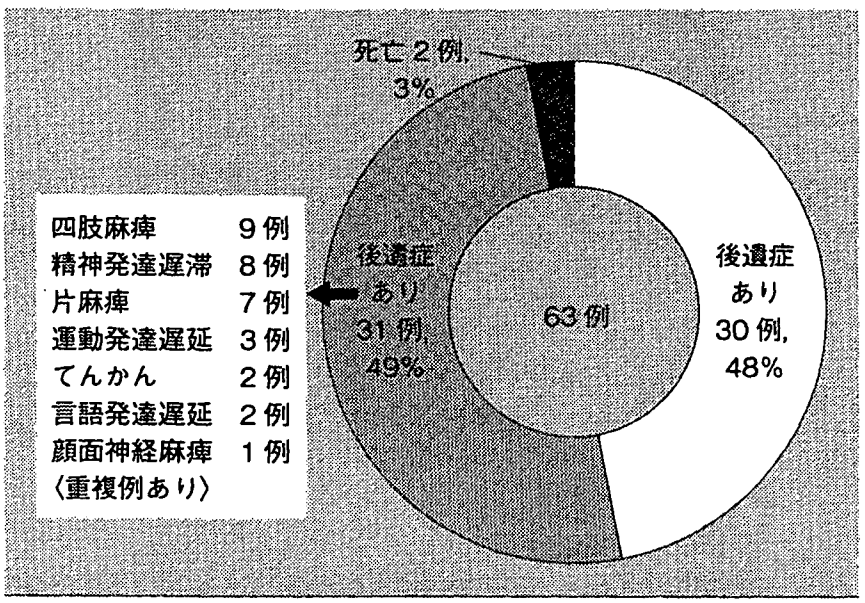

図4 HHV-6関連脳炎・脳症の予後

の一部は伝染性単核球症を起こす．典型例は発熱が持続し，扁桃炎，リンパ節腫脹，肝機能障害を伴う肝脾腫を呈する．正確な頻度は不明ではあるが，伝染性単核球症の際にも脳炎を合併することがある．

再活性化と回帰発症

潜伏感染ならびに再活性化はヘルペスウイルスのもつ最も興味深い生物学的特徴であるが，そのメカニズムについてはいまだ明確な答えはでていない．各亜科によりそのメカニズムは異なっているが，基本的には潜伏感染している細胞へさまざまなシグナルが入ることにより再活性化は高率に起きていることが予想され，それを宿主免疫機構が感知しウイルス増殖を抑制し回帰発症（再活性化に伴い臨床症状を呈すること）を防いでいると考えられる．よって，再活性化に引き続き回帰発症を起こすためには，宿主免疫能の低下が必要である．原発性あるいは後天性の細胞性免疫不全（エイズ患者，臓器移植患者など）や，加齢に伴う免疫能の低下が回帰発症の危険因子である．

各ウイルスの再活性化時の臨床像についても表3に示した．αヘルペスウイルス亜科に属するHSVやVZVはともに神経節に潜伏感染し，そこで再活性化する特徴をもつ．HSVは主に三叉神経や坐骨神経，VZVは脊髄後根神経節に潜伏感染し，それぞれ再活性化に伴う回帰発症として各神経支配領域に病変を生ずる．HSV−1は主に三叉神経領域で再活性化し再発性口唇ヘルペスを起こし，HSV−2は主に坐骨神経で再活性化し再発性性器ヘルペスの原因となる．さらに，HSV，VZVの再活性化がそれぞれBell麻痺やRamsay Hunt症候群といった耳鼻科的疾患や，急性網膜壊死という眼科疾患の原因となっていることも明らかになっている．VZVは65歳以上の高齢者や免疫抑制療法を必要とする臓器移植患者，悪性腫瘍患者，自己免疫疾患患者などで再活性化し帯状疱疹を起こす．抗ウイルス薬投与により以前に比べ治療は容易になったが，帯状疱疹後神経痛はいまだ大きな問題である．最近，高齢者の帯状疱疹予防を目的とした水痘ワクチン接種の有効性が報告され，注目を集めている[5]．

3種類のβヘルペスウイルス（CMV，HHV−6，HHV−7）は，臓器移植患者やエイズ患者での再活性化が問題になる．CMVはそのような患者において間質性肺炎，肝炎，拒絶反応増悪，脳炎，腸炎，網膜炎などさまざまな疾患を起こす．HHV−6も同様に，発熱，acute GVHD（移植片対宿主疾患）様の皮疹，acute GVHDの増悪，肝炎，間質性肺炎，脳炎などを起こす[6]．HHV−7につ

いては，いまだ解明が不十分で明確な答えがでていないのが現状である．

γヘルペスウイルスは，生物学の項で述べたようにトランスフォーメーション活性があるため，種々の悪性腫瘍の原因となる．宿主の細胞性免疫能が低下している種々の臓器移植患者において，潜伏感染していたあるいは移植時に感染したEBVの増殖により，移植後リンパ球増殖症が起こる．現状では末梢血中のEBV-DNA量をモニタリングしながら，免疫抑制薬の投与量を加減することによりウイルス増殖を抑制し，リンパ腫発症を抑える．さらにリンパ腫発症後の治療法として，化学療法に加えCD20モノクローナル抗体（リツキシマブ）による治療が導入され，予後が改善されつつある．

ヘルペスウイルスの臨床ウイルス学的診断

ヘルペスウイルス感染症の診断としては，ほかのウイルス感染症と同様，ウイルス分離，血清診断のgolden standardに加え，ウイルス抗原検出，PCR法などによるウイルス遺伝子検出，病理組織を用いたウイルス抗原（免疫組織化学法）あるいは遺伝子（*in situ* hybridization法）の検出法があげられる．

ウイルス分離は，活動性感染のよい指標となる点，薬剤感受性解析が可能な点などのため，その重要性は高い．しかしながら，それぞれのヒトヘルペスウイルスを分離するためにはおのおの適切な感受性細胞が必要なうえ，一部のウイルスは増殖速度が遅くウイルス分離判定までに時間を要することから，臨床現場で容易に行うことはできない．また，血清診断も有意な抗体価の上昇を確認するために急性期と回復期のペア血清を採取する必要があり，ベッドサイドでの迅速診断という観点では不向きである．よって現時点では，PCR法あるいはreal-time PCR法によるウイルスDNA検出が主流となっている．とくに本書の主眼である中枢神経感染症の診断においては，髄液からのウイルスDNA検出がきわめて有用である．残念ながら，ヘルペスウイルスに関する遺伝子検査法はいずれも保険収載されていないが，コマーシャルラボラトリーや筆者らの施設を含む各地の研究施設で実施可能である．迅速診断という面においては，CMVのアンチゲネミアアッセイがあげられる．これは，好中球に貪食されたCMVのpp65抗原を検出する方法で，活動性感染との高い相関が認められている．

