髄膜炎

Meningitis

[1] ウイルス性髄膜炎	
①グリセオール(200mL)	2V，分2，点滴静注
[2] 細菌性髄膜炎	
■原因菌が不明のとき：①または③と②を併用	
①ロセフィン	4g/日，分2，点滴静注
②ビクシリン	6～8g/日，分3～4，点滴静注
③セフォタックス	6～8g/日，分3～4，点滴静注
■グラム陽性菌	
①ビクシリン	6～8g/日，分3～4，点滴静注
■グラム陰性菌	
①セフォタックス	6～8g/日，分3～4，点滴静注
■ペニシリン耐性肺炎球菌	
①カルベニン	2g/日，分2，点滴静注
■メチシリン耐性黄色ブドウ球菌(MRSA)	
①塩酸バンコマイシン	2g/日，分4または分2，点滴静注
[3] 結核性髄膜炎	
①②③と④または⑤	
①イスコチン	400mg，分1
②リファジン	450mg，分1
③ピラマイド	1.5g，分2
④硫酸ストレプトマイシン	1g，毎日より始め，2～3カ月後2～3回/週，筋注
⑤エサンブトール	750mg，分1
[4] 真菌性(とくにクリプトコッカス)髄膜炎	
①に②を併用，③も選択される。	
①ファンギゾン	5～10mg/日より始め，約1週間で30～50mgまで増量
②アンコチル	8g/日，分4
③ジフルカン	200～400mg，分1～2，点滴静注

処方のポイント

• ウイルス性髄膜炎には対症療法が中心で，通常特別な治療を必要としない。単純ヘルペスウイルス，水痘・帯状疱疹ウイルスによる髄膜炎が疑われる場合，ゾビラックスによる治療を行う(624頁参照)。

• 細菌性髄膜炎の治療にあたっては，臨床所見，髄液検査より細菌性髄膜炎が疑われた場合には起炎菌の同定結果を待つことなく抗生剤投与を開始する。

• 起炎菌の判明前には，抗菌スペクトラムが広く，髄液への移行のよいロセフィン(セフトリアキソンナトリウム：CTRX)，またはクラフォラン(セフォタキシムナトリウム：CTX)とリステリア菌をカバーするビクシリン(アンピシリン：ABPC)の併用が一般的である。このほか，抗脳浮腫薬，抗痙攣薬，鎮痛・解熱薬の投与を行う。

• 結核性髄膜炎の治療にはイスコチン(イソニアジド：INH)，リファジン(リファンピシン：RFP)，ピラマイド(ピラジナミド：PZA)，ストレプトマイシン(SM)またはエサンブトール(EB)の4者併用が標準的な組み合わせである。重症例では，副腎皮質ステロイド30mg/日併用。一般に症状の回復に3カ月以上長期になることが多く，抗結核薬の副作用にも十分注意を払う。

• 真菌性(とくにクリプトコッカス)髄膜炎はエイズなどでの日和見感染症として注目されているが，ファンギゾンにアンコチルを併用する。ファンギゾンは5％ブドウ糖に溶解し3～6時間で点滴静注する。副作用の少ない点からジフルカンの投与も一般化しており，点滴静注，あるいは経口投与も可能である。

使用上の注意・禁忌

• 処方例で示した抗生剤の注意すべき点は，ショック，過敏症反応の発熱，発疹，抗酸球増加，ときに無顆粒球症，溶血性貧血がみられることである。肝障害，腎障害も出現する。一般使用量に比較して大量投与であり，血算，生化学検査を頻回に行い副作用の発現に注意する。

• INH, RFPの肝機能障害，INHの末梢神経障害(ビタミンB_6の投与)，SMの第8脳神経障害(聴力障害，平衡機能障害)には1カ月ごとに聴力検査が必要である。PZAは肝障害，高尿酸血症，発疹。EBによる視神経障害には問診と定期的眼科受診を行う。

• ファンギゾンの場合，投与直後の副作用とし過敏反応(アナフィラキシー)，発熱，悪心・嘔吐，食欲不振，頭痛，低血圧，静脈炎など。少量のステロイドの静注や制吐剤を併用する。経過中にみられる副作用に，貧血・顆粒球減少，低カリウム血症，腎機能障害があり，血算，尿検査，血清K, BUN, クレアチニンを頻回に施行し，十分注意する。

相互作用

セフォタックス，ファンギゾンは利尿薬で腎障害が助長される。塩酸バンコマイシンとアミノグリコシド系，ファンギゾンとの併用は聴力障害や腎障害を増強する。リファジンはエサンブトールの視力低下を増強する。

[庄司紘史]

脳炎，単純ヘルペス脳炎

Encephalitis, Herpes simplex encephalitis

[1] 単純ヘルペス脳炎が疑われる場合	
①が第一選択	
①ゾビラックス	10mg/kg，3回/日，200mLの輸液に混じ，1時間以上かけて点滴静注，14日間
②アラセナ-A	15mg/kg，1回/日，500mLの輸液に混入して点滴静注，14日間
[2] 痙攣発作がみられる場合	
①，②または③	
①セルシンまたはホリゾン	10～20mg，ゆっくり静注
②フェノバール	100～200mg，筋注
③アレビアチン	125～250mg，静注
[3] 脳浮腫を伴う場合	
①グリセオール	400～600mL/日，分2～3，点滴静注
[4] ウイルス感染後の急性散在性脳脊髄炎	
①ソル・メドロール	500～1,000mg/日，3日間点滴静注，症状によっては1～2クール追加

処方のポイント

•脳炎は高熱，痙攣，意識障害などの重篤な臨床所見を示す。臨床像，髄液所見，脳波，頭部CT・MRI所見などを参考にして診断を進める。原因にはウイルス，細菌，原虫，プリオンなど多岐にわたるが，ウイルス性脳炎を主体に解説する。抗体検査，PCR (polymerase chain reaction)検査(単純ヘルペス，水痘・帯状ヘルペス，日本脳炎，エンテロ，ムンプス，風疹ウイルスなど)による病因決定には日数を要するが，入院後迅速に(約半日)ウイルス性脳炎としての診断を確立し処方に入る。
•単純ヘルペス脳炎が疑われる場合，アシクロビル(ゾビラックスなど)の点滴静注に踏み切る。この脳炎は主として単純ヘルペスウイルス1型によるが，2型による髄膜炎・脳炎，水痘，帯状疱疹に伴う脳炎にも適応になる。
•遷延例や再発例に対しては，アラセナ-Aを追加する。
•呼吸器，尿路系の二次感染予防にペニシリン系，セフェム系薬剤の投与を行う。
•痙攣発作に対し早めに抗痙攣薬を投与する。セルシン，フェノバール，アレビアチンなどの静注，または筋注。抑制できない痙攣重積には，呼吸管理下でドルニカムの持続点滴投与を行う。
•脳浮腫に対しては10%グリセオール，20%マンニットールを用いる。
•日本脳炎には，レベトールなどの投与が試みられている。
•サイトメガロウイルス脳炎(脳室炎)に対しては，デノシン5mg/kg，2回/日，点滴静注。
•二次性脳炎，急性散在性脳脊髄炎(acute disseminated encephalomyelitis；ADEM)：麻疹，風疹，ムンプスなどに伴って意識障害，痙攣を認める場合，二次性脳炎が考えられる。また，ADEMに対し副腎皮質ステロイドの適応がある。
•トキソプラズマ脳炎には，ピリメサミンとスルファジアジンを用いるが，日本では未承認である。エイズ症例ではエイズ治療薬研究班(www.iijnet.or.jp/aidsdrugmhw/)に依頼できる。

