い点に留意する必要がある．

混合性結合組織病

mixed connective tissue disease；MCTD

1. 基礎事項

SLE, SSc, PM などの病像が混在し，血清中の抗 RNP 抗体が高値を示すことを特徴とする．レイノー現象またはソーセージ様指の腫脹も必発である．本邦で約 3,000 人の患者数があり，90％以上が女性で，発病のピークは 30 歳代である．肺高血圧の合併が予後を左右する．

2. 症例 4　67 歳，女性

主訴：両手指関節痛

1999 年頃より両手指関節痛，朝のこわばりが出現した．2003 年 2 月，両手指のしびれが出現した．近医を受診し，手根管症候群と診断され，手術を受けた．手術後よりレイノー症状がみられるようになった．2004 年 2 月頃より両手指関節痛と朝のこわばりが増悪し，両足かかとの痛みも出現したため，某院整形外科を受診した．両側第 1 指 MP 関節と CM 関節に骨びらんは認められるも（図 3-a），抗核抗体 2,560 倍 Sp 型が判明し，内科へ紹介となる．両手第 2～4 指にしびれがあり，手指のソーセージ様浮腫，両側手指，手関節，足関節に疼痛，発赤，腫脹が認められた．両側鼠径部に径 1 cm の弾性軟のリンパ節腫脹が認められた．CRP 1.38 mg/dl，抗 RNP 抗体 156.9（正常：＜22.0），リウマトイド因子 606 U/ml（正常：＜15.0 U/ml）．以上より両第 1 指 MP 関節と CM 関節の骨びらんは認めたものの，MCTD と診断した．プレドニゾロン 30 mg/日より開始したところ，症状は軽快し，その後徐々に減量した．プレドニゾロン 8 mg/日になった時点で，CRP 1.14 mg/dl に上昇するとともに両手指のこわばりと腫脹，左大腿部痛が出現した．以上より RA の合併を疑い，メトトレキサート 8 mg/週を追加するも十分な改善がみられず，4 カ月経過しても CRP は 1.71 mg/dl と高値のままであった．抗 CCP 抗体も 1.0 U/ml と上昇しておらず，骨 X 線像でも骨破壊の進展はみられなかったことから（図 3-b），MCTD の増悪と診断した．プレドニゾロンを 10 mg/日に増量したところ症状は改善し，その後 2 年間は安定している．

3. 診療のポイント

1）表 3 に厚生（労働）省研究班の MCTD の診断基準を示す．表 3 にはあげられていないが，労作時呼吸困難は，本症の予後を左右する肺高血圧の初発症状として注意する必要がある．SLE 様の所見の中で，リンパ節腫脹は ACR の SLE の分類基準に含まれないので，注意が必要である．

2）抗核抗体や抗 RNP 抗体は本症例のように著明な高値を示す場合が多い．

3）本症例のように，MCTD の中には RA との鑑別が極めて困難なものが少なからず存在する．

本症例においても抗 CCP 抗体は RA との鑑別に有用であったと考えられる．

リウマチ性多発筋痛症

polymyalgia rheumatica；PMR

1. 基礎事項

頸・肩（上肢帯）や腰・大腿（下肢帯）の痛みやこわばりを主徴とする疾患で，発熱や体重減少を伴うこともある．50 歳以上の高齢者に多い．時に滑膜炎を合併し，RA との鑑別が問題となることがある．本邦での有病率は明らかではないが，米国では 50 歳以上で人口 10 万人あたり約 500 人（0.5％）といわれている．

2. 症例 5　46 歳，男性

主訴：多関節痛

2004 年 4 月初めより，両股関節，両肩の痛みが出現し，近医整形外科を受診した．骨 X 線像にて異常はなく，湿布で対応していたが軽快しなかった．4 月下旬～5 月初めに両膝関節の疼痛が出現した．特に，立って歩き始める時に痛みのため動きづらくなった．さらに，両肩の疼痛も出現したため，某院内科を受診した．5 月中旬より血液検査にて CRP 2.69 mg/dl となり，RA を疑われてロキソニンを投与されたが，5 月下旬より 38℃ 台の発熱も出現するようになってきたため，当科を紹介され受診し，精査のため入院した．入院時 38℃

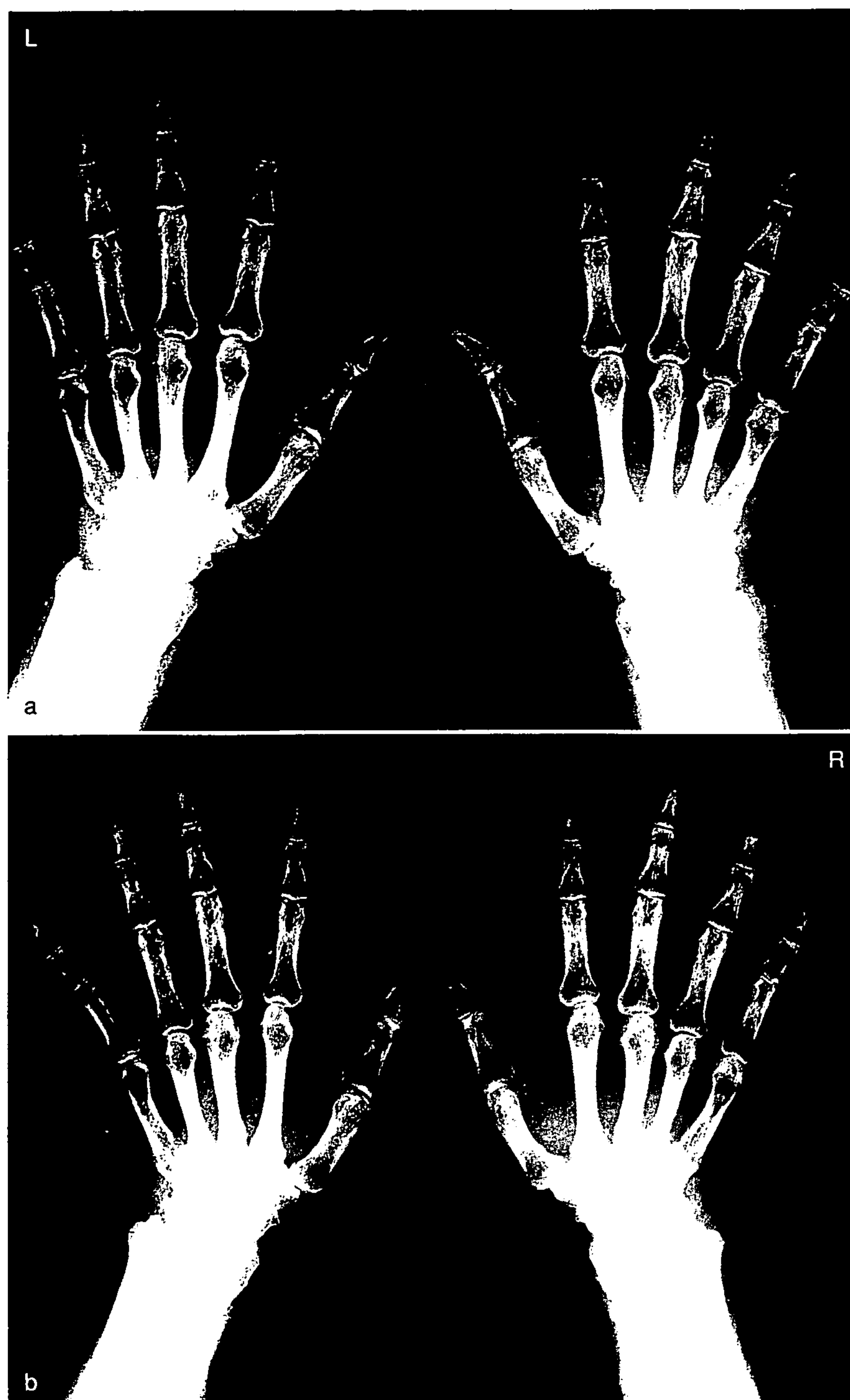

図 3　MCTD 患者の両手指 X 線像
a：2004 年 3 月　**b**：2005 年 6 月
大きな変化はみられない．

台の発熱および両肩関節，両股関節，両膝関節に疼痛(圧痛，運動痛)を認めた．また，両上下肢の近位筋の圧痛と軽度の筋力低下を認めた．血沈 78 mm/1 時間，CRP 5.37 mg/d*l*，リウマトイド因子 11.1 U/m*l*(正常：<15.0 U/m*l*)，抗核抗体陰性，P-ANCA 陰性，クレアチンキナーゼ(CK) 67 IU(正常：62〜287)，アルドラーゼ 4.1(正常：1.8〜5.8 IU/*l*)，ミオグロビン 24 ng/m*l*(正常：<60)，ミオシン軽鎖<1.0 ng/m*l*(正常：<2.4)．結節性多発動脈炎，多発筋炎を疑って筋生検を行ったが，全く

表 3　混合性結合組織病診断の手引き

Ⅰ．共通所見	
1．レイノー現象	(98)
2．指ないし手背の腫脹	(77)
Ⅱ．抗 RNP 抗体陽性	(100)
Ⅲ．混合所見	
A．全身性エリテマトーデス様所見	
1．多発関節痛	(79)
2．リンパ節腫脹	(30)
3．顔面紅斑	(33)
4．心膜炎または	(11)
胸膜炎	(11)
5．白血球減少症(4,000/mm^{3}以下)または	(47)
血小板減少症(100,000/mm^{3}以下)	(12)
B．強皮症様所見	
1．手指に限局した皮膚硬化	(47)
2．肺線維症,	(28)
肺拘束性障害(%VC 80%以下)または	(27)
肺拡散能低下(DLco 70%以下)	(37)
3．食道の蠕動低下または拡張	(25)
C．多発筋炎様所見	
1．筋力低下	(41)
2．筋原性酵素(CK)の上昇	(35)
3．筋電図における筋原性異常所見	(40)
[診断]	
1．Ⅰの1所見以上が陽性	
2．Ⅱの所見が陽性	
3．ⅢのA，B，C項のうち，2項目以上につきそれぞれ1所見以上が陽性	
以上の3項目を満たす場合を混合性組織病と診断する	