前述のように，分子生物学的手法によるヘルペスウイルスDNAの検出が現在の迅速診断の主流となっているが，初感染，再活性化時の臨床像が多彩な

め，さまざまな臨床検体からウイルスDNAを検出することになる．末梢血や血清に加え，水疱性皮膚疾患患者における病変部拭い液，中枢神経症状を呈した患者の髄液，急性網膜壊死患者の前房水など多岐にわたる．このような各種臨床検体からDNAを抽出後，目的とするウイルスDNAをPCR法で検出することになるが，ヘルペスウイルス感染症の診断上最も大きな問題は，ウイルスが潜伏感染するために常に潜伏感染と再活性化（活動性感染）を区別する必要がある点である．たとえば，βヘルペスウイルスやγヘルペスウイルスは末梢血単核球に潜伏感染するため，単核球からこれらのウイルスDNAが検出されたとしても，必ずしも活動性感染とは結びつかない．活動性感染と診断するためには，経時的なウイルスDNA量の推移を確認すること，潜伏感染細胞を取り除いたcell freeの成分（血清，髄液上清など）からウイルスDNAを検出することが重要である（図5）．

このように，ヘルペスウイルス感染症の分子生物学的診断においては，ウイルスDNA量の定量的評価が重要であり，その点，定性PCR法に比べreal-time PCR法の有用性が高い．しかしながら，real-time PCR法実施には高価な機器が必要なため，ベッドサイドでの迅速診断法として広く一般的に使われるまでには至っていない．筆者らはより安価で簡便な機器で実施可能なloop-mediated isothermal amplification（LAMP）法によるヘルペスウイルスDNA増幅法を開発した．定量性の点ではreal-time PCR法に劣るが，安価な器械でより迅速（40～60分）に結果判定が可能であり，現在，水疱性皮膚疾患患者

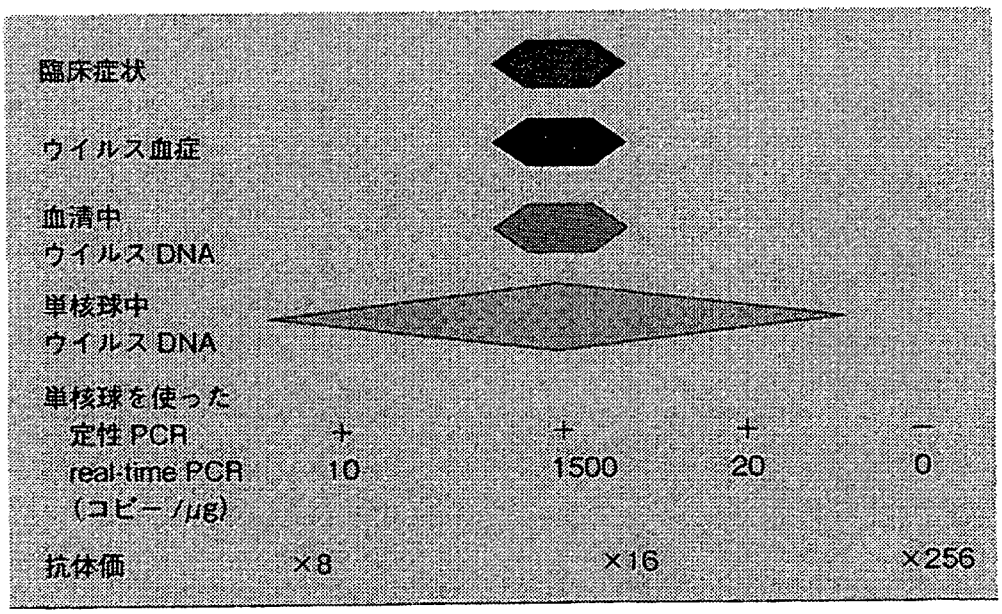

図5　CMV，HHV-6，HHV-7再活性化時の診断法の注意点（潜伏感染と活動性感染の鑑別）

病変部拭い液，性器ヘルペス疑いの病変部拭い液[7]，突発疹疑いの患児血清[8]などから直接（DNA抽出せず）ウイルスDNAを検出することが可能となった．この方法を用いることにより，ベッドサイドで患者から検体を採取した後1時間ほどで結果判定が可能である．

おわりに

ヘルペスウイルス感染症は多彩な臨床像と関与しており，さまざまな臨床科の医師が遭遇することになる．近年の医療技術の進歩に伴い，医原性の免疫不全宿主の数は増加の一途をたどっている．よって，このような患者におけるヘルペスウイルス再活性化が大きな問題になることが予想される．本書のターゲットである中枢神経感染症においても，HSVだけでなくHHV-6やCMV，EBVの関与する疾患も多い．ヘルペスウイルス属の関与する中枢神経感染症患者の診療に際し，ヘルペスウイルス共通の生物学的特徴（潜伏感染と再活性化）をよく理解し，各ウイルスの臨床像に関する基本的知識をもって望むことで，よりよい診療ならびに今後の新たな病態解明が進むことを期待する．

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Longitudinal Analysis of Cytokines and Chemokines in the Cerebrospinal Fluid of a Patient with Neuro-Sweet Disease Presenting with Recurrent Encephalomeningitis

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Abstract

Background Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration. However, the pathogenesis of this disease remains unknown. Neutrophil and other inflammatory cell activities are influenced by many cytokines and chemokines, but to date, no studies have examined the levels of these factors in patients with NSD.

Patient and Methods The patient presented with encephalomeningitis twice in one year and was diagnosed with NSD. We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) and chemokines (i.e., CCL2, CCL3, CCL5, CXCL8, CXCL10 and GM-CSF) in 10 CSF samples from the patient longitudinally for one year including those during two episodes of encephalomeningitis.

Results The elevations of IL-6, IFN-γ, CXCL8 (IL8) and CXCL10 (IP10) were markedly higher than the levels in uninfected control subjects with neurological disorders. The levels of these cytokines and chemokines were statistically correlated with total CSF cell counts ($p < 0.01$).

Conclusion CD4+ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns, and IFN-γ and IP10 are the Th1-type cytokine and chemokine indicating the involvement of Th1 cells in NSD. In addition, the level of IL8, a specific neutrophil chemoattractant, correlated well with the neutrophil cell counts in CSF. Our data suggest the important roles of Th1 cells and IL8 in the pathogenesis of NSD.

Key words: CXCL8 (IL-8), CXCL10 (IP-10), IL-6, IFN-γ, neutrophil cell, Th1 cell

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Introduction

Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration (1, 2). Patients present with painful erythematous plaques on their skin and histological examination of the plaques shows dense dermal infiltration of neutrophils with no signs of vasculitis. This characteristic finding, together with HLA B51 negativity, is important in distinguishing NSD from neuro-Behçet disease (NBD) (2, 3). Japanese patients with NSD also typically show high levels of HLAs B 54 and CW1 (2).