使用上の注意・禁忌

•ゾビラックスの使用上注意すべき点は，①結晶析出による腎障害を防ぐため，1時間以上かけての点滴静注。②少数例でGOT，GPTなどの軽度上昇がみられるが，継続投与可能である。腎機能障害患者では血中濃度が異常に高くなることが予想され，ときに脳症などを引き起こす。用量の減量など慎重投与。
•アラセナ-Aは，①難溶性で500～700mLの輸液量を必要とする，②白血球，血小板の減少，GOT，GPT，γ-GTPの上昇，少数例で振戦，構音障害などの神経症状の報告がある。これらの薬剤は妊婦，授乳婦，本剤に対し過敏症の既往のある場合，禁忌である。
•セルシンは呼吸抑制に注意，また，脳圧降下薬(グリセオール，マンニットール)は電解質異常に注意する。
•ソル・メドロールでは感染症の誘発，消化管出血に注意，生ワクチン，弱毒性生ワクチンは併用禁忌である。

相互作用

ゾビラックスはアルカリ性を呈するため，他剤との混注を避ける。　[庄司紘史]

1. 痴呆を伴うパーキンソン病，アルツハイマー病，レビー小体型痴呆の疫学

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はじめに

パーキンソン病（Parkinson's disease：PD），痴呆を伴うパーキンソン病（Parkinson's disease with dementia：PDD），レビー小体型痴呆（Dementia with Lewy bodies：DLB），アルツハイマー病（Alzheimer's disease：AD）は，高齢者の増加に伴って増えている．高齢社会におけるこれらの疾患の重要性はさらに高まっているが，実態は不明な点が多い．

A．PDD・DLB・AD などの痴呆性疾患の疫学における問題点

まず，診断基準の問題がある．各疾患の診断基準が設けられているが，それぞれの診断基準を使用して診断した場合，異なった複数の疾患の診断基準を満たすこともある．Stevens ら[1]は，地域住民における 65 歳以上の高齢者 1085 名について，臨床診断基準，すなわち，DSM-IV[2]，ICD-10[3]，INCDS-ADRDA[4]，NINDS-AIREN[5]，DLB consensus[6]，Gregory & Hodges criteria[7]，consensus on clinical diagnostic criteria of frontotemporal lobar degeneration[8]を用いてそれぞれの痴呆性疾患を診断した場合，いくつかの異なった疾患の診断基準を重なって示す症例が多く存在することを指摘した（図 1）．

一方，PDD・DLB・AD の頻度調査においては，調査により調査方法・調査対象が異なる．また，調査により母集団の対象人口年齢構成も異なる．剖検においては，症例収集にバイアスが入り易い．痴呆は頻度が多く，多くの疫学調査においてその臨床的な原因診断が必ずしも十分でない問題点がある．このような点から，痴呆疾患の疫学的調査結果の比較は難しい．

痴呆の疫学調査では，高齢者のなかでの患者数や有病率を求めることが多く，例えば，65 歳以上の住民での調査などが多い．AD の調査においては，痴呆患者調査と同時にされることも多く，65 歳以上の住民を調査対象とした高齢者の中での患者数や有病率を求める調査が多い．一方，PD においては一般住民中の患者数や有病率を算出する調査が多く，人口 10 万人あたりの頻度がしばしば用いられる．また，調査対象も，高齢者を対象とした調査もあるが，全人口を対象とすることが多い．PDD に関しては，PD 中における痴呆を有する PD 症例を確認し，PD 症例の中で痴呆を有する PD 症例数によりその割合を求めるものが多い．DLB においては，その診断的な特徴から剖検数における DLB 患者の占める割合を求める調査が多い．例えば，剖検症例中の DLB

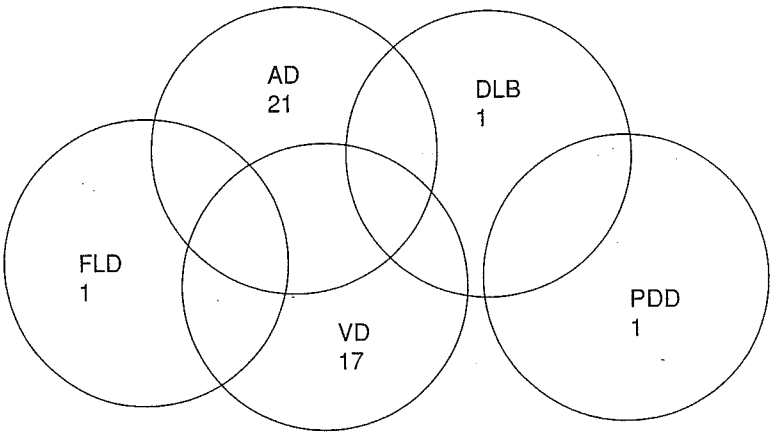

図1 Overlap of diagnosis（文献1より引用，一部改変）
数字は症例数を示す．AD：Alzheimer's disease. DLB：dementia with Lewy bodies. PDD：dementia in Parkinson's disease. VD：vascular dementia. FLD：frontal lobe dementia

症例数，あるいは剖検した痴呆患者症例のうちでDLBの占める割合を求めた報告が多い．また，臨床的に診断された痴呆症例のうちでDLB症例の割合を調べたものや，ADとの比較で算出した調査もある．しかし，これらの痴呆症例を中心にした調査の場合には，PD症状を主体にしたPDDに近い症例を見逃している可能性も考えられ，DLBの疫学調査においては調査の困難さが考えられる．このように，これまでの痴呆に関する疫学調査の多くは，その原因診断が必ずしも十分とは言えないこと，それぞれの報告で調査方法などに差があり調査成績を単純に比較することができないなどの問題点があり，PDD・DLB・ADの疫学的比較には困難さがある．

■B．ADの疫学

一般に，ADの疫学調査ではまず痴呆の有無を確認する．次に，脳血管障害や，PDなどの神経変性疾患，慢性硬膜下血腫や脳腫瘍などの記憶と認知に進行性の障害を引き起こす他の中枢神経疾患や，甲状腺機能低下症，各種のビタミン欠乏症や感染症などの痴呆を引き起こすことが知られている全身性疾患を否定することにより，ADと診断する．しかし，通常のADの疫学調査においては，画像検査も実施されず，十分な血液検査も施行されないことが多いところから，必ずしも十分な鑑別診断がなされているとは言いがたい．このため，疫学調査においてADと診断された症例の中にはDLBなどの他の痴呆を示す疾患が混在している可能性も考えられる．