(　)中の数字は出現頻度(%)　(厚生省混合性結合組織病調査研究班，1984年)

異常を認めなかった．PMRと診断し，プレドニソロン20 mg/日の投与を開始したところ，12時間以内に症状の劇的な改善を認め，炎症所見も消失した．その後，プレドニソロンを徐々に減量し，2006年5月にはプレドニソロン2 mg/日の内服で症状はなく，CRP 0.05 mg/dl未満，血沈6 mm/1時間と安定している．

表 4　リウマチ性多発筋痛症の診断基準(Birdら1979年)

1．両肩の疼痛および/またはこわばり
2．2週間以内の急性発症
3．赤沈の亢進(40 mm/h以上)
4．1時間以上持続する朝のこわばり
5．年齢が65歳以上
6．抑うつ症状および/または体重減少
7．両側上腕筋の圧痛

上記7項目中3項目以上ある場合，または上記の1項目以上，および臨床的，病理的に側頭動脈の異常を認めた場合，疑診例でかつプレドニソロンが有効であれば確診例となる(文献1を改変)．

3. 診断のポイント

1）高齢者(特に60歳以上)の不明熱，上肢帯・下肢帯の痛み・こわばりをみた際にはPMRの存在を疑う必要がある．悪性腫瘍，感染症，RAを含めた膠原病などとの鑑別診断が重要である．

2）表4にBirdらの診断基準を示す[1]．抑うつ状態を示すことも少なくない．少量のプレドニソロン(10 mg/日程度)が劇的に奏功するので，診断に迷った場合は試みる価値がある．

3）本症例では，年齢が50歳以下であり，PMRよりもまず結節性多発動脈炎が強く疑われた．筋生検結果と少量のプレドニゾロンに対して劇的に反応したことが診断の決め手となった．

4）PMRには側頭動脈炎の合併がしばしばみられることに注意しておく必要がある．

おわりに

以上，RAと鑑別を要する膠原病として，SLE・SSc・PM/DM・MCTDの4疾患と膠原病類縁疾患としてPMRについてその診療の実際とポイントについて概説した．これらの疾患は原因不明の発熱・関節痛を呈することが少なくない．その診断にあたっては，まずその存在を想起することが最も重要であることを重ねて強調したい．本稿で示した症例のうち多くは，整形外科医により抗核抗体などの検査がなされた結果に膠原病が疑われて，紹介受診されたものであることを付記しておきたい．治療については紙面の都合上その概略を述べるにとどまったため，詳細は他の専門書を参照していただきたい．

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第7回　エピドラスコピー研究会

開催日程：2006年10月28日(土)午前9：45〜11：45
開催場所：旭川市民文化会館(日本臨床麻酔学会第26回大会会場)
会　　長：野口隆之(大分大学医学部　脳・神経機能統御講座麻酔学　教授)
プログラム：①招待講演　演者　Young K. Choi M. D., F. A. C. P. M.
Medical Director, New Jersey Pain Management
Clinical Associate Professor
Robert Wood Johnson Medical School
演題　Spinal Endoscopy
Diagnostic versus Therapeutic Intervetion
②一般口演(募集中)
一般演題募集要項：テーマを問わず，症例報告など広く演題を募集．
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Evidence of the efficacy of radiation synovectomy with yttrium-90: comment on the article by Jahangier et al

To the Editor:

The study by Jahangier et al on the efficacy of yttrium-90 radiation synovectomy (radiosynoviorthesis [RSO]) versus intraarticular glucocortocoids (GCs) in the treatment of knee arthritis (Jahangier ZN, Jacobs JW, Lafeber FP, Moolenburgh JD, Swen WA, Bruyn GA, et al. Is radiation synovectomy for arthritis of the knee more effective than intraarticular treatment with glucocorticoids? Results of an eighteen-month, randomized, double-blind, placebo-controlled, crossover trial. Arthritis Rheum 2005;52:3391–402) is important because of the shortage of evidence-based studies on this topic. Contrary to the results and conclusions in a previous (2001) study by the same group (Jahangier ZN, Moolenburgh JD, Jacobs JW, Serdijn H, Bijlsma JW. The effect of radiation synovectomy in patients with persistent arthritis: a prospective study. Clin Exp Rheumatol 2001;19:417–24), Jahangier and colleagues drew very unfavorable conclusions regarding ^{90}Y RSO from the results of their recent study. However, there are striking differences between the 2 studies. The patients in the recent study were, on average, at a more advanced stage of disease (mean ± SD duration of synovitis 38 ± 38 months [range 6–240] in the RSO plus GC arm and 35 ± 32 months [range 2–120] in the placebo plus GC arm in the 2005 study, but 17 ± 13 months [range 1–60] in the 2001 study).

We believe the new trial does in fact show considerable evidence for the efficacy of ^{90}Y RSO. In the case of the secondary end point, duration of remission, there was a striking difference between the two randomized groups in favor of RSO: mean ± SD 27 ± 29 months in the RSO plus GC group versus 18 ± 25 months in the placebo plus GC group.

Figure 1 of this publication provides the most convincing evidence for the efficacy of RSO in this trial. The figure, which lists the mean composite change index—an index of unproven value—presents data on patients who remained in the study. Data on patients in both treatment arms, in both the randomized and the crossover portions of the study, are included. In the comparison of the 2 randomized treatment groups, the figure shows a striking difference in favor of RSO at all time points, which becomes very prominent at 12 months and 18 months. At 12 months the mean composite change index in the placebo plus GC group was ~4.9, while in the RSO plus GC group it was ~7.7, ~60% higher. A similar picture was seen at 18 months, with a mean composite change index of ~4.4 in the placebo plus GC group and ~7.7, or 75% higher, in the RSO plus GC group. The interpretation of the data in the 2 crossover groups is not as straightforward. In the group that started on placebo but was switched to ^{90}Y RSO, there was still considerable progress at 12 months and 18 months, although not as much as was seen in the randomized RSO group (mean composite change index ~6 at 12 months and ~6.7 at 18 months, considerably higher than in the randomized placebo group). In patients who started on RSO but were switched to placebo by the investigators after ≥6 months because RSO therapy did not appear successful, good improvement was seen at 12 months and 18 months, with results similar to those in the original randomized RSO group. What is not immediately evident from Figure 1 is the fact that the month 0 time point refers to the start of the placebo treatment, but is in fact also 6 months after the original ^{90}Y RSO treatment in these patients. We attribute the improvements seen in this group at 12 months and 18 months after the crossover placebo treatment (and thus 18 months and 24 months after RSO treatment) to the long-term effect of RSO in this group and not to the placebo treatment, with which one would expect only short-term improvement (up to 3 months).

In summary, we find considerable evidence for the efficacy of RSO in the trial described by Jahangier et al. Consequently, we arrive at the opposite conclusion from that drawn by the authors.

Drs. Mödder and Langer have received speaking fees (less than $10,000) from Schering AG.

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Specificity of enzyme-linked immunosorbent assay for IgG anti-NR2 glutamate receptor antibodies: comment on the concise communication by Yoshio et al

To the Editor:

We read with interest the concise communication by Yoshio et al (1) describing an association of IgG anti-NR2 glutamate receptor antibodies in cerebrospinal fluid (CSF) with neuropsychiatric systemic lupus erythematosus (NPSLE). DeGiorgio et al first demonstrated that IgG anti-NR2 glutamate receptor antibodies caused neuronal death in mice, when injected into the brain (2). They also reported the presence of these antibodies in CSF from an SLE patient with progressive cognitive decline (2). It was further demonstrated by investigators in that group that the presence of IgG anti-NR2 glutamate receptor antibodies within the brain resulted in cognitive decline in mice (3). These findings suggested that these antibodies might cause diffuse psychiatric/neurologic syndromes in human SLE. However, Yoshio and colleagues found that, compared with lupus patients without NPSLE, CSF IgG anti-NR2 glutamate receptor antibody levels were increased in lupus patients with neurologic syndromes of the central nervous system, but not in those with diffuse psychiatric/neuropsychological syndromes alone (1).

To measure IgG anti-NR2 glutamate receptor antibodies in sera and CSF, Yoshio et al used an enzyme-linked immunosorbent assay (ELISA) with the synthetic DWEYSVWLSN peptide conjugated to bovine serum albumin (BSA) as antigen (1). Our group previously demonstrated that sera from SLE patients frequently express antibodies to human serum albumin (HSA), BSA, and ovalbumin (4). Therefore, subtracting binding activity to these carrier proteins would be mandatory in order to determine specific activities of antibodies to peptides conjugated to carrier proteins, including BSA and HSA (4). However, Yoshio and colleagues did not subtract

Table 1. Measurement of IgG anti-NR2 glutamate receptor antibodies (optical density at 492 nm) using enzyme-linked immunosorbent assay*

Sample	CSF or serum	Maxisorp		Pro-Bind	
		HSA–NR2	HSA	HSA–NR2	HSA
1	CSF	0.146	0.065	0.090	0.067
2	CSF	0.223	0.068	0.147	0.124
3	CSF	0.206	0.278	0.053	0.090
4	CSF	0.428	0.521	0.050	0.060
5	CSF	0.974	0.480	0.088	0.082
6	CSF	0.205	0.323	0.224	0.280
7	CSF	0.248	0.139	0.259	0.249
8	CSF	0.044	0.135	0.101	0.139
9	CSF	0.332	0.137	0.030	0.020
10	Serum	0.628	0.686	0.094	0.116
11	Serum	0.434	0.795	0.100	0.180
12	Serum	0.272	0.093	0.050	0.044
13	Serum	0.181	0.209	0.082	0.099
14	Serum	0.333	0.339	0.057	0.221
15	Serum	0.280	0.139	0.051	0.050
16	Serum	0.878	0.464	0.616	0.522

* Cerebrospinal fluid (CSF) or serum samples from systemic lupus erythematosus patients were assayed for IgG anti-NR2 glutamate receptor antibodies by enzyme-linked immunosorbent assay as described in the text. Peroxidase-conjugated $F(ab')_2$ goat anti-human IgG was used at 1:10,000 (Maxisorp plates) and at 1:5,000 (Pro-Bind plates). HSA–NR2 = human serum albumin conjugated with synthetic DWEYSVWLSN peptide.