Encephalitis and meningitis are common neurological manifestations of NSD (1). Systemic corticosteroid therapy is highly effective and most patients recover from their neurological deficits without sequelae (1, 2, 4, 5). Despite effective treatment, however, some patients have recurrent episodes indicating that more effective therapies are still needed. A clearly defined pathogenesis for NSD and reliable laboratory markers reflecting disease activity remain elusive. Here, we report the first longitudinal analysis of the levels of cytokines and chemokines in the cerebrospinal fluid

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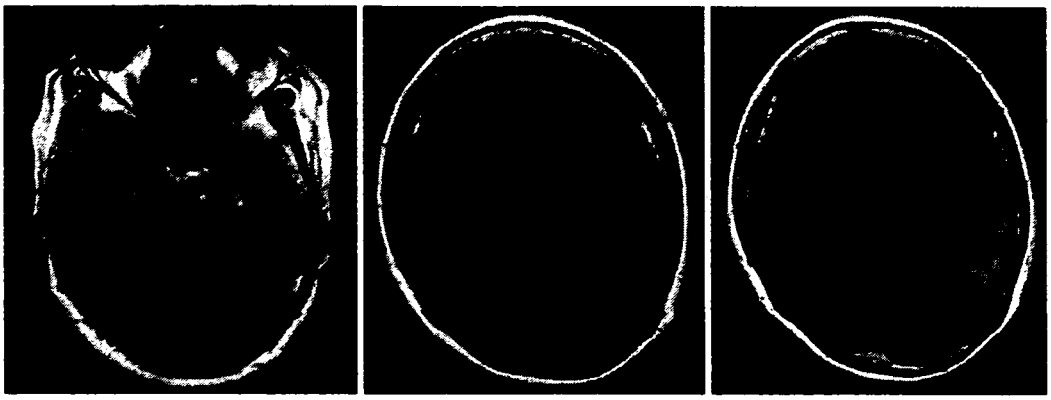

1-1 1-2 1-3

Figure 1. Magnetic resonance images on admission. FLAIR images show high-intensity lesion in the brainstem (1-1), right thalamus and caudate nucleus (1-2) at first hospitalization, and high-intensity lesion in the cortex and subcortical white matter of the left temporal lobe at second hospitalization (1-3).

(CSF) of a patient with NSD. Our results provide important clues to the pathogenesis of NSD and may contribute to the formulation of more effective preventative NSD therapies.

Patient and Methods

Patient

At first hospitalization

A 59-year-old woman had a sore throat and a fever in late August 2005. Four days later, she visited a local hospital. She was diagnosed with acute tonsilitis, admitted to a hospital and treated with antibiotic therapy in early September 2005. The day after admission, she became drowsy and she was transferred to our hospital. She had a history of acute hepatitis B viral infection. She had a temperature of 36.6℃, a pulse of 78/min, and a blood pressure of 148/65 mm Hg. She had erythematous plaques on both legs. On neurological examination, her consciousness level was semicoma and she presented with right pupillary dilatation and delayed light reflex. The deep tendon reflexes of all four limbs were hyperactive except for the bilateral Achilles tendon reflexes. Laboratory evaluation revealed increased numbers of peripheral blood leukocytes and neutrophils: white blood cell (WBC) count, $15.1\times10^3/\mu l$ (normal range: $3.3\times10^3\sim7.9\times10^3/\mu l$) and neutrophil cell count, $13.9\times10^3/\mu l$ (normal range $1.5\times10^3\sim5.9\times10^3/\mu l$). Her serum C reactive protein (CRP) level was 18.8 mg/dl (normal <0.20 mg/dl). CSF examination showed 341 cells/mm^3 (mononuclear cells, 298; neutrophilic cells, 43) and a total protein concentration of 172 mg/dl. A culture of a CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus were absent and PCR analysis also showed no herpes simplex virus. A brain MRI scan showed increased signal intensities on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images in the brainstem (Fig. 1-1), right thalamus and caudate nucleus (Fig. 1-2). The electroencephalogram showed slow basic rhythm and diffuse θ activity. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Subsequently, the disturbance of consciousness became progressively worse and mechanical respiratory management was required two days after admission. She suffered a generalized tonic seizure and was treated with phenytoin. The seizures were difficult to control, however, and required treatment with the anesthetic agent propofol. Because a brain MRI scan showed increased signal intensities on T2-weighted and FLAIR images in various subcortical brain structures, a diagnosis of acute disseminated encephalomyelitis (ADEM) was suspected. Thus, four days after admission intravenous dexamethasone (12 mg/day for 5 days) was administered for 4 days and then, ten days later, methylprednisolone (1,000 mg/day for 3 days) was administered for three days. Her condition gradually improved and she did not require respiratory management. However four weeks after admission a brain MRI scan showed an abnormal signal intensity lesion in the periventricular white matter of the left parietal lobe and expansion of the brainstem lesion. Then her symptoms and abnormal brain MRI findings gradually improved and she was discharged from the hospital without any sequelae in early November 2005.

At second hospitalization

The patient had a sore throat and a fever in mid-January 2006. Five days later, she consulted an otolaryngologist and was diagnosed with acute tonsilitis. She was treated with an intravenous infusion of antibiotics. Five days later she suffered a sudden, generalized tonic seizure during infusion and was referred to our department. She had a temperature of 37.8℃, a pulse of 95/min, and a blood pressure of 153/83 mm Hg. Her throat was reddish and the palatal tonsil was swelling with velaque. Erythematous plaques were apparent on her cheek, forearms and legs. On neurological examination, she was disoriented and could not remember her name

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and birthday correctly. The deep tendon reflexes of all four limbs were hyperactive predominantly in left upper and lower limbs. She presented with bilateral Hoffman reflexes and spasticity of the lower limbs. Laboratory tests revealed increased numbers of peripheral blood leukocytes and neutrophils: WBC count, $13.7\times10^3/\mu l$ and neutrophil cell count, $11.5\times10^3/\mu l$. Her serum CRP level was elevated at 11.3 mg/dl (normal <0.20 mg/dl). The serum rheumatoid factor and antibodies including antinuclear, anti-SS-A, anti-SS-B, anti-DNA, anti-Sm, and anti-RNP antibodies, and the perinuclear anti-neutrophil cytoplasmic antibody (P-ANCA), and the cytoplasmic anti-neutrophil cytoplasmic antibody (C-ANCA) were all absent. Human leukocyte antigen (HLA) typing showed B-54 and CW1. CSF examination showed 108 cells/mm^3 (mononuclear cells, 91; neutrophilic cells, 17), a total protein concentration of 41 mg/dl. A culture of the CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus, varicella zoster virus and toxoplasma were negative. PCR analysis also showed no herpes simplex virus. A brain MRI revealed increased signal intensity on T2-weighted and FLAIR images in the cortex and subcortical white matter of the left temporal lobe (Fig. 1-3). ^{99m}Tc-HMPAO SPECT performed on the third day of hospitalization revealed hyperperfusion in the left temporal lobe. An electroencephalogram showed diffuse slow activity with small spikes and sharp waves in the left temporal region. There were no ocular lesions such as uveitis, episcleritis and conjunctivitis. Neither oral aphthae nor genital ulcers were obserbed. We performed a malignancy survey including a whole-body CT, an examination by gastrointestinal endoscopy, a bone marrow aspiration study, and a gynecological consultation, all of which showed negative results. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Her consciousness was progressively disturbed and she suffered frequent generalized tonic seizures; therefore, at ten days after admission she required propofol treatment and mechanical respiratory management. A skin biopsy of the erythema on her right forearm was performed. Histological examination showed dense dermal infiltration of neutrophils with no signs of vasculitis, and as a result she was diagnosed with Sweet's disease. Corticosteroid therapy was initiated with an intravenous administration of methylprednisolone (1,000 mg/day for 3 days) from the tenth day of admission, followed by 50 mg of prednisolone administrated orally. Her symptoms gradually improved by the end of January 2006 she no longer required mechanical ventilation. However, she continued suffering from a slight fever, and elevated levels of CRP and WBCs without signs of infection and presented with aphasia. As a result, she was treated with a second intravenous administration of methylprednisolone (1,000 mg/day for 3 days) in early Feburary 2006. Subsequently, her symptoms and laboratory data improved, and she was discharged from the hospital without any sequelae about three weeks later.