1990年以降の65歳以上の住民における痴呆やADの有病率に関する成績[9-16]をみてみると，本邦と欧米との差も少なく，痴呆は3.5～10.8，多くが6～7%程度である（表1）．一方，ADは1.2～6.5の値を示し，約3%程度である．すなわち，65歳以上の住民における痴呆の頻度は6～7%程度で，そのうちの20～60%，住民の約3%程度がADと考えられる．

我々は，鳥取県大山町において老年期痴呆の有病率を1990年から10年毎に調査しているが，痴呆やADの頻度は他の報告にほぼ一致していた．さらに経時的にみてみると，患者数（1980年：56名，1990年：82名，2000年：137名）や粗有病率（65歳以上の住民における有病率（%）

表1　1990年以降の痴呆・ADの有病率（65歳以上）

報告者	年	国	痴呆	AD
本邦				
Kawano et al	1990	Japan	5.8	1.2
Hasegawa	1990	Japan	4.8	1.2
Fukunishi et al	1991	Japan	4.1	1.6
Ueda et al	1992	Japan	6.7	1.7
Kiyohara et al	1994	Japan	6.5	1.3
Shiba et al	1995	Japan	8.5	3.5
Yamada et al	2000	Japan	3.8	2.1
Meguro et al	2002	Japan	8.5	
アジア				
Zhang et al	1990	China	4.6	3.0
Lin et al	1993	Taiwan	3.7	2.0
Liu et al	1994	China	3.5	2.8
Park et al	1994	Korea	10.8	6.5
欧米				
Rocca et al	1990	Italy	7.9	3.3
Folstein	1991	USA	4.5	2.0
Dartigues et al	1991	France	3.6	2.6
Bachman et al	1992	USA	4.1	2.3
CSHA	1994	Canada	8.5	5.1
Stevens	2002	UK	9.9	3.1

1980年：4.4，1990年：4.9，2000年：7.5）のみならず，1980年の人口を標準人口として年齢を補正した訂正有病率をみても，1990年が4.5，2000年が5.9と増加しており，痴呆患者の増加が確認された．痴呆の病型別でみてみると，AD患者の増加が明らかであった．また，痴呆の程度を軽症と中等度以上に分けて検討してみると，軽症痴呆例，特に軽症ADが増えていた．高齢化とともに痴呆の絶対数が増加し，その痴呆患者の増加には罹病期間の延長も関与していると考えられる．また，画像など診断法の進歩や痴呆に関する認知度の上昇などにより，軽症例の発見が増加している可能性も考えられる．痴呆の発症率自体が上昇しているかは，今後の課題である．2002年に日本神経学会から痴呆疾患の治療ガイドラインが出された[17]が，そこには，“後期高齢者における痴呆の増加はアルツハイマー型痴呆の増加によるものであると考えられるが，病因・病理所見の異なる他の痴呆性疾患を含んでいる可能性があり，今後それらを臨床的に区別した疫学調査を行なう必要がある．”と記載されており，痴呆の疫学調査には，各痴呆性疾患の原因疾患に関する診断基準の問題や，画像を含めた諸検査を行って確実に診断していくことが重要である．

ADの発症危険因子として，アポE4などの遺伝子的要因，痴呆やパーキンソン病の家族歴，ダウン症候群，出生時母年齢や加齢などとともに，後天的要因として頭部外傷，甲状腺機能低下症，うつ病，生活習慣，非喫煙，無趣味，あまり運動しない，不活発な精神生活，栄養・食餌，低血圧，アルミニウム，パーキンソニズム，などが指摘されている[18,19]．EURODEMでは11の患者

表2　ADの発症危険因子：EURODEM Case-control studies
（文献18より引用）

	Odds Ratio	95% CI
Family history of dementia	3.5	2.6-6.9
Family History of PD	2.4	1.0-5.8
Head injury	1.8	1.3-2.7
Head injury in sporadic cases	2.3	1.2-4.8
Maternal age＞40	1.7	1.0-2.9
Thyroid disease	1.0	0.8-1.3
Hypothyroidism	2.3	1.0-5.4
Down syndrome	2.7	1.2-5.7
Depression in late-onset（≧70）cases	2.4	1.4-4.4
Ever smoked	0.8	0.6-0.98

対照研究を再解析し（表2），ADの危険因子として痴呆の家族歴，PDの家族歴，頭部外傷，出産時母親の年齢，甲状腺機能低下症，ダウン症候群，うつ，非喫煙歴などが指摘されている[18]．ADの発症危険因子にはPDの危険因子と共通のものも多く，また，パーキンソニズム症状の存在がADの発症危険因子になることも指摘されている．Wilsonら（2003）[20]は，痴呆のないPD患者でもない高齢者824例を8年間追跡し，解析初期のUnified Parkinson's Disease Rating Scale（UPDRS）と痴呆発症が関連していること，UPDRSの進行の早い者は高率にADを発症することを示した．彼らは，黒質にAD病変が存在する可能性や，Lewy bodiesや脳血管障害との関連を指摘している．ADや痴呆の進行とLewy bodiesなどPD病変との関連はDLBとの関連も含め，さらに検討される必要がある．

■C．PDの疫学

本邦におけるPDの有病率は1990年代は10万人あたり100程度であったが，人口高齢化とともに患者が増加して，2000年代には120～130程度になっている[21]．その値を諸外国からの報告と比較してみると著明な差は認められず，人種差は大きくないと考えられている[21-23]．

一方，発症年齢についてみると，高齢発症患者の増加と共に，若年発症者が減少している可能性がある[21-23]．Caradoc-Daviesら[24]は，ニュージーランドにおける1962年・1984年の調査と比較して1990年の調査では若年PD患者が減少していることを報告した．彼らは，若年PD患者発症の減少と共に，大量のL-dopa投与により疾患が進行して早期に死亡している可能性などを指摘した．

他方，PDにおいては男女差がみられ，本邦では女性PD患者が多い．欧米からの報告では男性に多い傾向があり，この男女差の理由は明らかでない[21-23]．殺虫剤や農薬などの影響など，職業との関連も考えられる．また，PDと非喫煙の関連が指摘されているが，本邦女性は非喫煙者が多いことも関連する可能性も考えられる．さらに，一般に女性が長寿であるところからPDを発症する年齢の住民は女性が多いこと，男性はPDに罹患あるいは診断する前に死亡してしまっ

表3　パーキンソン病の発症要因（文献25より引用）

1．加齢
2．遺伝
3．環境要因
　1）MPTP，工業・化学物質
　2）重金属
　3）田舎での生活，農業活動，井戸水の飲水
　4）農薬・除草剤・殺虫剤
4．生活習慣
　1）頭部外傷
　2）非喫煙
　3）飲酒
　4）食餌
　5）慢性便秘
　6）発症前性格
　7）精神的ストレス
　8）運動
　9）腫瘍などの疾患との関連性
5．遺伝的要因と環境要因の相互作用，多因子遺伝
6．脳内在性神経毒
7．ウイルス感染