BSA binding activities. It appears that all sera with anti-HSA also contain antibodies to BSA (4). We therefore reexamined whether CSF and sera from SLE patients contain antibodies to HSA, using the method described by Yoshio et al.

Wells of a 96-well microtiter plate (Falcon Pro-Bind; Becton Dickinson, Lincoln Park, NJ or Nunc-immuno module F8 Maxisorp; Nunc, Roskilde, Denmark) were coated with HSA (Miles, Elkhart, IN) or HSA conjugated (at a 1:1 weight ratio) with highly purified synthetic DWEYSVWLSN peptide (purity >95%) (HSA–NR2 peptide), at 20 μg/ml in phosphate buffered saline (PBS), overnight at 4°C. The wells were blocked with Block Ace (Dainippon, Osaka, Japan) for Falcon Pro-Bind plates or with PBS containing 1% BSA (Miles) for Nunc Maxisorp plates, for 2 hours at room temperature. Before being added to the wells, serum and CSF samples were diluted 1:200 and 1:2, respectively, in PBS containing 1% BSA. After incubation at 37°C for 1 hour, bound IgG anti-NR2 glutamate receptor antibody was detected with peroxidase-conjugated $F(ab')_2$ fragments of goat anti-human IgG (Cappel, Cochranville, PA). Binding activity was expressed as optical density at 492 nm (OD_{492}) as measured in a 2-wavelength microplate photometer (MTP-450; Corona Electric, Ibaraki, Japan).

As seen in Table 1, all 16 samples exhibited positive binding to HSA–NR2 peptide in Falcon Pro-Bind plates (Yoshio and colleagues' method) as well as in Nunc Maxisorp plates. However, 8 of the 16 samples showed higher binding activity (OD_{492}) to HSA alone than to HSA–NR2, indicating that those samples would yield false-positive results for IgG anti-NR2 glutamate receptor antibodies unless nonspecific binding to HSA alone were subtracted. In addition, the levels of binding activity obtained with Falcon Pro-Bind plates were ~20–30% of those with Nunc Maxisorp plates, even though peroxidase-conjugated $F(ab')_2$ goat anti-human IgG was used at 1:5,000 in the former plates and at 1:10,000 in the latter. Therefore, it would be preferable to use Nunc Maxicorp plates. Nonetheless, these results confirm that ~50% of serum and CSF samples contain antibodies to HSA (and presumably to BSA as well, since it was previously shown that all sera positive for anti-HSA contained antibodies to BSA [4]).

These findings raise serious concern about the specificity of the ELISA used by Yoshio et al. It is highly likely that the presence of anti-BSA antibodies would have significantly influenced their results and conclusions. Therefore, their conclusion that IgG anti-NR2 glutamate receptor antibodies in CSF may cause focal neurologic damage such as seizure disorders, aseptic meningitis, and transverse myelopathy is not supported.

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Cortisol metabolism by 11β-hydroxysteroid dehydrogenase as a novel target in the treatment of inflammation- or immune-mediated bone loss: comment on the article by Makrygiannakis et al

To the Editor:

We read the report by Makrygiannakis et al (1), on the effects of intraarticular corticosteroids on bone biology regulation, with great interest, since it may have major consequences regarding the development of novel treatment concepts in rheumatoid arthritis (RA). In Makrygiannakis and colleagues' study, evaluation of RANKL, osteoprotegerin (OPG), and surface marker expression in synovium of arthritis patients or in human osteoblast-like cells showed that a decrease in inflammation is accompanied by down-modulation of markers of bone destruction. These findings offer a ration-

Research article

Association of cerebrospinal fluid anti-ribosomal P protein antibodies with diffuse psychiatric/neuropsychological syndromes in systemic lupus erythematosus

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Abstract

We explored the relationship of antibodies to the whole ribosomal P proteins (P0, P1, and P2) in cerebrospinal fluid (CSF) with diffuse psychiatric/neuropsychological syndromes in systemic lupus erythematosus (SLE). CSF samples were obtained from 71 SLE patients (52 patients with diffuse psychiatric/neuropsychological syndromes [diffuse NP-SLE] and 19 patients with neurological syndromes or peripheral neuropathy [focal NP-SLE]) as well as from 24 patients with non-inflammatory neurological disease. Immunoglobulin G (IgG) antibodies to the C-terminal 22-amino acid ribosomal P synthetic peptide (anti-P_{C22}) and those to purified bovine ribosomal P proteins (P0, P1, and P2) (anti-whole P) were determined by enzyme-linked immunosorbent assay; affinity-purified IgG anti-P_{C22} were used as the standard. The concentrations of antibodies to epitopes other than the C-terminal 22 amino acids of ribosomal P proteins were calculated by subtracting anti-P_{C22} from anti-whole P (anti-$P_{EX.C22}$). CSF anti-whole P levels were significantly elevated in diffuse NP-SLE compared with focal NP-SLE or control patients. By contrast, there were no significant differences in CSF anti-P_{C22} levels among the three groups. Of note, CSF anti-$P_{EX.C22}$ levels were significantly elevated in diffuse NP-SLE compared with the other two groups. CSF anti-$P_{EX.C22}$ levels were not significantly correlated with CSF anti-P_{C22} levels, but with CSF antibodies against the recombinant ribosomal P0 protein lacking the C-terminal 22 amino acids (C22-depleted rP0). Moreover, levels of CSF anti-$P_{EX.C22}$ or CSF anti-C22-depleted rP0, but not CSF anti-P_{C22}, were significantly correlated with CSF anti-neuronal cell antibodies (anti-N). These results indicate that CSF IgG antibodies to the epitopes other than the C-terminal 22 amino acids of ribosomal P proteins, which might contain one of the major targets of CSF anti-N, are associated with the development of diffuse NP-SLE.

Introduction

Central nervous system (CNS) involvement is a relatively common and serious complication of systemic lupus erythematosus (SLE) [1,2]. Previous studies have demonstrated the association of serum antibodies directed against the C-terminal 22-amino acid sequences of ribosomal P protein (anti-P_{C22}) with CNS involvement in patients with SLE (neuropsychiatric SLE [NP-SLE]), especially diffuse psychiatric/neuropsychological syndromes (diffuse NP-SLE) [3-5]. However, the mechanism by which serum anti-P_{C22} leads to the development of diffuse NP-SLE has not yet been elucidated. In fact, the role of anti-P_{C22} in the cerebrospinal fluid (CSF) in the

ACR = American College of Rheumatology; anti-C22-depleted rP0 = antibodies directed against recombinant ribosomal P0 protein lacking the C-terminal 22 amino acids; anti-N = anti-neuronal cell antibodies; anti-P_{C22} = antibodies directed against the C-terminal 22-amino acid sequences of ribosomal P protein; anti-$P_{EX.C22}$ = autoantibodies directed against the ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence; anti-whole P = antibodies to the whole ribosomal P proteins; C22-depleted rP0 = recombinant ribosomal P0 fusion protein lacking the C-terminal 22 amino acids; CNS = central nervous system; CSF = cerebrospinal fluid; ELISA = enzyme-linked immunosorbent assay; HSA = human serum albumin; IgG = immunoglobulin G; IL-6 = interleukin-6; NMDA = *N*-methyl-d-aspartate; non-CNS SLE = systemic lupus erythematosus without neuropsychiatric manifestations; NP-SLE = neuropsychiatric systemic lupus erythematosus; OD_{492} = optical density at 492 nm; PBS = phosphate-buffered saline; SLE = systemic lupus erythematosus.

pathogenesis with diffuse NP-SLE or even their presence in the CSF remains uncertain. Thus, Golombek and colleagues [6] detected the presence of CSF anti-P_{C22} in all four of the patients with lupus psychosis in their studies, whereas others did not [3,4,7].

On the other hand, autoantibodies, which react with the neuronal cell lines or brain tissue, have been reported in the sera of patients with NP-SLE [8-10]. However, they have been shown to be present in SLE patients with no clinical evidence of CNS involvement [10]. In fact, in a cross-sectional study of SLE patients, no significant association was found between serum lymphocyte/brain cross-reacting antibodies and NP-SLE (present in 32% of cases with NP-SLE and 23% of those without NP-SLE) [10]. Of note, using a radioimmunoassay with the SK-N-SH neuroblastoma cell as a target, Bluestein and colleagues [11] demonstrated that immunoglobulin G (IgG) anti-neuronal cell antibodies (anti-N) were present in much higher concentrations in the CSF from patients with active NP-SLE than in the CSF from SLE patients without active CNS involvement. Using a cell enzyme-linked immunosorbent assay (ELISA) with SK-N-MC neuroblastoma cell lines fixed with paraformaldehyde, we also confirmed that CSF IgG anti-N levels were significantly elevated in patients with diffuse NP-SLE compared with those in SLE patients without diffuse NP-SLE [7]. However, the fine epitopes to which CSF anti-N were directed have not yet been delineated.