Methods

Analysis of levels of cytokines and chemokines

We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) and chemokines (i.e., CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL8/IL-8, CXCL10/IP-10 and GM-CSF) in 10 CSF samples from the patient throughout the clinical course. We also measured the levels of those cytokines and chemokines in CSF samples from the control subjects. The control subjects for cytokines were 21 noninfected patients with neurological disorders (epilepsy, 8; psychomotor delay, 5; psychogenic response, 5; functional headache, 1; myopathy, 1; agenesis of corpus callosum, 1) and the control subjects for chemokines were 10 noninfected subjects with neurological disorders (functional headache, 3; Parkinson disease, 1; normal pressure hydrocephalus, 2; spinocerebellar degenetration, 2; amyotrophic lateral sclerosis, 2). CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts. All upper values of control subjects are expressed as mean + 3SD.

Determination of cytokine levels

The levels of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA) as previously described (6-8), with the exception that data analysis was performed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1 pg/mL, 2.8 pg/mL, 2.6 pg/mL, 2.6 pg/mL, 2.5 pg/mL, and 2.8 pg/mL, respectively.

Determination of chemokine levels

The levels of CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL8/IL-8, and GM-CSF were measured using ELISA kits (Endogen, Woburn, MA, USA), and the concentration of CXCL10/IP-10 was measured using an ELISA kit (R&D Systems, Minneapolis, MN, USA) on the basis of the quantitative sandwich enzyme immunoassay technique, as previously described (9). The sensitivity of these assays was 10 pg/mL.

Statistical analysis

The Spearman rank correlation was calculated to assess the correlation between the levels of cytokines and total CSF cell counts, and the levels of chemokines and total CSF cell counts.

Results

Clinical course (Fig. 2)

Clinical manifestations and brain MRI findings correlated

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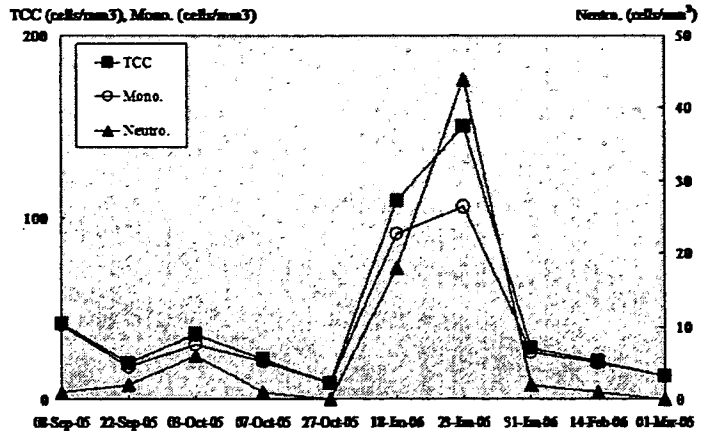

Figure 2. Cell counts [total cell count (TCC), mononuclear cell count (Mono.), neutrophilic cell count (Neutro.), cells/mm³] in CSF.

Table 1. The Levels (pg/mL) of Cytokines (1-1), Chemokines (1-2) and Total Cell Count [(TCC), Cells/mm³] in CSF

(1-1)

Date	8-Sep 2005	22-Sep 2005	3-Oct 2005	7-Oct 2005	27-Oct 2005	18-Jan 2006	23-Jan 2006	31-Jan 2006	14-Feb 2006	1-Mar 2006	*P value*
IL-6 (<12.1)*	209	14.5	255.8	12.3	9.3	2417.2	1329.4	182.7	13.6	12.6	<0.01
IL-4 (<14.3)*	7	<2.5	6	5	<2.5	17.6	13.2	4.4	<2.5	<2.5	<0.01
IL-2 (<5.5)*	2.7	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	ns
IFN-γ (<60.3)*	22.6	12.5	29.1	<7.1	<7.1	134.6	463.7	58	<7.1	<7.1	<0.01
TNF-α (<7.2)*	2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	ns
IL-10 (<7.2)*	4	3.3	4.6	<2.8	<2.8	5.9	5.3	2.9	<2.8	<2.8	<0.01
TCC	42	20	36	22	9	109	150	28	21	13	

(1-2)

Date	8-Sep 2005	22-Sep 2005	3-Oct 2005	7-Oct 2005	27-Oct 2005	18-Jan 2006	23-Jan 2006	31-Jan 2006	14-Feb 2006	1-Mar 2006	*P value*
MCP-1 (<1380)*	348.5	740.8	1344.6	730.6	962.5	1403.2	1217.2	689.9	1097.5	1235.1	ns
IL-8 (<55.23)*	198.5	146.2	283.6	96	86.5	449.4	441.4	264	50.9	80.7	<0.01
MIP-1α (<10.05)*	24.4	18.5	25.2	17.5	17.5	23.7	49.6	26.7	14.5	25.9	ns
RANTES (<7.22)*	40	56	48	26.7	21.3	13.3	50.7	37.3	42.7	50.7	ns
IP-10 (<579.2)*	1880.5	632.1	2417.8	1511.8	605.8	3076.2	3176.3	2639	358.2	932.2	<0.01
TCC	42	20	36	22	9	109	150	28	21	13	

* the levels of CSF cytokines and chemokines of the control subjects (< mean + 3SD)

well with CSF cell counts. The disease activity was divided into active and inactive phases. September 8, 2005, October 3, 2005, January 18, 2006, and January 23, 2006 correspond to the active phases.