ている可能性，配偶者あるいは嫁といった介護者が誰であるかといった影響などの社会的な要因も考えられる．

ところで，パーキンソン病の発症危険因子についても疫学的に検討されてきている（表3）[25]．加齢，遺伝，環境要因としての工業・化学物質，井戸水の飲水，農薬・除草剤・殺虫剤，生活習慣としての非喫煙・食餌・慢性便秘や発症前性格，精神的ストレス，運動や腫瘍などとの関連性，遺伝的要因と環境要因の相互作用や多因子遺伝の考え方，脳内在性神経毒，ウイルス感染などが指摘されている[24]が，これらのいくつかは前述のAD発症危険因子と一致する．

■D．PDDの疫学

PDにおけるPDDの頻度も検討されており，その多くは20%から40%程度である（表4）[26-37]．1992年の米子市PD調査における年代別有病率では，65歳以上におけるPDの頻度は0.644%である．PDDがPDの20〜40%とすると，PDDの頻度は0.13〜0.26%となるが，PDDが高齢PDに多いことを勘案すればさらに若干多い可能性もあり，約0.3%程度と推定するのが妥当のように思われる．McKeithら（2004）は，65歳以上の住民におけるPDDの頻度を0.3%であるとの報告を指摘している[38]．この値は，前述の米子市におけるPD疫学調査から算出した値にほぼ一致する．

PDにおけるPDD発症の要因は，年齢，高齢PD患者，発症年齢が高齢，罹病期間が長い，在宅よりも施設入所，PDであること自体や錐体外路系症状，特に，寡動症状，幻覚症状，痴呆の

表4　PDにおけるPDDの頻度（1990年以降）

Mayeux et al	1990	USA	26.1%
Ebmeier et al	1990	UK	23.6%
Mayeux et al	1992	USA	40.9%
Biggins et al	1992	UK	19.0%
Stern et al	1993	USA	29.6%
Friedman et al	1994	Poland	21.6%
Marder et al	1995	USA	19.2%
Jacobs et al	1995	USA	20.7%
Tison et al	1995	France	17.6% (65歳以上)
Aarsland et al	1996	Norway	27.7%
Mahieux et al	1998	France	23.5%
Aarsland et al	2003	Norway	26%

家族歴，うつ症状，精神的ストレス，社会経済的レベルが低い，低教育レベル，遺伝子的特徴としてのApoE4などが指摘されている[39]．これらのうちのいくつかはADの発症危険因子に一致する．

■E．DLBの疫学

DLBの頻度は明らかでない．DLBの頻度調査は報告により調査対象や調査方法が異なり，それらの比較が困難である．Akatsuら（2002）は病院での痴呆患者の剖検例で18%[40]，Wakisakaら（2003）は地域住民における剖検例で22.5%，剖検痴呆患者で41.4%[41]，Imamuraら（1999）は臨床的に痴呆と診断された例で4.6%[42]，Stevensら（2002）は65歳以上の痴呆患者の中で10.9%[1]，Rahkonenら（2003）は75歳以上の住民において5.0%，75歳以上の痴呆患者のなかでは22%[43]と報告している．McKeithら[38]は，DLBは高齢痴呆患者の原因としての神経変性疾患のなかではADに次いで二番目に多く，65歳以上の住民ではDLBが0.7%でPDDが0.3%であり，DLBはPDDの倍になることを指摘した．前述のごとく痴呆患者が6〜7%，ADが約3%であるところから，DLBは痴呆の約10%，ADの1/4の頻度ということになる．

DLBにおける年齢・男女差・発症危険因子などの古典的な詳細な疫学調査は，いまだ報告されていないことも指摘されており[38]，今後これらの検討が必要である．

■F．PDD，DLB，ADの今後の疫学調査

痴呆の疫学においては，画像を含めたより詳細な原因診断に基づいた調査が必要である．各痴呆性疾患がオーバーラップしないような診断基準の確立，臨床的診断に基づく地域調査と病理学的診断を行なう剖検例調査結果が一致するような方向への努力も必要であろう．また，PDの疫学においては，痴呆，すなわちPDDやDLBの可能性にも着目した地域疫学調査が必要であり，PD例における痴呆発症に向けて経過を追っての継続的な調査を実施していかねばならない．

DLB の疫学においては，厳密な原因診断を行った上での痴呆・AD 調査や，痴呆症例も含めた詳細な PD 調査を行って広く DLB の可能性のある症例を収集すると共に，これらの症例を長期に渡って観察して診断精度を高めて調査していかねばならない．そのようにして正確な頻度調査を実施することにより，DLB の疫学的・臨床的特徴を明らかにすることが可能となる．さらに，DLB の危険因子においても検討していく重要である．このようにして，DLB と PDD や AD との関連性や相違点などをさらに明確していくことができるものと考える．

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Autoantibodies against the amino terminal of α-enolase are a useful diagnostic marker of Hashimoto's encephalopathy

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Abstract

We investigated autoantibodies and their epitope(s) in Hashimoto's encephalopathy associated with Hashimoto's thyroiditis. In a proteomic analysis, they proved to recognize α-enolase. We further searched the epitope region in α-enolase using different regions of recombinant proteins expressed in cultured human cells. The amino terminal region was recognized by autobodies from a much higher proportion of patients with Hashimoto's encephalopathy (83.3%; 5/6) than from patients with Hashimoto's thyroiditis (11.8%; 2/17), and not at all by sera from controls (25 healthy individuals and 25 controls with other neurological disorders) (0%; 0/50). Neither the carboxyl terminal nor the mid-region of α-enolase showed specificity for Hashimoto's encephalopathy. Autoantibodies against the amino terminal of α-enolase are a useful diagnostic marker for Hashimoto's encephalopathy.

Keywords: Hashimoto's encephalopathy; Autoantibody; Epitope; α-Enolase; Amino terminal

1. Introduction

A neurological disorder associated with Hashimoto's thyroiditis (HT) has recently come to be regarded as a clinical entity, and named Hashimoto's encephalopathy (HE). HE was originally described by Brain et al., who speculated that an unknown autoimmune mechanism underlies its pathogenesis (Brain et al., 1966). Having carefully observed the relationship between the clinical signs, the change in titers of anti-thyroid antibodies and the endocrine dysfunction in a patient over a long period, Brain and colleagues proposed a new disease entity, HE, which is related to the anti-thyroid antibodies and is clearly distinct from myxoedema encephalopathy associated with hypothyroidism (Brain et al., 1966). Accumulated case reports support this distinction (Shaw et al., 1991; Kothbauer-Margreiter et al., 1996; Peschen-Rosin et al., 1999; Chong et al., 2003; Ferracci et al., 2004).