The presence of the immunodominant C-terminal epitope of ribosomal P proteins was demonstrated to be present on the surface of human neuroblastoma cells [12]. However, CSF anti-P_{C22} could be detected in only a fraction of patients with diffuse NP-SLE, whereas almost all the patients with diffuse NP-SLE expressed CSF anti-N [7]. Of note, previous studies also demonstrated the presence of a 38-kDa protein that is closely related to, or identical with, ribosomal P0 protein in purified human plasma membranes [12]. In addition, it has been shown that autoantibodies directed against the ribosomal P proteins are not only directed against the common C-terminal 22 amino acids, but against the N-terminal sequence of the ribosomal P2 or P1 proteins [13]. In fact, recent studies have revealed that measurement of CSF IgG anti-ribosomal P protein antibodies with Western blotting using purified ribosomes, containing whole ribosomal P0, P1, and P2 proteins, was more sensitive [14]. Because ribosomal P0 protein contains epitopes other than the C-terminal 22 amino acids, it is possible that CSF from patients with diffuse NP-SLE contains antibodies to such epitopes. The current studies, therefore, were carried out to compare the CSF levels of antibodies to the whole ribosomal P proteins (anti-whole P) in patients with diffuse NP-SLE and in patients with focal NP-SLE or non-SLE non-inflammatory neurological disorders.

Materials and methods

Patients and samples

One hundred and three patients with SLE were included in the present study. All patients fulfilled the American College of Rheumatology (ACR) 1982 revised criteria for the classification of SLE [15]. Of the 103 patients with SLE, 52 showed diffuse psychiatric/neurological syndromes (diffuse NP-SLE) according to the 1999 ACR definition of NP-SLE [16], 19 patients showed CNS manifestations other than diffuse NP-SLE (focal NP-SLE), and 32 patients showed no CNS manifestations (non-CNS SLE). Ten of the 52 patients with diffuse NP-SLE also presented seizures. Because of the difficulties in confirming the neurological diagnosis and in assigning the cause to SLE, we defined NP-SLE as (a) the presence of neuropsychiatric manifestations and (b) the elevation of CSF Ig indices [17,18] and/or the elevation of CSF interleukin-6 (IL-6) levels [19]. Thus, the 52 patients all showed increased CSF Ig indices and/or CSF IL-6 in the present study. In addition, 24 patients with non-SLE non-inflammatory neurological diseases (9 cerebrovascular diseases, 8 cervical spondylosis, 4 degenerative diseases, 2 diabetic neuropathy, and 1 epilepsy) were studied as a control. The 127 patients all gave informed consent, and the study was approved by the institutional ethical committee of Teikyo University School of Medicine (Tokyo). The detail and demographic features of the 127 patients are shown in Table 1. CSF specimens were obtained by a lumbar puncture when the patients showed active disease. These samples were kept frozen at -20°C until assayed. All assays were performed without knowledge of the diagnosis or clinical presentations.

Human anti-P_{C22} sera and affinity purification of anti-P_{C22}

IgG fractions were purified from the anti-P_{C22}-positive sera of SLE patients by means of a protein G-Sepharose 4FF column (Amersham Pharmacia Biotech, now part of GE Healthcare, Little Chalfont, Buckinghamshire, UK). Anti-P_{C22} were purified from the IgG fractions of SLE sera by means of an *N*-hydroxysuccinimide-activated Sepharose HP column (GE Healthcare) coupled with synthetic ribosomal P peptide-human serum albumin (HSA) conjugates as previously described [20]. Anti-P_{C22} thus purified reacted strongly with ribosomal P peptide-HSA conjugates, but not with HSA alone in an ELISA. It was also confirmed on Western blot analysis that purified anti-P_{C22} reacted with native ribosomal P proteins (P0, P1, and P2) (data not shown).

Measurement of autoantibodies to ribosomal P proteins

Antibodies for the C-terminal 22-amino acid ribosomal P synthetic peptide (anti-P_{C22}) in sera and CSF and those for purified whole ribosomal P proteins (anti-whole P) in CSF were determined by specific ELISA using the highly purified synthetic C-terminal 22-amino acid ribosomal P peptide conjugated to HSA as an antigen as previously described [5] and highly purified bovine ribosomal P proteins (P0, P1, and P2) (purity of more than 90%) (Arotec Diagnostics Limited, Wel-

Table 1

Profiles of the patients studied

Diagnosis	Number of patients	Gender (male/female)	Age in years (mean ± SD)
SLE	103		
Diffuse NP-SLE	52	4/48	37.8 ± 14.2
Acute confusional state	20		
Anxiety disorder	3		
Cognitive dysfunction	10[a]		
Mood disorder	12[b]		
Psychosis	7		
Focal NP-SLE	19	2/17	39.0 ± 14.8
Cerebrovascular disease	6		
Headache	2		
Movement disorder	1		
Seizure disorder	6		
Polyneuropathy	4		
Non-CNS SLE	32	3/29	42.7 ± 13.9
Non-SLE control	24	22/2	48.0 ± 13.7

[a]One patient also presented mood disorder. [b]One patient also presented cognitive dysfunction. Non-CNS SLE, systemic lupus erythematosus without neuropsychiatric manifestations; NP-SLE, neuropsychiatric systemic lupus erythematosus; SD, standard deviation; SLE, systemic lupus erythematosus.

lington, New Zealand). Antibodies for the epitope representing regions of the ribosomal P proteins other than P_{C22} were similarly determined by ELISA using recombinant ribosomal P0 fusion protein lacking the C-terminal 22 amino acids (C22-depleted rP0) as previously described [21].

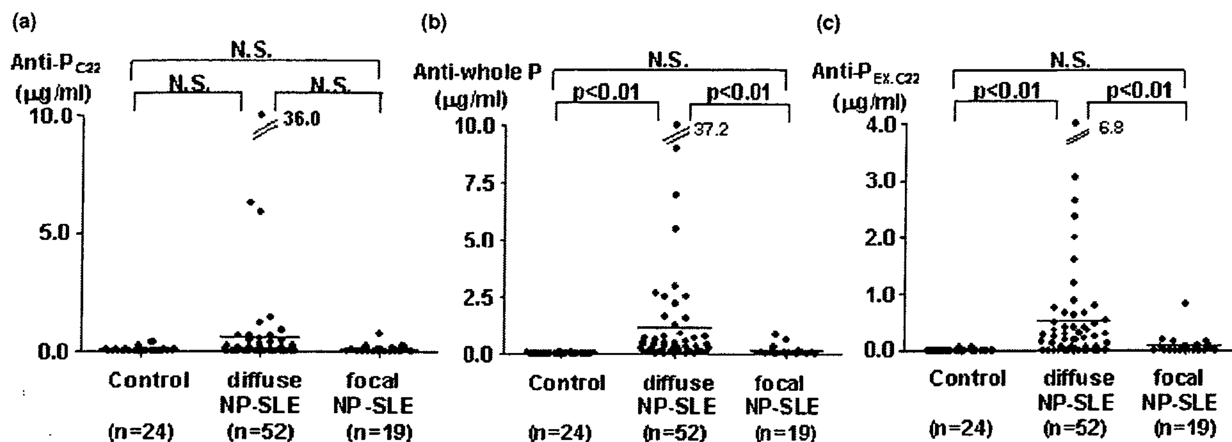
Briefly, wells of a 96-well microtiter plate were coated with ribosomal P peptide-HSA conjugates at 15 μg/ml or highly purified bovine ribosomal P proteins at 1.0 μg/ml in phosphate-buffered saline (PBS) (pH 7.2) or C22-depleted rP0 at 5 μg/ml in 6 M urea/10 mM Tris-HCl (pH 7.5) with 2 mM 2-mercaptoethanol (coating buffer) at 4°C overnight. Each well was then overcoated with Block Ace (Dainippon Pharmaceutical, Osaka, Japan), diluted 1:4 with PBS. Prior to being added to the antigen-coated wells, serum and CSF samples were usually diluted 1:200 and 1:2, respectively, in PBS containing 1% bovine serum albumin (Miles, now part of Bayer Corp., Emeryville, CA, USA). Bound antibody was detected with peroxidase-conjugated F $(ab')_2$ fragments of goat anti-human IgG (MP Biochemicals, Solon, OH, USA). After incubation with substrate solution containing 60 mg of *o*-phenylenediamine and 10 μl of 30% H_2O_2 in 100 ml of 0.05 M citrate phosphate buffer (pH 4.8) at 37°C for 30 minutes, the reaction was stopped by addition of 5 N H_2SO_4, and the absorbance (optical density) at 492 nm (OD_{492}) was read with a two-wavelength microplate photometer (MTP-120; Corona Electric Co., Ltd., Ibaraki, Japan). Determinations of OD_{492} were normalized to affinity-purified anti-P_{C22} such that anti-P_{C22} and anti-whole P activity might be converted to micrograms per milliliter of IgG. Antibodies directed against C22-depleted rP0 (anti-C22-depleted rP0) were expressed by arbitrary unit designation using a standard serum.

Non-specific binding activities to HSA for anti-P_{C22} or those to wells with PBS alone or coating buffer alone for anti-whole P or anti-C22-depleted rP0 were also determined in reference to the standard curves for binding activities to ribosomal P peptide (P_{C22})-HSA conjugates, highly purified ribosomal P proteins, or C22-depleted rP0. The specific anti-P_{C22}, anti-whole P, or anti-C22-depleted rP0 activities were thus determined by subtracting the values for the non-specific binding activity from those for binding activity to P_{C22}-HSA conjugates or to highly purified ribosomal P proteins or C22-depleted rP0. The intra-assay and interassay variances (coefficient of variation values) for anti-whole P were 13.8% and 15.7%, respectively, and those for anti-P_{C22} were previously described [7].