Cytokine levels (Table 1-1, Fig. 3)

The levels of IL-6 and IFN-γ in CSF were statistically correlated with total CSF cell counts ($p < 0.01$). The elevations of these cytokines were markedly higher than the lev-

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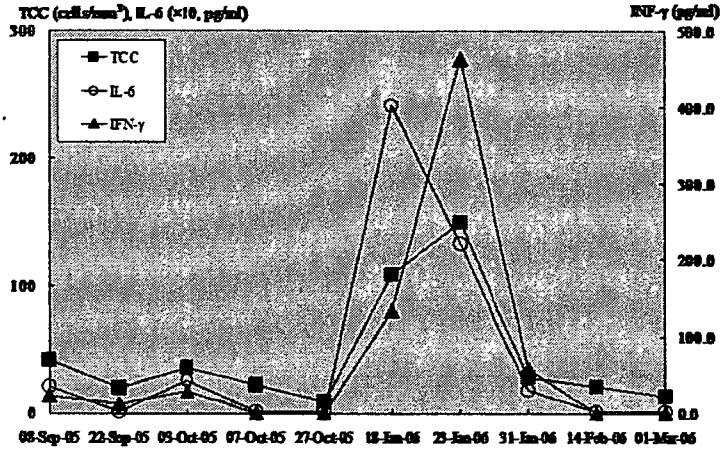

Figure 3. Levels of IL-6, IFN-γ (pg/mL) and total cell count [(TCC), cells/mm³] in CSF.

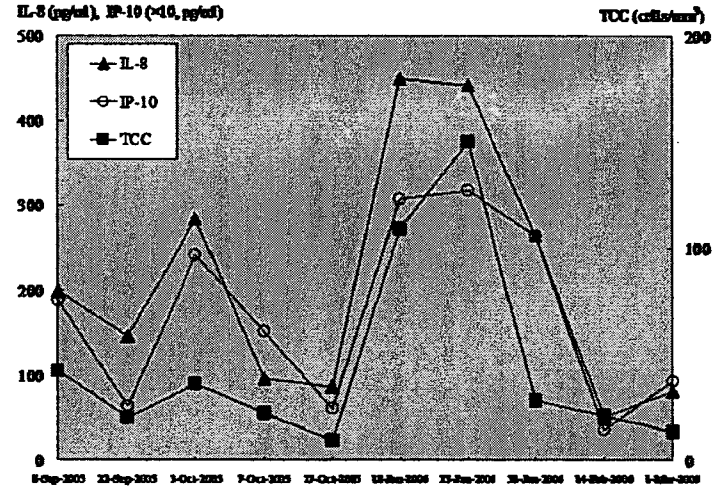

Figure 4. Levels of IL-8, IP 10 (pg/mL) and total cell count [(TCC), cells/mm³] in CSF.

els in 21 uninfected subjects with neurological disorders. The levels of IL-4 and IL-10 in CSF were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. The levels of IL-2 and TNF-α in CSF were equal to or below the detection limits. The levels of CSF cytokines of the control subjects are shown in Table 1-1.

Chemokine levels (Table 1-2, Fig. 4)

The levels of IL-8 and IP-10 in CSF were statistically correlated with total CSF cell counts ($p < 0.01$). The elevations of these chemokines were markedly higher than the levels in 10 uninfected subjects with neurological disorders. The levels of other chemokines in CSF also showed various changes during the follow-up period; however, there was no significant correlation between these levels and total CSF cell counts. The levels of GM-CSF in all of the CSF samples were below the detection limits. The levels of CSF chemokines of the control subjects are shown in Table 1-2.

Correlations between level of IL-8 in CSF and neutrophilic cell counts in peripheral blood and CSF

The level of IL-8 in CSF correlated with the neutrophilic cell count in CSF (Fig. 5-1). The level of IL-8 in CSF also correlated with the peripheral neutrophilic cell count except for during the active phase (October 3, 2005) at the first time hospitalization (Fig. 5-2).

Discussion

The patient's symptoms are compatible with probable NSD consistent with the criteria advocated by Hisanaga et al and the Neuro-Sweet Disease Study Group (2). The present patient's clinical features are summarized according to the following findings: 1. She presented with recurrent encephalomeningitis with subsequent acute pharyngitis and tonsilitis. 2. She had erythematous plaques on her cheek, forearms and legs. A histological examination of the skin biopsy revealed predominant neutrophilic infiltration of the dermis, spared epidermis, and the absence of leukocytoclastic vasculitis. 3. On HLA typing, B-54 and CW1 were positive, but B-51

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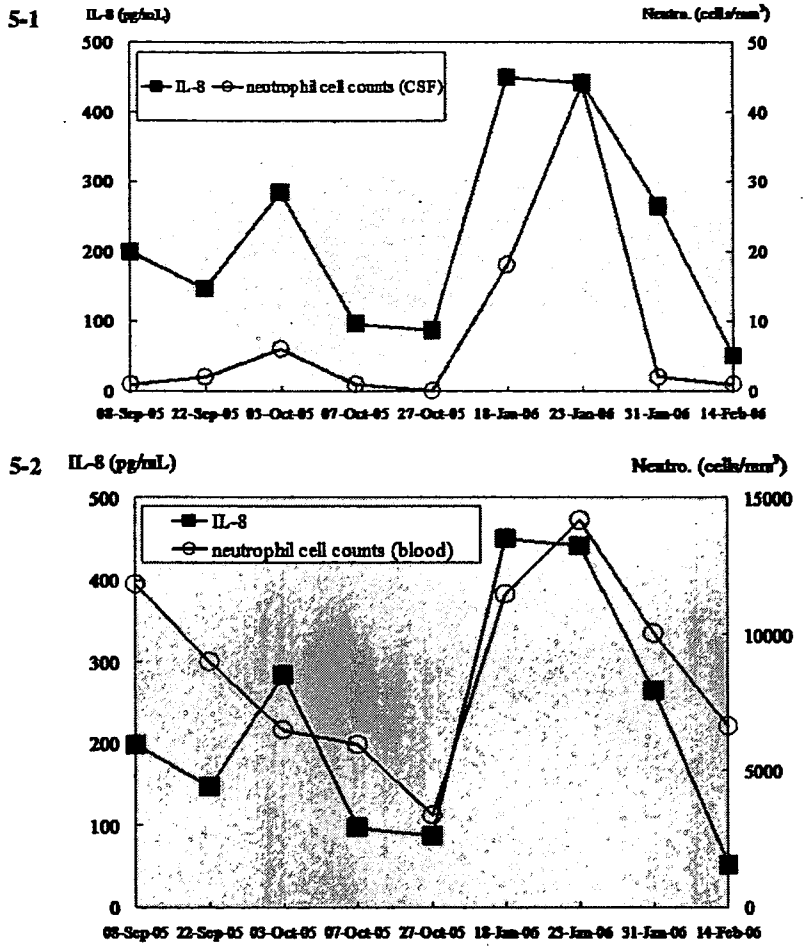

Figure 5. Correlation between level of IL-8 (pg/mL) in CSF and neutrophilic cell counts [(Neutro.), cells/mm³] in CSF (5-1) and peripheral blood (5-2).

was negative. 4. Antibiotics and antiviral therapy were not effective, but systemic glucocorticoids were so effective that the neurologic symptoms and laboratory findings markedly improved. 5. She did not display cutaneous vasculitis and thrombosis, which are seen in Behçet's disease. 6. Abnormal signal intensities on MRI were demonstrated in various CNS regions without site predilection.