HE is usually diagnosed based on a combination of neurological findings, the presence of anti-thyroid antibodies, and/or steroid-responsiveness. However, it has a wide spectrum of clinical features including hypertonia, tremors, myoclonus, choreoathetosis, ataxia, seizures, dementia, psychiatric symptoms and stroke. Thus, HE can be underdiagnosed or misdiagnosed as myxoedema encephalopathy, encephalitis, encephalopathy with collagen diseases, paraneoplastic neurological syndromes, cerebrovascular disease, schizophrenia, or even Creutzfeldt–Jakob disease (CJD) (Ghika-Schmid et al., 1996; Wilhelm-Gos-

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sling et al., 1998; Cossu et al., 2003). Abnormalities of brain MRI/CT and EEG are frequent but heterogeneous in HE, and lack the specificity needed for diagnosing the disease (Shaw et al., 1991; Henchey et al., 1995; Ghika-Schmid et al., 1996; Peschen-Rosin et al., 1999), so more specific diagnostic markers are needed.

An anti-neural antibody against an unknown 34 kDa protein was reported in serum from a HE patient (Oide et al., 2004). Anti-thyroid antibodies and immune complexes were reported in the CSF of HE patients (Ferracci et al., 2003). Thus, antibody-mediated autoimmunity has been speculated to be important in the pathogenesis of HE. Ochi et al. recently identified α-enolase as a candidate target for HE autoantibodies in a proteomic analysis (Ochi et al., 2002). Their report demonstrated that autoantibodies against the full-length recombinant α-enolase expressed in *E. coli* are highly specific in HE. Our own proteomic analysis has also identified α-enolase as a candidate target. We now report that HE autoantibodies specifically recognize its amino terminal and are a useful marker for the diagnosis of HE.

2. Patients and methods

2.1. Patients

The clinical findings of six HE patients are summarized in Table 1. All patients presented with a deterioration in cognition/consciousness, epilepsy, ataxia, tremors, myoclonus or strokes, had serum anti-thyroid antibodies, and responded to steroid therapy. Serum samples from HE patients were studied, and compared with sera from 17 HT patients without any neurological symptoms, 25 healthy individuals and 25 controls with other neurological disorders (5 with encephalitis, 7 with collagen diseases with neurological symptoms, 2 with paraneoplastic neurological syndromes, 4 with multiple sclerosis, 2 with myasthenia gravis, 1 with Wernicke's encephalopathy, 3 with cerebrovascular disease and 1 with CJD). The ethics committee of the University of Fukui approved this research.

2.2. Preparation of proteins for immunological analyses

Human tissues including cerebral white matter, cerebral cortex, cerebellum, liver and thyroid gland were obtained from a 70-year-old woman who died of cerebral embolism in the left middle cerebral artery and underwent an autopsy 3 h postmortem. The unaffected cerebral hemisphere and cerebellum were used for this analysis. Brain tissue was homogenized in 10 mM Tris–HCl, 1 mM ethylene glycol tetraacetic acid (EGTA), with 1 μM phenylmethylsulfonyl fluoride (PMSF), a protease inhibitor.

2.3. SDS–PAGE and immunoblotting

Immunoblotting analysis of the patient's serum against different human tissue lysates including those of cerebral white matter, cerebral cortex, cerebellum, liver and thyroid gland was carried out with 12% sodium lauryl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) using a gel electrophoresis system (BE-220, BIO CRAFT, Tokyo, Japan). The proteins on the gel were Western-blotted onto polyvinylidene difluoride (PVDF) membrane (Hybond-P, Amersham Biosciences, NJ) with a blotting apparatus (KS-8453, Oriental Instrument, Tokyo, Japan) at 0.3 mA/cm^2 for 8 h at 4 °C. For detection of the band specific to HE patients, serum was applied to the membrane and incubated in 1% gelatin for 1 h at room temperature, then horseradish peroxidase (HRP)-conjugated anti-human goat IgG Fc (ICN Pharmateuticals, OH) was applied to the membrane as the secondary antibody, fluoresced, and developed on X-ray films (BioMax, Kodak, NY). The serum from a normal individual was used as a control.

2.4. Two-dimensional electrophoresis (2-DE) and immunoblotting

Two-dimensional electrophoresis (2-DE) was carried out using lysate of autopsied human cerebellum in a horizontal electrophoresis system (Multiphor II Electrophoresis Unit, Amersham Biosciences, CA) for the first-dimension isoelectric focusing using an Immobiline DryStrip 7 cm long for the gel, and a linear gradient of p*I* 3–10 (Amersham Biosciences, CA), and was followed by a gel electrophoresis (BE-220, BIO CRAFT, Tokyo, Japan) for the second-dimension SDS–PAGE (12% gel). After the first dimension, the Immobiline gel was incubated in an equilibration buffer (0.05 M Tris–HCl, 6 M urea, 30% glycerol, 1% SDS, 16 mM DTT, and 240 mM iodoacetamide), and subjected to the second-dimension SDS–PAGE (9.5 × 8.5 × 0.1 cm). Immu-

Table 1
The clinical findings of six patients with HE

Number	Patients	Clinical signs	Anti-thyroid Ab	Thyroid function	Protein in CSF	Steroid response
1	57 years, female	disorientation, ataxia, chorea	TPO	euthyroid	normal	excellent
2	44 years, female	disorientation, chorea	TPO	euthyroid	increased	excellent
3	71 years, female	disorientation, seizures	TPO, Tg	euthyroid	increased	excellent
4	71 years, female	coma, strokes	Tg	euthyroid	not examined	fair
5	69 years, female	somnolence, seizures	TPO	euthyroid	increased	good
6	63 years, female	coma, ataxia, myoclonus	Tg	euthyroid	normal	excellent

Ab, antibodies; TPO, anti-thyroid peroxidase Ab; Tg, anti-thyroglobulin Ab.

noblotting was carried out using sera from the HE patients, HT patients and controls as the primary antibody.

2.5. MALDI-TOF/mass spectroscopic analysis

Brain proteins separated by 2-DE were stained with a dye (Zinc Stain kit, BIO-RAD, CA), and the protein spots recognized by serum from an HE patient were removed from the gel, and denatured. The gels were digested with a protease (trypsin), then subjected to a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)/mass spectroscopic analysis (Autoflex, Bruker Daltonics, Bremen, Germany or Voyger-DEPR, ABI, CA) for peptide mass fingerprinting (PMF). Protein identification was carried out using a Mascot search (Matrix science, MA) by sending as a query of the PMF data.

2.6. Preparation of recombinant α-enolase proteins in E. coli or cultured human cells

The full-length α-enolase c-DNA was amplified from a human brain cDNA library (Human Brain, whole Marathon-Ready cDNA, CLONTECH, CA) by PCR using a pair of primers encompassing the selected region. The amplified α-enolase cDNA was subcloned into a pGEX plasmid vector (Amasherm Biosciences, CA), Glutathione *S*-transferase (GST)-fusion protein for expression in *E. coli*, or a pcDNA3.1 plasmid vector (Invitrogen, CA) for expression in cultured human cells. The nucleotide sequences of the inserts and cloning junctions were confirmed with a Dye Primer Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, CT). For the preparation of the α-enolase recombinant proteins in an *E. coli* strain (DH5α), 1 μg of the plasmid was transformed by a heat-shock method. The recombinant α-enolase protein was purified using a glutathione sepharose column with a GST Purification kit (Amersham Biosciences, CA), and digested with thrombin to cleave the recombinant protein from the GST.