Measurement of anti-N

Anti-N in the CSF samples were determined by a cell ELISA using human neuroblastoma cell line SK-N-MC as previously

Figure 1



Cerebrospinal fluid antibodies to various components of ribosomal P proteins. CSF antibodies to the C-terminal 22-amino acid sequence of ribosomal P protein (anti-P_{C22}), highly purified ribosomal P proteins (anti-whole P), and epitopes other than the C-terminal 22-amino acid sequence (anti-$P_{EX.C22}$). Anti-P_{C22} **(a)**, anti-whole P **(b)**, and anti-$P_{EX.C22}$ **(c)** in CSF from patients with non-inflammatory neurological diseases (Control), with diffuse neuropsychiatric systemic lupus erythematosus (NP-SLE), or with focal NP-SLE were compared. Horizontal lines indicate the mean values. Statistical analysis was performed by Kruskal-Wallis test with multiple comparisons (Scheffé's method). CSF, cerebrospinal fluid; N.S., not significant.

described [7]. Briefly, SK-N-MC cells were seeded at a density of 5×10^4 per well in wells of a flat-bottomed 96-well tissue culture plate (no. 3596; Costar, now part of Corning Life Sciences, Acton, MA, USA) for 48 hours, after which the cells were fixed with 1% paraformaldehyde in PBS for 5 minutes at 37°C. After three washes with PBS containing 0.05% Tween 20, 50 μl of the appropriately diluted samples or various concentrations of standard sera were added and the plates were incubated for 1 hour at 37°C. Bound IgG anti-N were detected with peroxidase-conjugated $F(ab')_2$ fragments of goat anti-human IgG as previously described [7]. Determination of OD_{492} was normalized to standard sera for anti-N obtained from patients with diffuse NP-SLE such that anti-N activity might be converted to an arbitrary unit scale. The concentration of anti-N that produced half of the maximal absorbance at 492 nm, given by the saturating concentration of anti-N in the cell ELISA plate, was arbitrarily defined as 1 U/ml [7].

Statistical analysis

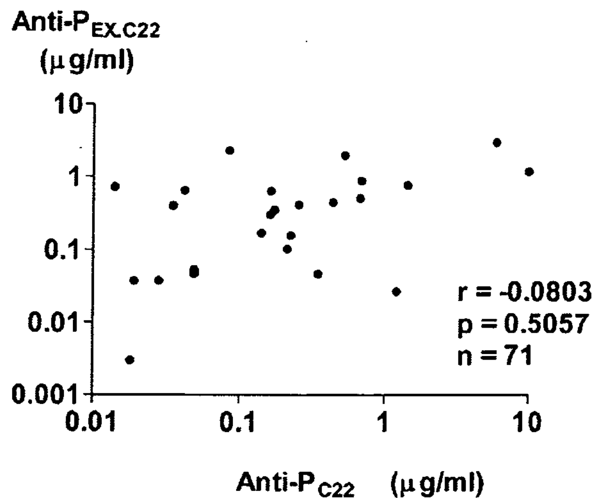
Differences in CSF anti-P_{C22}, anti-whole P, anti-$P_{EX.C22}$, and anti-C22-depleted rP0 among various groups were analyzed by Kruskal-Wallis test with multiple comparison (Scheffe's method). The correlation of anti-P_{C22} levels with anti-$P_{EX.C22}$ or anti-C22-depleted rP0 levels and the correlation of anti-N levels with anti-P_{C22}, anti-$P_{EX.C22}$, or anti-C22-depleted rP0 levels were evaluated by Spearman rank correlation test. Differences in serum anti-P_{C22}, anti-whole P, and anti-$P_{EX.C22}$ levels between non-CNS SLE and NP-SLE were analyzed by Welch's *t* test.

Results

Initial experiments examined CSF anti-P_{C22} levels in the three groups of patients. Although anti-P_{C22} levels in CSF appeared to be higher in diffuse NP-SLE, there were no significant differences in their levels among the three groups, including diffuse NP-SLE, focal NP-SLE, and non-inflammatory neurological control (Figure 1a). The results therefore confirm the previous observation that CSF anti-P_{C22} might not be prevalent in diffuse NP-SLE. By contrast, anti-whole P levels in CSF from patients with diffuse NP-SLE were significantly elevated compared with those from patients with focal NP-SLE or with non-inflammatory neurological diseases (Figure 1b). In addition, it should be noted that CSF anti-whole P levels were significantly higher than CSF anti-P_{C22} levels in 67 patients with diffuse NP-SLE and focal NP-SLE ($P < 0.0001$ as evaluated by Wilcoxon signed rank test). These results suggest that in addition to anti-P_{C22}, CSF from patients with NP-SLE might contain autoantibodies that recognize ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence.

To explore in detail the prevalence of the autoantibodies directed against the ribosomal P protein, epitopes other than the C-terminal 22-amino acid sequence (anti-$P_{EX.C22}$) were calculated by subtracting anti-P_{C22} from anti-whole P. As can be seen in Figure 1c, anti-$P_{EX.C22}$ levels in CSF from patients with diffuse NP-SLE were significantly elevated compared with those from patients with focal NP-SLE or with non-inflammatory neurological diseases. As shown in Figure 2, there was no significant correlation between CSF anti-P_{C22} and CSF anti-$P_{EX.C22}$ levels, obviating the possibility that CSF anti-$P_{EX.C22}$ activities might result from contamination of CSF anti-P_{C22} in patients with SLE. These results indicate that autoantibodies directed against ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence are strongly associated with the development of diffuse NP-SLE. Moreover, the data indicate that the expression of such

Figure 2



Correlation between autoantibodies to various components of ribosomal P proteins. The correlation between antibodies to the C-terminal 22-amino acid sequence of ribosomal P protein (anti-P_{C22}) and those to the ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence (anti-$P_{EX.C22}$) in cerebrospinal fluid from patients with systemic lupus erythematosus (SLE), including 52 patients with diffuse neuropsychiatric SLE (NP-SLE) and 19 patients with focal NP-SLE, was analyzed. Statistical analysis was performed by Spearman rank correlation test.

autoantibodies in CSF is not related to the presence of anti-P_{C22} in CSF.

To confirm the presence of autoantibodies to ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence, IgG antibodies to recombinant ribosomal P0 protein lacking the C-terminal 22 amino acids (C22-depleted rP0) were examined in CSF from 65 SLE patients with neuropsychiatric manifestations. Affinity-purified anti-P_{C22} reacted with ribosomal P peptide-HSA conjugates, but not with C22-depleted rP0, confirming the lack of the C-terminal 22-amino acid sequence in the C22-depleted rP0 (Figure 3). As shown in Figure 4, CSF anti-C22-depleted rP0 levels were significantly correlated with CSF anti-$P_{EX.C22}$ levels in these 65 patients. In addition, anti-C22-depleted rP0 levels in CSF from patients with diffuse NP-SLE were significantly elevated compared with those from patients with focal NP-SLE or with non-inflammatory neurological diseases (Figure 5). Accordingly, the frequency of positive expression of anti-C22-depleted rP0 in CSF from patients with diffuse NP-SLE was higher than that in CSF from patients with focal NP-SLE or with non-inflammatory neurological diseases (Table 2). These results confirm the presence of autoantibodies to ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence.

We next examined whether CSF anti-whole P might account for anti-N activities in CSF from patients with NP-SLE. As shown in Table 3, levels of CSF anti-whole P and anti-PC22 as well as CSF anti-N were decreased when CSF was incubated with paraformaldehyde-fixed SK-N-MC cells for 120 minutes at room temperature, confirming that CSF anti-whole P or anti-PC22 are constituents of CSF anti-N. However, as shown in Figure 6a, CSF anti-N levels were not significantly correlated with CSF anti-PC22 levels in SLE patients, including those with diffuse NP-SLE and focal NP-SLE. By contrast, CSF anti-N levels were significantly correlated with CSF anti-PEX.C22 or CSF anti-C22-depleted rP0 levels (Figure 6b,c

Finally, we examined serum levels of anti-P_{C22}, anti-whole P, and anti-$P_{EX.C22}$ in patients with non-CNS SLE or with NP-SLE. The values of anti-P_{C22}, anti-whole P, and anti-$P_{EX.C22}$ in 24 patients with non-SLE non-inflammatory neurological diseases were 2.44 ± 2.92 μg/ml, 4.92 ± 6.51 μg/ml, and 3.41 ± 6.06 μg/ml (mean ± standard deviation), respectively. As shown in Figure 7, serum anti-P_{C22} as well as anti-whole P levels in NP-SLE were significantly elevated compared with those in non-CNS SLE, which is consistent with previous studies [3-5]. Serum anti-P_{C22} and anti-whole P levels appeared to be higher in diffuse NP-SLE than those in focal NP-SLE, although

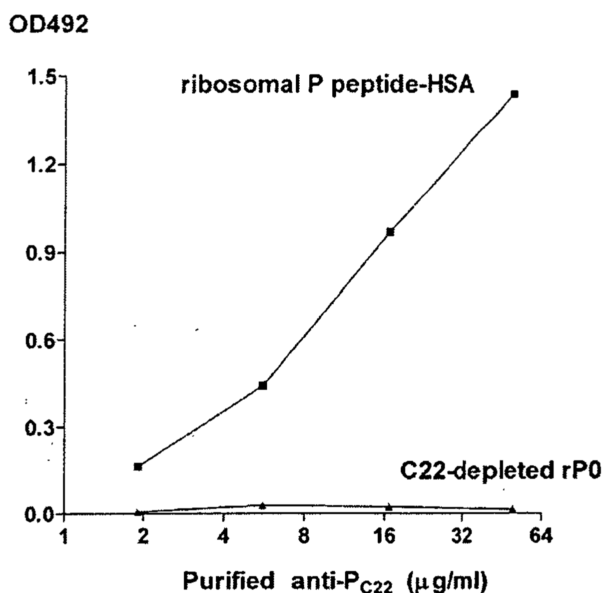
Table 2

Summary of the frequency of positive expression of antibodies to various ribosomal P protein components in cerebrospinal fluid[a]

	Percentage positive[b]		
	Control	Diffuse NP-SLE	Focal NP-SLE
Anti-P_{C22}	4.2% (1/24)	23.1% (12/52)	5.3% (1/19)
Anti-whole P	0% (0/24)	78.8% (41/52)	31.6% (6/19)
Anti-$P_{EX.C22}$	4.2% (1/24)	65.4% (34/52)	26.3% (5/19)
Anti-C22-depleted rP0	5.3% (1/19)	44.7% (21/47)	5.6% (1/18)

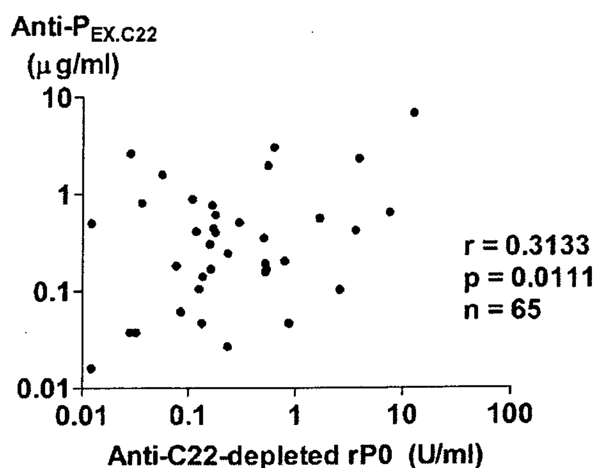
[a]Antibodies to the C-terminal 22-amino acid sequence of ribosomal P protein (anti-P_{C22}), to highly purified ribosomal P proteins (anti-whole P), to the epitopes other than the C-terminal 22-amino acid sequence (anti-$P_{EX.C22}$), and to recombinant ribosomal P0 protein lacking the C-terminal 22-amino acid sequence (anti-C22-depleted rP0) in cerebrospinal fluid from patients with non-inflammatory neurological diseases (Control), with diffuse neuropsychiatric systemic lupus erythematosus (NP-SLE), or with focal NP-SLE were compared. [b]Cutoff values were set as the mean + 3 standard deviations of the values in control group. Values in parenthesis mean (numbers of patients with positive results/total patient numbers) in each group.