The cytokines and chemokines in CSF that correlated well with the clinical state and total CSF cell counts were IL-6, IFN-γ, IL-8 and IP-10. CD4+ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns (10-12). IFN-γ and IP-10, the levels of which increased in our patient, are Th1-type cytokines. Coincidentally, Th1-type cytokines have previously been implicated as mediators of the pathogenesis of Sweet's disease (13, 14). Our data suggest an important role of Th1 cells in the pathogenesis of NSD which is Sweet disease with CNS involvement. The levels of IL-4 and IL-10, which are Th2-type cytokines, were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. It is known that these cytokines in turn cause a decrease in the release of the Th1-type cytokines, thereby regulating the inflammatory response. We thought that the elevations of these cytokines were induced by the elevations of the Th1-type cytokines. IFN-γ causes overexpression of adhesion molecules, responsible for neutrophilic adherence and diapedesis (13). There are multiple reports that suggest that neutrophil chemotactic dysfunction may be the basis of Sweet disease (15-17). In this study, the level of IL-8, a specific neutrophil chemoattractant, correlated with the neutrophil cell count in CSF indicating that NSD may also result from neutrophil chemotactic dysfunction. The level of GM-CSF, a neutrophil chemoattractant similar to IL-8, was below the detection limits. We were therefore unable to show any correlation for this chemokine. The increases in the levels of cytokines and chemokines in the CSF of the patient at the second hospitalization were generally higher than those at the first hospitalization. This finding may be attributed to the delay of the systemic glucocorticoid therapy at the second hospitalization.

Recently, the differences between NSD and NBD have been discussed (2, 18, 19). The present patient did not fulfill the criteria of BD (20) and the HLA type (Cw1 and B54) and histology of a skin biopsy from our patient corresponded to NSD, but not to NBD (2). There are several reports of BD that demonstrate an elevation in the levels of Th1-type cytokines in the serum of patients in the active

phase (21, 22) and in turn suggest that IL-8 could be a serological marker of disease activity (23, 24). In addition, elevated levels of IFN-γ and IL-6 in CSF are detectable in patients in the active phase of NBD (25, 26). Our cytokine data suggested that there are common aspect of pathogenesis between NSD and NBD.

Therapy with systemic glucocorticoids is usually effective in improving the neurologic symptoms in patients with NSD; however, like our patient, some patients occasionally experience recurrent episodes of neurological manifestations after glucocorticoid therapy is discontinued (1). Preventive therapies have not been established, but our study demonstrates that the levels of the Th1 cytokines, IL-6 and IL-8 in CSF are important markers of disease activity in patients with NSD. It is known that the treatment with IFN-β reduces the amount of Th1 proinflammatory cytokines and shifts the immune response toward a Th2 profile (27). Therefore, this treatment might have the potential to prevent the reccurence of NSD. We believe that these results provide useful information for clarifying the pathogenesis of NSD, which may contribute to the development of future therapeutic strategies.

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PAPER

Proteomic analysis of autoantibodies in neuropsychiatric systemic lupus erythematosus patient with white matter hyperintensities on brain MRI

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The pathogenesis of neuropsychiatric systemic lupus erythematosus (NPSLE) may be related to autoantibody-mediated neural dysfunction, vasculopathy and coagulopathy. We encountered an NPSLE patient whose brain showed characteristic diffuse symmetrical hyperintensity lesions in the cerebral white matter, cerebellum and middle cerebellar peduncles on T2-weighted magnetic resonance (MR) images. In this study, we investigated all the antigens that reacted strongly with autoantibodies in this patient's serum by two-dimensional electrophoresis (2DE), followed by western blotting (WB) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) using rat brain proteins as the antigen source. As a result, we identified four antigens as beta-actin, alpha-internexin, 60 kDa heat-shock protein (Hsp60) and glial fibrillary acidic protein (GFAP). There are several reports on the detection of anti-endothelial cell antibodies (AECAs) in an SLE patients. Recently, one of the antigens reacting with AECAs in SLE patient's sera has been identified as human Hsp60. We speculated that the abnormal findings on brain MR images of our patient may be due to impairment of microcirculation associated with vascular endothelial cell injury mediated by the antibody against Hsp60. This proteomic analysis is a useful tool for identifying autoantigens in autoimmune diseases involving autoantibodies. *Lupus* (2008) **17**, 16–20.

Key words: endothelial cell; 60 kDa heat shock protein (Hsp60); neuropsychiatric systemic lupus erythematosus (NPSLE); proteome; white matter hyperintensity (WMH)

Introduction

Patients with neuropsychiatric systemic lupus erythematosus (NPSLE) frequently show various abnormal findings including white matter hyperintensities (WMHs) on T2-weighted brain magnetic resonance (MR) images.[1–3] White matter hyperintensities appear to represent asymptomatic cerebral small vessel disease (SVD).[4] There is accumulating evidence that WMHs are associated with several impairments such as cognitive deficits.[5–7] The pathogenesis of cerebral SVD is poorly understood, but endothelial activation and dysfunction may play a causal role.[4]

Here, we report the case of an NPSLE patient, whose brain MRI showed characteristic WMHs on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images. We examined the reactivity of his serum antibodies against rat brain antigens using the two-dimensional immunoblotting method and identified the antigens that reacted with these autoantibodies by the proteomic method.

Materials and methods

Patient and serum samples

Serum samples were collected from an untreated 69-year old male patient with NPSLE. His clinical features are summarized as follows:

1. He showed slowly progressive polyneuropathy predominantly in the lower limbs and subsequent

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encephalopathy one year after the onset of polyneuropathy.