To locate the epitope of the autoantibody in HE, we expressed regions of α-enolase in HEK cells which may also permit post-translational modifications that would not occur in *E. coli*. The amino (NH_2)-terminal (1–157 amino acids [a.a.]), carboxyl (COOH)-terminal (246–436 a.a.), or mid-region (148–304 a.a.) of α-enolase cDNAs was subcloned into pcDNA3.1 (Invitrogen, CA), a 6xHis-fusion protein expression plasmid vector. Then, 10 μg of the plasmid was precipitated with calcium phosphate and transfected into 5×10^6 cells of HEK293, a human embryonic kidney cell line, in a 10-cm culture dish with Lipofectamine (Invitrogen, CA). Purification of the recombinant α-enolase protein was done through a His column (ProBond Protein Purification kit, Invitrogen, CA).

2.7. Comparison of immunoreactivity with different regions of α-enolase among HE patients, HT patients and controls

Appropriate amounts of different regions of α-enolase expressed in human cells were subjected to SDS–PAGE and immunoblotting. 2-DE was employed for the mid-region

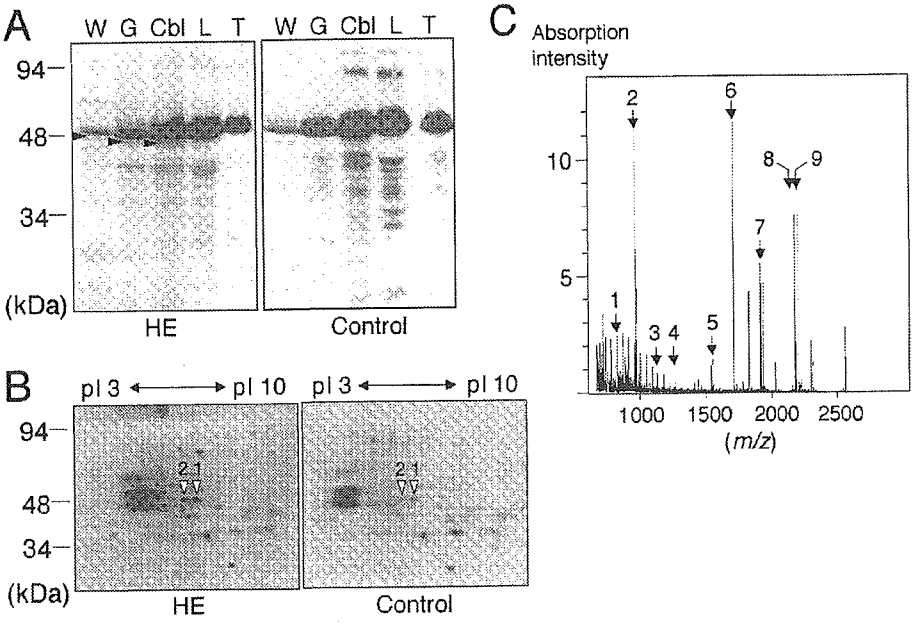

Fig. 1. (A) SDS–PAGE and immunoblotting for the presence of anti-neural antibody in HE. W, brain white matter. G, brain grey matter. Cbl, cerebellum. L, liver. T, thyroid gland. Arrowheads indicate the bands immunoreactive with serum from HE patient 1 in Table 1. (B) Detection of spots immunoreactive with serum from the HE patient on 2-DE. Open arrowheads indicate spot-1 and -2 which have a stronger intensity in HE than in control. (C) MALDI-TOF/mass spectroscopic pattern of the immunoreactive spot-1 digested by trypsin. Nine common signals of mono-isotopic peptides (numbers 1–9 in Table 2), which were obtained by removal of the background noise and repetitive analyses, are indicated by arrows.

Table 2
Mass-fit analysis for typical fragments of the immunoreactive spot in HE

Signal numbers	*m/z* observed	*m/z* expected	Start–end amino acid numbers	Peptide sequences
1	806.41	805.40	309–314	YNQLLR
2	959.53	958.52	329–336	NFRNPLAK
3	1118.69	1117.68	306–314	LAKYNQLLR
4	1259.73	1258.72	23–34	AGAVEKGVPLYR
5	1540.80	1539.79	142–155	VVIGMDVAASEFFR
6	1691.91	1690.90	309–322	YNQLLRIEEELGSK
7	1907.98	1906.98	65–81	LAMQEFMILPVGAANFR
8	2176.08	2175.07	136–155	AGYTDKVVIGMDVAASEFFR
9	2189.16	2188.15	209–229	FTASAGIQVVGDDLTVTNPKR

Selected mass signals for tryptic peptides of spot-1 detected by MALDI-TOF/mass-spectroscopic analysis completely matched the database sequence of tryptic fragments of human α-enolase. The peptide sequences shown in the table were obtained from database sequences of α-enolase, the peptide masses of which matched the search query of tryptic peptide-finger printing. The sequence-matched tryptic peptides covered with 28% of α-enolase. The mass-fit analysis for spot-2 also gave the same results as that for spot-1.

because the signal occasionally overlapped with proteins derived from the cultured human cells from which it was barely distinguishable.

3. Results

3.1. Screening and identification of the target molecule of an HE patient's autoantibodies

To screen for the target molecule that reacted selectively with serum from HE patient 1 in Table 1, SDS–PAGE and immunoblotting were employed. In Fig. 1A, a 48 kDa protein shows stronger blotting signals with serum from the HE patient than from a control. It appears to be restricted to brain tissues. The cerebellum protein lysate was subjected to 2-DE, and two spots with the same molecular weight and different p*I* values were identified that gave stronger blotting intensities with the HE than the control serum (Fig. 1B). These two spots were further subjected to MALDI-TOF/mass spectroscopic analysis. Fig. 1C and Table 2 show the MALDI-TOF mass spectra of immunoreactive spot-1 and its mass-fit analysis data, respectively. α-Enolase was identified in two spots with a slightly different p*I*, probably due to post-translational modifications. These results demonstrated that α-enolase was a candidate target molecule reactive with serum from the HE patient.

3.2. Immunoreactivity of sera from HE patients, HT patients and controls with full-length recombinant α-enolase expressed in E. coli or cultured human cells

All of the sera from HE patients, HT patients and controls reacted with the "full-length" α-enolase expressed in *E. coli* with varying signal intensities (Fig. 2A). The sera from HE and HT patients also reacted with the full-length α-enolase expressed in cultured human cells, and there was even discordant reactivity with the control sera (Fig. 2B). However, the same serum sometimes reacted with recombinant proteins from *E. coli* and cultured human cells. All recombinant full-length α-enolase expressed in *E. coli* reacted with control sera ($n=15$), whereas some of the expressed one in cultured human cells reacted with control sera (2/8; 25%).