Figure 3



Differential reactivity of purified antibodies to the C-terminal 22 amino acids of ribosomal P protein. Differential reactivity of purified antibodies to the C-terminal 22-amino acid sequence of ribosomal P protein (anti-P_{C22}) with ribosomal P peptide-human serum albumin (HSA) conjugates and with recombinant ribosomal P0 protein lacking the C-terminal 22-amino acid sequence (C22-depleted rP0). Purified anti-P_{C22} react with ribosomal P peptide-HSA conjugates, but not with C22-depleted rP0 on enzyme-linked immunosorbent assay plates. OD492 (optical density at 492 nm) values that are subtracted by non-specific binding activities are plotted.

Figure 4



Correlation between autoantibodies to various components of ribosomal P proteins. The correlation between antibodies to recombinant ribosomal P0 protein lacking the C-terminal 22-amino acid sequence (anti-C22-depleted rP0) and those to the ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence (anti-$P_{EX.C22}$) in cerebrospinal fluid patients, including 47 patients with diffuse neuropsychiatric systemic lupus erythematosus (NP-SLE) and 18 patients with focal NP-SLE, was analyzed. Statistical analysis was performed by Spearman rank correlation test.

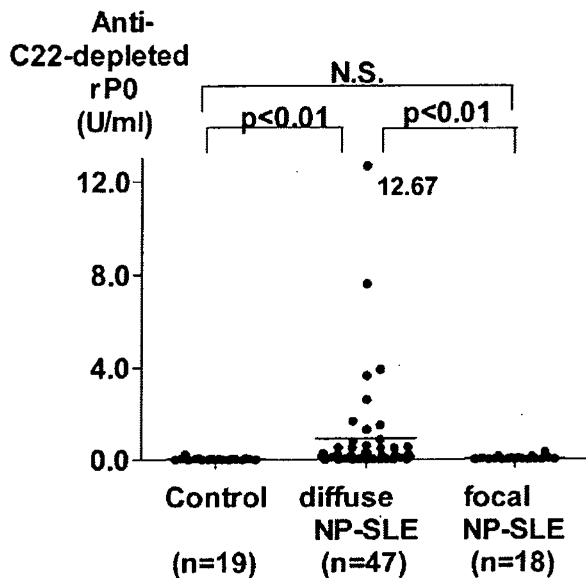
there were no statistical significances by Kruskal-Wallis test with multiple comparisons. Of note, there were no significant differences in serum anti-$P_{EX.C22}$ levels between non-CNS SLE and NP-SLE. These results suggest that in contrast with the CSF results, serum anti-P_{C22}, but not serum anti-P). The data therefore suggest that C22-depleted rP0 might contain one of the major targets, against which CSF anti-N are directed.$_{EX.C22}$, are associated with NP-SLE, especially diffuse NP-SLE.

Discussion

A number of studies have suggested that CSF anti-N play an important role in the pathogenesis of diffuse NP-SLE [7,11]. However, the epitopes to which CSF anti-N are directed have not been delineated. Of note, previous studies have demonstrated that epitopes antigenically related to ribosomal P proteins are present on the surface of SK-N-MC neuroblastoma cells [12]. Although anti-P_{C22} have been shown to be major autoantibodies to ribosomal P proteins [3,4,22], the frequency of their detection in CSF from patients with diffuse NP-SLE was not high enough to ensure their involvement in the pathogenesis of this disease [3,4,7]. Therefore, it was suggested that anti-P_{C22} might not be a major constituent of anti-N in CSF from patients with diffuse NP-SLE. Consistently, the data in the current studies indicated that CSF anti-P_{C22} levels were not significantly elevated in patients with diffuse NP-SLE compared with those in patients with focal NP-SLE or with non-inflammatory neurological diseases. However, it was still possible that CSF autoantibodies directed to ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence were more prevalent. Thus, the results in the current studies have also demonstrated that levels of CSF anti-whole P as well as CSF anti-$P_{EX.C22}$ were significantly higher in patients with diffuse NP-SLE than in patients with focal NP-SLE or non-inflammatory neurological diseases. The data therefore indicate that CSF antibodies to ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence are associated with diffuse NP-SLE.

To confirm the presence of antibodies for the epitopes representing regions of the ribosomal P proteins other than the C-terminal 22-amino acid sequence, antibodies to recombinant ribosomal P0 protein lacking the C-terminal 22 amino acids (C22-depleted rP0) [21] were evaluated. The results clearly demonstrate that CSF anti-C22-depleted rP0 levels were significantly correlated with CSF anti-$P_{EX.C22}$ levels. In addition, levels of CSF anti-C22-depleted rP0 as well as CSF anti-$P_{EX.C22}$ were significantly elevated in diffuse NP-SLE. The data therefore confirm that CSF antibodies to ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence play a role in the pathogenesis of diffuse NP-SLE, but further studies are required to identify the fine epitopes.

Figure 5



Cerebrospinal fluid antibodies to recombinant ribosomal P0 protein lacking the C-terminal 22-amino acid sequence. Antibodies to recombinant ribosomal P0 protein lacking the C-terminal 22-amino acid sequence (anti-C22-depleted rP0) (U/ml) in cerebrospinal fluid from patients with non-inflammatory neurological diseases (Control), with diffuse neuropsychiatric systemic lupus erythematosus (NP-SLE), or with focal NP-SLE were compared. Horizontal lines indicate the mean values. Statistical analysis was performed by Kruskal-Wallis test with multiple comparisons (Scheffé's method). N.S., not significant.

In has been demonstrated that purified human plasma membranes contain a 38-kDa protein that is closely related or identical to ribosomal P0 proteins [12]. Therefore, it was suggested that autoantibodies to ribosomal P proteins, especially those directed to epitopes other than the C-terminal 22-amino acid sequence, might be involved (at least in part) in CSF anti-N activities. In fact, levels of CSF anti-$P_{EX.C22}$ as well as CSF anti-P_{C22} or CSF anti-whole P were decreased after incubation of CSF with paraformaldehyde-fixed SK-N-MC cells, confirming that CSF anti-$P_{EX.C22}$ as well as anti-P_{C22} are constituents of CSF anti-N. However, CSF anti-P_{C22} levels were not significantly correlated with CSF anti-N levels in the present study. By contrast, CSF anti-$P_{EX.C22}$ or CSF anti-C22-depleted rP0 levels were significantly correlated with CSF anti-N levels. These results indicate that ribosomal P0 proteins contain one of the major targets of CSF anti-N in their portions other than the C-terminal 22-amino acid sequence. Of note, recent studies have demonstrated that autoantibodies directed against the *N*-methyl-d-aspartate (NMDA) receptor mediated apoptotic death of neurons *in vivo* and *in vitro* in murine systems [23]. Of note, anti-NMDA receptor antibodies were also detected in CSF from a single patient with SLE [22]. It is therefore likely that anti-NMDA receptor antibodies might also be involved in CSF anti-N activities and thus play a pivotal role in the pathogenesis of diffuse NP-SLE. Further studies with a large number of patients are required to confirm the involvement of anti-NMDA receptor antibodies in diffuse NP-SLE and to explore its relationship with anti-N.