2. Proteinuria was detected by urinalysis and membranous nephropathy was demonstrated by renal biopsy.
3. Our patient presented delusion and hallucination without insight. Disorientation of time and place were noted. His recent memory was impaired, as he was unable to recall any of three objects after 5 min. He showed impairment of complex attention [disability of digit span (backward)]. Our patient's neurological symptoms were cognitive dysfunction, psychosis and polyneuropathy, as determined on the basis of American College of Rheumatology (ACR) Nomenclature on the NPSLE.[8]
4. Laboratory tests revealed the presence of several autoantibodies [anti-nuclear antibody, anti-DNA antibody, anti-Sm antibody, anti-RNP antibody and lupus anti-coagulant (dRVVT 1.31: normal <1.3)]; hyperglobulinemia [IgG (3448 mg/dL, normal 890–1850 mg/dL)]; decreases in the levels of complements [CH50 (16.9 CH50U/mL, normal 23–46 CH50U/mL) and C4 (2 mg/dL, normal 12–30 mg/dL)], white blood cell count (3620/μl, normal 3400–9200/μl) and lymphocyte cell count (1340/μl, normal 646–4177/μl); and coagulation-fibrinolysis abnormalities [increases in the levels of fibrinogen/fibrin degradation products (FDP) (11.3 μg/mL, normal ≤4.0 μg/mL), D-dimer (1.5 μg/mL, normal ≤1.0 μg/mL) and alpha2-plasmin inhibitor-plasmin complex (PIC) (1.4 μg/mL, normal ≤0.8 μg/mL) and decreases in the value of the thrombotest (48%, normal 70–150%), fibrinogen level (150 mg/dL, normal 150–350 mg/dL), anti-thrombin III activity (71%, normal 80–130%), protein C activity (58%, normal 64–146%), protein C antigen level (61%, normal 70–150%) and protein S activity (52%, normal 60–150%)].
5. Brain MRI showed characteristic diffuse symmetrical hyperintensity lesions in the cerebral white matter, cerebellum and middle cerebellar peduncles on T2-weighted and FLAIR images (Figure 1). Diffusion-weighted images (DWIs) showed high intensities in the bilateral middle cerebellar peduncles with decreased apparent diffusion coefficient (ADC) values. These findings on DWIs and the ADC map suggest that the lesions represent cytotoxic edema caused by ischemic changes.
6. The findings of a nerve conduction study revealed sensory motor axonal degeneration predominantly in the lower limb.

The above-mentioned findings fulfilled the ACR criteria on SLE.[9] He had no risk factors for atherosclerosis, such as hypertension, hyperlipidemia and diabetes mellitus.

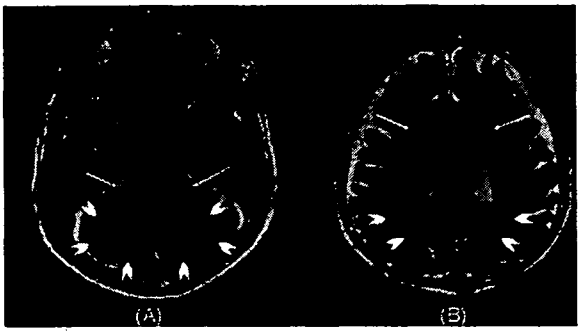

Figure 1 T2-weighted brain MR images (SE, TR/TE: 4080/100 ms). Brain MRI showed symmetrical hyperintensity lesions in bilateral cerebellar hemispheres (arrowheads), middle cerebellar peduncles (arrows) (A), periventricular white matter (arrows) and (B), deep white matter (arrowheads).

Preparation of tissue proteins

Under ether anesthesia, adult Sprague-Dawley rats were sacrificed. The cerebrums were immediately removed and frozen in dry-ice powder. The frozen brain tissue was homogenized with a tissue homogenizer in lysis solution, consisting of 20 mM Tris, 7M urea, 2M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol (DTT), 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride containing a cocktail of protease inhibitors (Calbiochem, San Diego, CA, USA). The homogenate was centrifuged at 150 000 × g for 45 min and the supernatant was used in all experiments. Protein concentration was determined by Bio-Rad Protein assay based on the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA).

Two-dimensional electrophoresis

The samples were dissolved in destreak rehydration solution (GE Healthcare, Buckinghamshire, UK) and loaded by in-gel rehydration into 7-cm long immobilized pH gradient dry strips (GE Healthcare, Buckinghamshire, UK). Up to 250 μg of extracted proteins was applied to the dry strips for Western blotting (WB). Isoelectric focusing was conducted at 20°C for 24 000 Vh at a maximum of 5000 V using a horizontal electrophoresis system, Multiphor III (GE Healthcare, Buckinghamshire, UK). Before separation in the second dimension, the IPG strips were equilibrated for 15 min in a buffer containing 2% SDS, 6M Urea, 30% v/v glycerol, 0.001% BPB and 50 mM Tris-HCl (pH 8.8) under reducing conditions with 65 mM DTT, followed by incubation for 15 min in the same buffer

under alkylating conditions with 140 mM iodoacetamide. Equilibrated IPG strips were transferred to a 12.5% polyacrylamide gel and run at 15 mA/gel. After the electrophoresis, the SDS-PAGE gels were stained with Coomassie Brilliant Blue (GelCode Blue Stain Reagent, Pierce) or used for protein transfer onto polyvinylidine difluoride (PVDF) membranes.

Immunoblotting

Separated proteins were electrophoretically transferred to a PVDF membrane at 50 volts for 3 h using a buffer transfer tank with cold equipment. The PVDF membrane was incubated in blocking solution (5% skim milk in 1 × TBST; 1 × TBS containing 0.1% Tween 20) overnight in a cold room and then reacted with the patient's serum diluted (1:1000) in 1% skim milk in 1 × TBST for 1 h at room temperature. The PVDF membrane was washed five times with 1 × TBST and reacted with peroxidase-conjugated goat anti-human Ig (A + G + M) antibodies (P.A.R.I.S, France) diluted (1:1000) with 1% skim milk in 1 × TBST for 1 h at room temperature. After six washes, the membrane was incubated with the ECL reagent for 1 min and then exposed to an x-ray film for 15–300 s.

Gel digestion and mass spectrometry

The target spot was excised from the gel and subjected to trypsin digestion and peptide fragments were analysed using a nanoscale capillary LC system (LV-VP, Shimadzu) and an ion trap tandem mass spectrometer (LCQ Advantage Max, Thermo Electron). Proteins were identified from MS/MS spectra using protein identification software (X calibur TM, Thermo Finnigan and MASCOT Search, Matrix Science).

Determination of anti-60 kDa heat shock protein antibodies

We determined by enzyme-linked immunosorbent assay (ELISA) the titers of anti-Hsp60 antibodies in sera from our patient, patients with NPSLE (n = 5; age range, 22–58; mean age, 42.4) without abnormal WMHs and healthy controls without abnormal WMHs (n = 7; age range, 17–67; mean age, 43.1). We carried out ELISA to analyse the reactivities of autoantibodies against human Hsp60, which were measured using an ELISA kit (Stressgen, Ann Arbor, MI, USA). Sera diluted 1:1000 in a dilution buffer were added to a precoated ready-to-use recombinant human Hsp60 immunoassay plate and then incubated for 2 h at room temperature (RT). After four washes, peroxidase-conjugated anti-human IgG, A or M was added to each well and then incubated for 1 h at RT. After four washes, a stabilized tetrametylbenzidine substrate was added to each well and then incubated for 15 min at RT. The reaction was stopped by adding acid stop solution and the plate was read at 450 nm on a microplate reader. The OD of control wells without Hsp60 was subtracted from the OD of Hsp60-coated wells. Serial dilutions of serum samples of healthy blood donors having high antibody levels against the tested Hsp60 were used as standards.