3.3. Comparison of immunoreactivity of various regions of α-enolase among HE patients, HT patients and controls

To locate the serological epitope(s) in the α-enolase, various regions of recombinant proteins were expressed in cultured human cells, purified on a Ni column, and subjected to SDS–PAGE (Fig. 3A). The NH_2-terminal, COOH-terminal and mid-regions of the recombinant α-enolase were clearly identified with an anti-His antibody, which verified their expression (Fig. 3A, left panel). A commercially available monoclonal antibody against α-enolase only reacted with the COOH-terminal region (Fig. 3A, 2nd left panel).

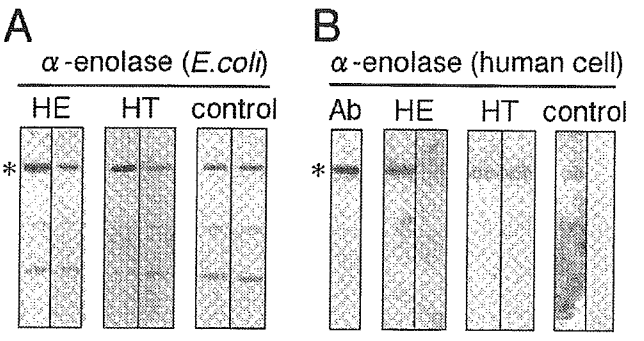

Fig. 2. (A) Immunoblotting of the full-length recombinant α-enolase expressed in *E. coli* with sera from HE patients 1 and 2 (Table 1), HT patients or healthy controls. An asterisk indicates the full-length recombinant α-enolase expressed in *E. coli*. (B) Immunoblotting of the full-length recombinant α-enolase expressed in cultured human cells with sera from HE patients 1 and 2, two HT patients or two controls. Ab, a commercially available monoclonal anti-α-enolase antibody (sc-7455, Santa Cruz Biotechnology, CA). An asterisk indicates the full-length recombinant α-enolase expressed in human cultured cells.

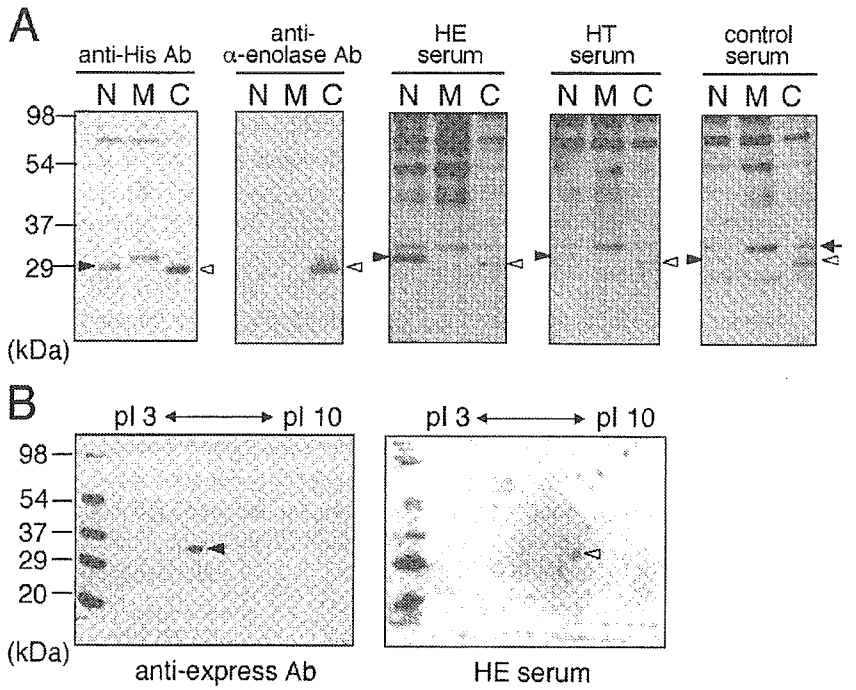

Fig. 3. (A) Immunoblotting of various regions of α-enolase with sera from HE and HT patients and a control. Recombinant α-enolase regions were expressed in cultured human cells. N, recombinant NH_2-terminal region of α-enolase. M, recombinant mid-region of α-enolase. C, recombinant COOH-terminal region of α-enolase. Anti-His Ab, immunoblotting with an anti-Histidine antibody. Anti-α-enolase Ab, immunoblotting with a commercially available monoclonal anti-α-enolase antibody (sc-7455, Santa Cruz Biotechnology, CA). HE serum, immunoblotting with serum from HE patient 1 (Table 1). HT serum, immunoblotting with serum from a patient with HT without any neurological signs. Control serum, immunoblotting with serum from a normal control. Arrowheads indicate the NH_2-terminal region. Open arrowheads indicate the COOH-terminal region. An arrow indicates signals derived from cultured human cells. (B) 2-DE of the recombinant mid-region of α-enolase expressed in cultured human cells, and lack of immunoreactivity with serum from a patient with HE. Anti-expression antibody, a commercially available monoclonal antibody recognizing the region between the His tag and cloning site (Anti-Xpress Antibody, Invitrogen, CA). An arrowhead indicates the recombinant mid-region of α-enolase. An open arrowhead indicates the band imunoreactive with serum from an HE patient.