A number of studies have indicated that serum anti-ribosomal P protein antibodies, including anti-P_{C22} or anti-whole P, are frequently observed in patients with NP-SLE [3-5,24]. Consistently, the results in the current studies have also disclosed that levels of serum anti-P_{C22} as well as serum anti-whole P are significantly higher in NP-SLE than those in non-CNS SLE. Of

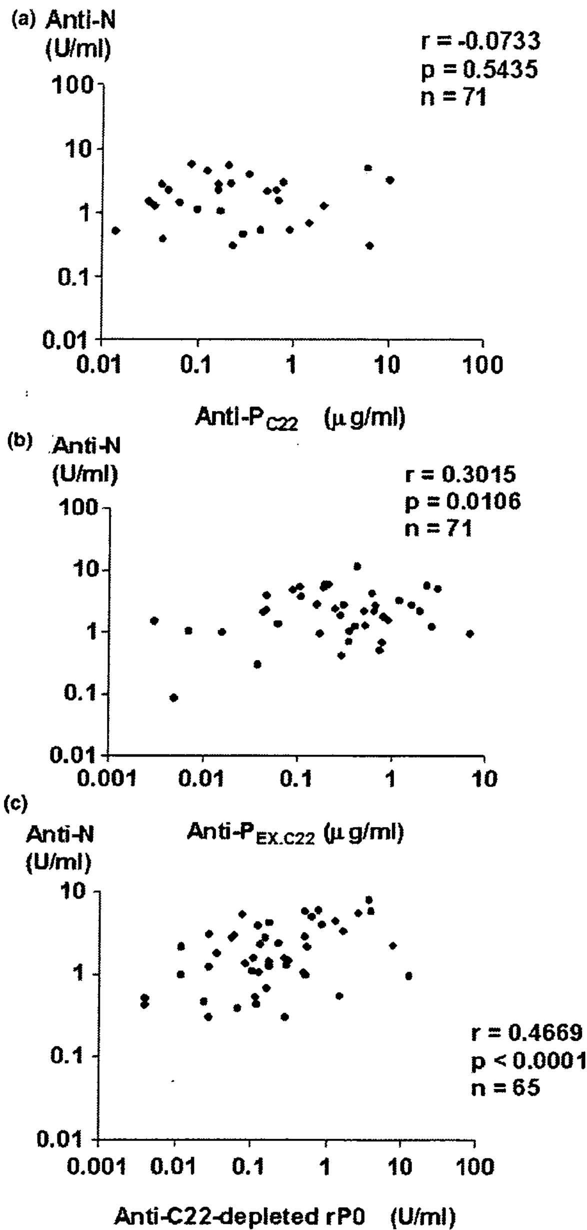
Table 3

Absorption of CSF autoantibodies to various components of ribosomal P proteins by neuronal cells

Patient	Autoantibodies	Without absorption	With absorption
1	Anti-whole P (μg/ml)	7.243	1.435
	Anti-P_{C22} (μg/ml)	3.456	1.019
	Anti-$P_{EX.C22}$ (μg/ml)	3.787	0.416
	Anti-N (U/ml)	4.083	1.950
2	Anti-whole P (μg/ml)	0.140	0.050
	Anti-P_{C22} (μg/ml)	0.070	0.042
	Anti-$P_{EX.C22}$ (μg/ml)	0.070	0.008
	Anti-N (U/ml)	0.789	0.588

Cerebrospinal fluid (CSF) samples (50 μl/well) were incubated in wells of a 96-well flat-bottomed microtiter plate with or without confluent SK-N-MC cells fixed with 1% paraformaldehyde at room temperature for 2 hours. After the incubation, CSF samples were recovered and were examined for anti-whole P, anti-P_{C22}, anti-$P_{EX.C22}$, and anti-N as described in Materials and methods. Anti-N, anti-neuronal cell antibodies; anti-P_{C22}, antibodies directed against the C-terminal 22-amino acid sequences of ribosomal P protein; anti-$P_{EX.C22}$, autoantibodies directed against the ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence; anti-whole P, antibodies to the whole ribosomal P proteins.

Figure 6



Correlation between autoantibodies to ribosomal P proteins and antineuronal cell antibodies. The correlation of antibodies to the C-terminal 22-amino acid sequence of ribosomal P proteins (anti-P_{C22}) **(a)**, those to the ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence (anti-$P_{EX.C22}$) **(b)**, or those to recombinant ribosomal P0 protein lacking the C-terminal 22-amino acid sequence (anti-C22-depleted rP0) **(c)** with anti-neuronal cell antibodies (anti-N) in cerebrospinal fluid from systemic lupus erythematosus (SLE) patients, including 52 patients **(a,b)** or 47 patients **(c)** with diffuse neuropsychiatric SLE (NP-SLE) and 19 patients **(a,b)** or 18 patients **(c)** with focal NP-SLE, was analyzed. Statistical analysis was performed by Spearman rank correlation test.

note, serum anti-$P_{EX.C22}$ levels were not significantly elevated in NP-SLE compared with those in non-CNS SLE. These findings contrast sharply with the results of CSF studies. Thus, in CSF, anti-$P_{EX.C22}$, but not anti-P_{C22}, were significantly associated with diffuse NP-SLE, whereas in serum, anti-P_{C22}, but not anti-$P_{EX.C22}$, were associated with NP-SLE.

The mechanism by which anti-whole P cause neuronal damage remains unclear. We previously reported that the expression of IL-6 mRNA in neurons was upregulated in the brain of an SLE patient who died of active diffuse NP-SLE [25]. Of note, we recently disclosed that anti-P_{C22} upregulate the expression of mRNAs for IL-6 and tumor necrosis factor-alpha in human peripheral blood monocytes [20]. It should be pointed out that anti-$P_{EX.C22}$ as well as anti-P_{C22} might be able to bind the ribosomal P protein on neuronal cells [12]. Taken together, these results suggest that anti-whole P or anti-$P_{EX.C22}$ might also upregulate the expression of IL-6 mRNA in neurons and thus result in the alteration of their functions. Further studies to explore the targets and the effects on their functions of anti-P_{C22} and anti-$P_{EX.C22}$ (or anti-P_{AA9}) would improve our understanding of the pathogenesis of NP-SLE.

In summary, the current studies have demonstrated that the expression of autoantibodies directed against the epitopes of ribosomal P proteins other than the C-terminal 22-amino acid sequence is increased in CSF from patients with diffuse NP-SLE. The presence of such autoantibodies might account for CSF anti-N activities, although there might be other antibodies that bind to neuronal cells, such as anti-NMDA receptor antibodies. Further studies to explore the whole spectrum of epitopes of neurons to which autoantibodies are directed as well as the mechanism by which such autoantibodies cause damage to neurons are needed for a complete understanding of the pathogenesis of diffuse NP-SLE.

Conclusion

The present study has disclosed that CSF IgG antibodies to the epitopes of ribosomal P0 proteins other than the C-terminal 22 amino acids are associated with the development of diffuse NP-SLE as one of the major CSF anti-N components.

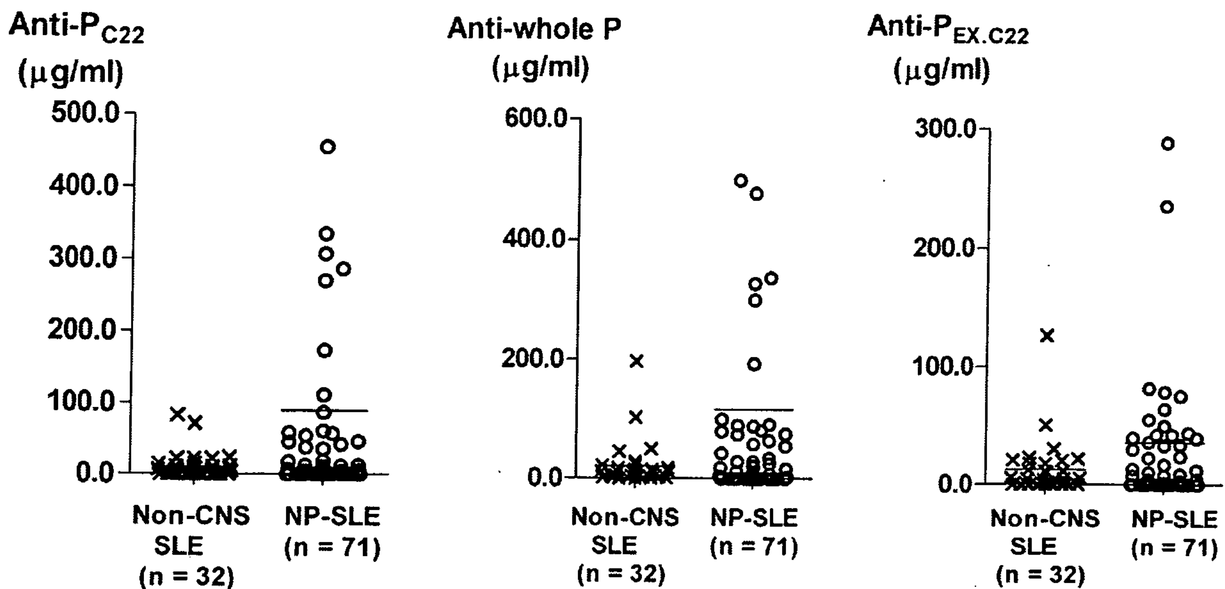
Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SH designed the study and participated in experimental procedures, collection, analysis, and interpretation of data and manuscript preparation. YA and MT contributed to the collection and analysis of data. TY helped to prepare C22-depleted rP0 and to develop ELISA for anti-C22-depleted rP0. All authors read and approved the final text before submission of the manuscript.

Figure 7



Serum autoantibodies to various components of ribosomal P proteins. Anti-P$_{C22}$, anti-whole P, and anti-P$_{EX.C22}$ in sera from SLE patients without neuropsychiatric manifestations (non-CNS SLE) (cross), with diffuse NP-SLE (open circle), or with focal NP-SLE (closed circle) were compared. Horizontal lines indicate the mean values. Statistical analysis between non-CNS SLE versus NP-SLE (focal + diffuse) was performed by Welch's *t* test. Anti-P$_{C22}$, antibodies directed against the C-terminal 22-amino acid sequences of ribosomal P protein; anti-P$_{EX.C22}$, autoantibodies directed against the ribosomal P protein epitopes other than the C-terminal 22-amino acid sequence; anti-whole P, antibodies to the whole ribosomal P proteins; non-CNS SLE, systemic lupus erythematosus without neuropsychiatric manifestations; NP-SLE, neuropsychiatric systemic lupus erythematosus; SLE, systemic lupus erythematosus.

Acknowledgements

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LETTERS

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Retirement rates and distribution of the United States rheumatology workforce: comment on the article by Deal et al

To the Editor:

I read with interest the report by Deal et al on the rheumatology workforce in the US (1). The model utilized in that study incorporates retirement calculations based on self-reported estimates. However, such self-reported estimates of physician retirement may not correlate with actual retirement rates. In fact, one study demonstrated that self-reported intention to leave clinical practice had 73.3% sensitivity but only 34.5% positive predictive value for actual departure from practice (2).