Results

Screening and identification of target proteins that reacted with autoantibodies in patient's serum

We detected nine spots (pI 4.2–124 kDa, pI 5.15–kDa, pI 5.3–53 kDa, pI 5.4–53 kDa, pI 5.5–53 kDa, pI 5.25–57 kDa, pI 5.4–57 kDa, pI 5.15–63 kDa, pI 8.0–35 kDa) that strongly reacted with autoantibodies in patient's serum on 2DE-WB (Figure 2). Five among the nine spots that matched proteins on 2-DE gels were analysed by LC-MS/MS. These immunoreactive proteins were identified as beta-actin (pI 5.15–46 kDa), alpha-internexin (pI 5.15–63 kDa), Hsp60 (pI 5.25–57 kDa and pI 5.4–57 kDa) and glial fibrillary acidic protein (GFAP) (pI 5.3–53 kDa) (Table 1).

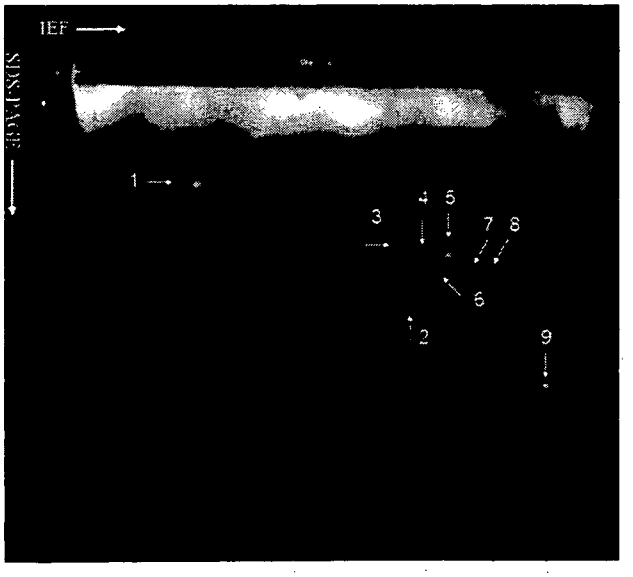

Figure 2 Two-dimensional electrophoresis (2DE) and western blotting (WB). Nine spots strongly reacted with autoantibodies in patient's serum on 2DE-WB. Five spots (No. 2–6) were analysed using mass spectrometry. No. 2: Beta actin; No. 3: Alpha-internexin; No. 4 and 5: 60 kD heat-shock protein (Hsp60); No. 6: Glial fibrillary acidic protein (GFAP); No. 1, 7, 8 and 9: no identification was made.

Table 1 Autoantigens identified using mass spectrometry

Spot Number[a]	*Protein name*	*Mascot score*	*Number of peptides*	*Coverage%*	*Observed M.W.(kDa) /pI*	*Calculated M.W.(kDa) /pI*
2	Beta-actin	421	16	41	46/5.15	42/5.29
3	Alpha-Inx[b]	203	5	12	63/5.15	56/5.20
4	Hsp 60[c]	112	3	6	57/5.25	61/5.91
5	Hsp 60	112	3	8	57/5.4	61/5.91
6	GFAP[d]	113	2	2	53/5.3	50/5.35

[a]Spot number corresponds to the number shown in Figure 2.
[b]Alpha-Inx, Alpha-internexin.
[c]Hsp 60, 60 kD heat-shock protein.
[d]GFAP, Glial fibrillary acidic protein.

Detection of anti-Hsp60 antibodies in our patient and controls

The titer of the anti-Hsp60 antibody in our patient was 133.6 ng/mL. The mean titer of this antibody in five NPSLE patients without WMHs on brain MR images was 19.72 (SD 10.65; range 9.0–32.2) ng/mL. The mean titer of this antibody in the seven healthy controls without WMHs on brain MR images was 15.36 (SD 11.85; range 5.7–39.5) ng/mL.

Discussion

In this study, we detected some autoantigenic proteins reacting with autoantibodies in a serum sample from a patient with NPSLE using the proteomic approach and we identified four autoantigens, namely, beta-actin, alpha-internexin, Hsp60 and GFAP. There are some previous studies demonstrating the association of autoantibodies in serum and cerebrospinal fluid (CSF) with central nervous system involvement in patients with NPSLE.[10–12] Anti-endothelial cell antibodies (AECAs) have been detected in SLE patients.[13,14] Recently, one of the antigens that reacted with AECAs in a SLE patient's sera has been identified as human Hsp60.[15] Human Hsp60 is a molecular chaperone that participates in the folding of mitochondrial proteins and facilitates proteolytic degradation of misfolded or denatured proteins.[16] However, it has also been reported that an enhanced expression of this protein on endothelial cells has been noted and antibodies against human Hsp60 induce endothelial cell toxicity.[15,17,18]

Our patient's brain MR images showed characteristic cerebral WMHs, which appear to represent cerebral SVD. The pathogenesis of cerebral SVD is poorly understood, but endothelial activation and dysfunction may play a causal role.[4] It has been reported that the anti-Hsp60 antibody is present in most patients with coronary artery disease that its titer correlates with disease severity[19] and that it may contribute to the initiation or amplification of vascular endothelial cell damage in atherosclerosis, which is considered a crucial event.[20] Our patient's laboratory findings showed a slightly high level of lupus anticoagulant and some coagulation-fibrinolysis abnormalities. It has been reported that the anti-Hsp60 antibody bind to endothelial cells and induce a thrombotic cascade following endothelial cell apoptosis in SLE patients with the anti-phospholipid antibody.[15] In this study, we determined by ELISA the titer of the anti-Hsp60 antibody in sera from our patient and controls without WMHs on brain MR images. The titer of this antibody in serum from our patient was markedly higher than the mean +2SD of NPSLE patients without WMH or that of healthy controls without WMHs. Thus, the abnormal WMH lesions on brain MR images in our patient may be at least partially due to the impairment of microcirculation associated with vascular endothelial cell dysfunction mediated by the antibody against Hsp60. Further studies using a large series of controls are required to clarify the relationship between the anti-Hsp60 antibody and WMHs on brain MR images.

On the other hand, the clinical significance of the anti-GFAP antibody in NPSLE remains controversial. There is a report showing that the anti-GFAP antibody is specific for NPSLE.[2] Another report suggested that GFAP might be a useful marker in the diagnosis and monitoring of NPSLE, because GFAP level increases in the CSF of NPSLE.[21] However, Valesini *et al.*[22] have reported that the presence of the anti-GFAP antibody in sera of SLE patients showed no significant correlation with neurologic or psychiatric morbidity. Further study will be necessary to clarify the association between the anti-GFAP antibody and NPSLE.

Previously, there were several reports, which described that the autoantibodies against beta-actin and alpha-internexin were detected from non-neurological diseases or healthy controls.[23,24] Therefore, we thought that these autoantibodies were not specifically related to NPSLE and are parts of the natural autoantibody repertoire.

In this study, we detected several autoantibodies from our NPSLE patient and identified the autoantigens that they reacted. Several autoantibodies are generated in systemic autoimmune diseases, and an understanding of the interaction among these