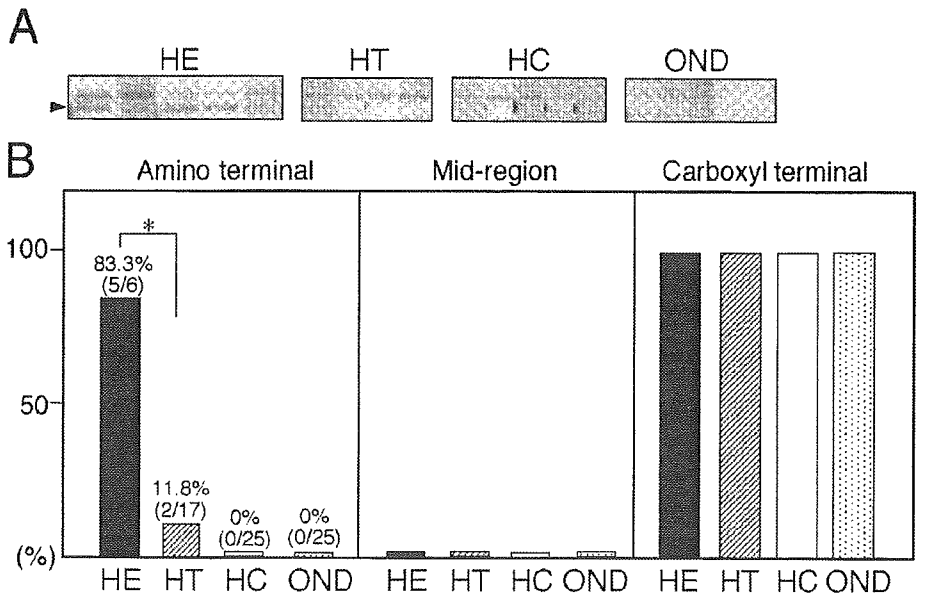

Fig. 4. (A) Immunoblotting of the recombinant NH_2-terminal region of α-enolase expressed in cultured human cells with sera from patients with HE or HT, or from controls. HE, sera from HE patients 1–5 in Table 1. HT, sera from HT patients. HC, sera from healthy controls. OND, sera from controls with other neurological disorders. OND includes patients with encephalitis, encephalopathy with collagen disease, multiple sclerosis, CJD and cerebrovascular disease. Arrowheads indicate the signals reactive with the NH_2-terminal region. (B) Percentages of sera from patients with HE or HT, or from controls, reactive with different regions of recombinant α-enolase expressed in cultured human cells. *$p<0.005$. HE, $n=6$; HT, $n=17$; HC, $n=25$; OND, $n=25$ in amino terminal region, $n=15$ in mid-region and carboxyl terminal region.

The NH_2-terminal of the recombinant α-enolase reacted with sera from HE patients, but rarely from HT patients or controls (Fig. 3A, the panels 3–5). By contrast, the COOH-terminal of the recombinant α-enolase reacted with all sera tested. The mid-region did not react with any human sera, though it gave a strong signal at the expected position with the commercially available monoclonal antibody at one end (Fig. 3B).

3.4. Percentages of serum samples from HE patients, HT patients and controls immunoreactive with various regions of α-enolase

The recombinant NH_2-terminal region of α-enolase was highly reactive in HE patients 1–5 (Table 1) (5 out of 6; 83.3%), much less reactive in HT (2 out of 17; 11.8%), and unreactive in 25 healthy controls and 25 controls with other neurological disorders (p value <0.005 between HE and HT; Fisher's exact test) (Fig. 4). In contrast, the COOH-terminal reacted with all sera, and the mid-region did not react with any of them (Fig. 4). These results demonstrate that the NH_2-terminal region of α-enolase contains an immunoreactive site.

4. Discussion

Searching for specific autoantibodies in sera from HE patients, we identified an antibody against a 48 kDa molecule in an HE patient on SDS–PAGE and 2-DE (Fig. 1A and B). The patient's serum reacted with the 48 kDa protein in brain tissues, suggesting the existence of an anti-neuronal antibody (Fig. 1A). Two-DE revealed two spots with the same molecular weight and slightly different p*I* values, which showed stronger reactivity with HE than the control sera (Fig. 1B). MALDI-TOF/mass spectroscopic analysis identified α-enolase sequences in these two spots (Fig. 1C; Table 2). This suggests that α-enolase is a candidate target for HE autoantibodies, which may recognize post-translationally modified forms. Recently, Ochi et al. also identified α-enolase as a possible target for antibodies in sera from patients with HE, in a proteomic analysis (Ochi et al., 2002).

To locate epitope(s) in α-enolase that react with the autoantibodies in HE patients, we tested the NH_2-terminal, COOH-terminal and mid-regions. These regions were expressed in cultured human cells because a nascent polypeptide undergoes natural post-translational modifications possibly influencing the conformation of the protein concerned with the autoimmunity, as compared with the absence in *E. coli*. Remarkably, the recombinant NH_2-terminal of α-enolase was recognized by sera from 5/6 HE patients (83.3%) but only 2/17 HT patients (11.8%) and 0% of healthy individuals and controls with other neurological disorders ($p<0.005$ between HE and HT) (Fig. 4). These results clearly demonstrate that the autoantibodies in HE patients specifically recognize a key epitope in the NH_2-terminal region of α-enolase.

In sharp contrast, the sera from all patients and controls apparently reacted non-specifically with the recombinant COOH-terminal region and full-length α-enolase, but did not react detectably with its mid-region (Fig. 4).

Notably, however, when Ochi et al. used full-length α-enolase expressed in *E. coli*, they detected autoantibodies more often in sera from HE patients (60%) than from HT patients (6%) or controls (0%) (Ochi et al., 2002). This apparently specific recognition contrasts starkly with the non-specific binding that we found between control sera and full-length recombinant α-enolase, even after expression in human cells, which argues against influences from *E. coli* components. The reasons are not clear, but the 'stickiness' appears to be localized to the region form 246 to 436. Possibly (a) this region somehow masks the specific epitope within 1–157 when full-length recombinant products are tested or (b) this masking was avoided by Ochi et al. because of differences in re-folding or in proteolysis. If post-translational modifications are involved, they must differ between human brain and *E. coli*. They might include the conversion of amide side-chains (i.e. Gln or Asn to Glu or Asp) in the protein, which is sometimes necessary for T cell recognition (Anderson et al., 2000), or glycosylation of the protein. The number of Gln is 1 in the amino terminal region, 2 in the mid-region and 9 in the carboxyl terminal region of α-enolase; while the number of Asn is 4 in the amino terminal region, 9 in the mid-region and 5 in the carboxyl terminal region.

The enolases are cytosolic enzymes and play an important role in the glycolytic pathway in all cells (Lebioda and Stec, 1991). The functional enzyme is a dimer made up of subunits referred to as α, β and γ. In mammals there are at least three isoforms of enolase characterized by different tissue distributions as well as by distinct biochemical and immunologic properties. The α-enolase is a nearly ubiquitous form, found in almost all tissues, and its expression precedes that of the other isoforms in the early stage of embryonic development. The β-enolase is present in adult skeletal muscle, and the γ-enolase is the major form found in mature neurons and in cells of neuronal origin (Lebioda and Stec, 1991). However, it is thought to be a multifunctional protein and also has potential roles in autoimmune disorders (Pancholi, 2001; Moscato et al., 2000; O'Dwyer et al., 2002). α-Enolase apparently serves as a plasminogen receptor on the surface of a variety of hematopoetic, epithelial and endothelial cells, suggesting that it plays an important role in the intravascular and pericellular fibrinolytic systems (Pancholi and Fischetti, 1998). Indeed, vasculitis was reported in an autopsy case of HE (Nolte et al., 2000). In addition, this protein can act as a heat-shock protein and bind cytoskeletal and chromatin structures, implying crucial roles in transcription and a variety of pathophysiological processes (Subramanian and Miller, 2000).

The NH_2-terminal region of α-enolase is located on the extruding part of the enzyme and is important for intermolecular interactions (Lebioda and Stec, 1991), and evidently includes a major serological epitope. Although it is unclear whether α-enolase, a cytosolic protein, is expressed on the cell surface or not, it could be released from the cytosol to the outside of cells by destruction of the thyroid gland (i.e. thyroiditis), and so come into contact with immune cells.

In conclusion, the NH_2-terminal region of α-enolase is a novel target for autoantibodies in HE, and may be subject to post-translational modifications. These autoantibodies appear to be a useful diagnostic marker of HE.

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