Several factors may lead rheumatologists to work beyond an estimated retirement age. First, rheumatology is not a physically taxing specialty, thus allowing older practitioners to remain in practice as long as cognitive abilities remain intact. Second, inflation and higher costs of retirement may prompt rheumatologists to continue working. Third, older rheumatologists who are grandfathered out of recertification requirements may have fewer barriers to continued practice. Fourth, generational work ethics may be associated with continued interest in work over retirement; a general survey by the American Association of Retired Persons in 2004 revealed that 79% of baby boomers plan to work in some capacity during their retirement years (3). Last, lengthening life expectancy will require greater savings for a longer retirement, as well as extend a clinician's potential working lifespan. If older rheumatologists do not retire as estimated, but continue working, could we witness an oversupply, or at least adequate supply, of rheumatologists, in contrast to the projections reported by Deal and colleagues?

Finally, Deal et al looked at national supply/demand figures, without addressing regional factors. Large metropolitan areas, especially those with large training programs, could potentially face an oversupply or adequate supply of rheumatologists due to a continued supply of new trainees remaining in the area, as well as inward migration. In contrast, more rural areas could experience an undersupply. While such regional discrepancies may prompt some migration into underserved areas, preferences for living in larger metropolitan areas may continue to promote a maldistribution of the rheumatology workforce.

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DOI 10.1002/art.22931

Correct citation regarding interleukin-6 levels in cerebrospinal fluid from patients with neuropsychiatric lupus: comment on the article by Fragoso-Loyo et al

To the Editor:

I read with interest the report by Fragoso-Loyo et al (1) describing the quantification of interleukin-6 (IL-6) and chemokines in cerebrospinal fluid (CSF) as an accurate indicator of neuropsychiatric involvement in systemic lupus erythematosus (NPSLE) that could be useful in the followup and evaluation of treatment response in these patients. It is always a pleasure when our observations are confirmed and extended by other investigators. However, Fragoso-Loyo and colleagues cited the wrong paper as their reference 10: our article in *Clinical Immunology and Immunopathology* (2), in which no data on NPSLE were included. We reported the elevated levels of IL-6 in CSF from patients with NPSLE in *Arthritis & Rheumatism* in 1990 (3). Therefore, Fragoso-Loyo et al should refer to this report instead of our paper in *Clinical Immunology and Immunopathology*, and provide comments on the relevance of our findings to theirs.

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Kanagawa, Japan

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DOI 10.1002/art.22937

Reply

To the Editor:

We apologize for this awful mistake on our part. Indeed, the correct reference is the report by Hirohata and Miyamoto (Hirohata S, Miyamoto T. Elevated levels of interleukin-6 in cerebrospinal fluid from patients with systemic lupus erythematosus and central nervous system involvement. Arthritis Rheum 1990;33:644–9), which was the first demonstration of elevated levels of IL-6 in CSF from NPSLE patients. They even showed that CSF IL-6 levels decreased when the central nervous system

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Histopathology of central nervous system lesions in Behçet's disease

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Abstract

Central nervous system (CNS) involvement in Behçet's disease, usually called neuro-Behçet's syndrome (NB), is one of the most serious complications of the disease. In the present study, we carried out immunohistological examination of biopsied or autopsied brain tissues from 3 patients with different types of NB, acute NB, chronic progressive NB, and NB in a long-term remission. Histopathology of mass lesion in acute NB revealed infiltration of mononuclear cells around small vessels, consisting of CD45RO+ T lymphocytes and CD68+ monocytes with few CD20+ B lymphocytes. Of interest, TUNNEL staining disclosed that most neurons were undergoing apoptosis in the inflammatory lesion. In chronic progressive NB, similar histopathological changes were noted in pons, cerebellum, medulla, internal capsule, and midbrain, although the degree of mononuclear cell infiltration was modest. There were also scattered foci of neurons undergoing apoptosis with formation of a few binucleated neurons. The most prominent feature of NB in a long-term remission was atrophy of basal pons with formation of cystic or moth-eaten lesions, consisting of isomorphic gliosis with viable neurons. There were still scattered foci of perivascular cuffing of T lymphocytes and monocytes. These results emphasize the common features throughout the courses of NB, perivascular cuffing of T lymphocytes and monocytes, irrespective of the clinical phenotypes. More importantly, it is suggested that soluble factors produced by infiltrating cells, including IL-6, might play a role in the induction of apoptosis of neurons in NB.
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Keywords: Behçet's disease; Central nervous system; Histopathology; Cerebrospinal fluid; IL-6

1. Introduction

Behçet's disease is a chronic relapsing inflammatory disease of unknown etiology, presenting recurrent aphthous stomatitis, uveitis, genital ulcers, and skin lesions. The predominant histopathological features in the inflamed tissues are infiltration of lymphocytes and monocytes, and sometimes polymorph nuclear leukocytes, around small veins without microscopic changes in the vessel walls. Thrombophilia or thrombophlebilis involving small and large veins is also common, whereas arteritis is rare. In these regards, Behçet's disease is unique compared with other vasculitides [1].

Central nervous system (CNS) involvement in Behçet's disease, usually called neuro-Behçet's syndrome (NB), is one of the most serious complications of the disease. CNS involvement in Behçet's disease is either caused by primary neural parenchymal lesions or is secondary to major vascular involvement [2,3]. The latter type is rarely complicated with the parenchymal lesions and should be called vasculo-Behçet's disease [2]. Of parenchymal CNS involvement, involvement of the brainstem was the most common, whereas spinal cord involvement, hemispheric involvement and meningoencephalitis also occurred [3–5]. Although cerebrospinal fluid analysis was performed in only a fraction of patients, most patients with brainstem disturbance showed abnormalities, including leukocytosis and an increase in protein concentration [3].

Although the role of small vessel vasculitis, mainly venular involvement, in the pathogenesis of NB has been suggested [6], the precise sequelae have not been delineated due to the paucity of autopsy studies. In addition, it remains unclear whether there are any differences in the histopathological characteristics among various clinical patterns of NB, including acute NB and chronic progressive NB [4,5,7]. In the present study, we carried out immunohistological examination of biopsied or autopsied brain tissues from 3

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Fig. 1. Histopathology of the mass lesion in acute NB (biopsy). H&E staining. Original magnification: ×25.

patients with different types of NB, one with acute NB, another who died of chronic progressive NB, and the other who died of myocardial infarction during remission of NB.

2. Patients and methods

2.1. Patients

Brain tissue was obtained from 3 patients with different types of NB.

2.1.1. Patient 1 (acute NB)

A 54-year-old Japanese man with Behçet's disease developed convulsion with subsequent left homonymous hemianopsia in 1989. CAT scan showed mass lesion with surrounding brain edema, which was hypovascular on cerebral angiography, in the right occipital lobe. A diagnosis of brain tumor was strongly suspected and open biopsy was performed on April 11, 1990. He was diagnosed as NB by biopsy, and improved by treatment with corticosteroid.

2.1.2. Patient 2 (chronic progressive NB)

A 38-year-old Japanese man developed progressive cerebellar ataxia, dysarthria, bladder–bowel incontinence, and dementia in 1974. Treatment with high doses of prednisolone started in December 1977. However, his neurological symptoms did not improve, and he died of aspiration pneumonia in July 1978.

2.1.3. Patient 3 (NB in a long-term remission)

A 62-year-old Japanese man had developed acute NB (headache and dysarthria) and had been successfully treated with low doses of prednisolone for 15 years before he developed acute myocardial infarction on November 24, 2001. He received aorto-coronary bypass operation in August 2002, but he died of congestive heart failure on September 2, 2002. After he developed myocardial infarction, there was no evidence for exacerbation of NB until he died of congestive heart failure.

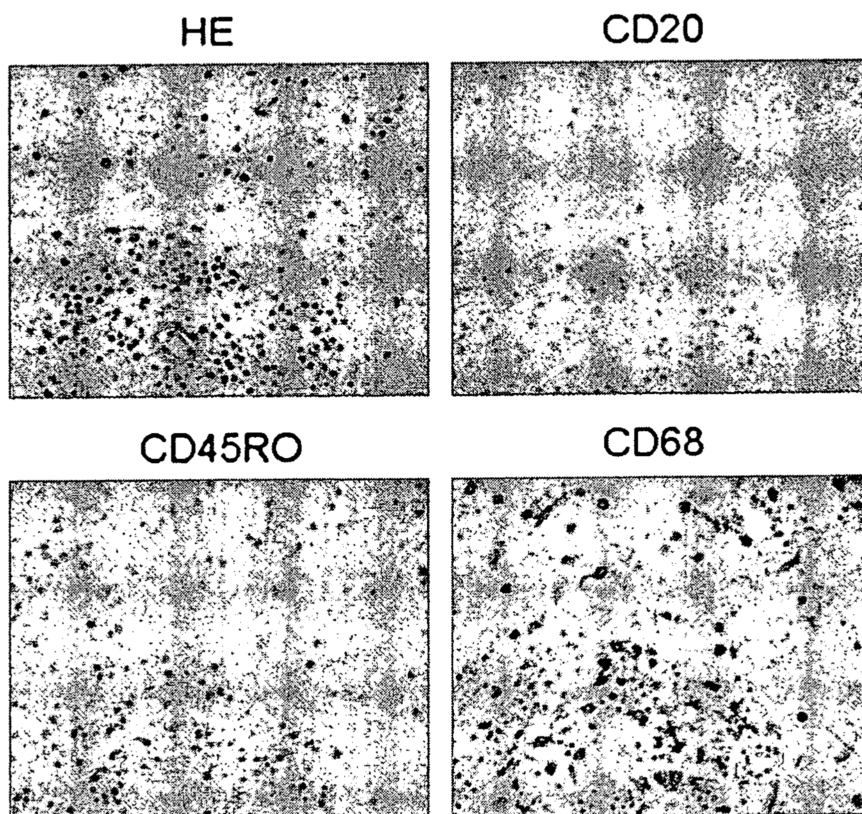

Fig. 2. Immunohistological examination of the infiltrated mononuclear cells in acute NB (biopsy). CD20 (L26), CD45RO (UCHL-1), CD68 (KP-1). Original magnification: ×50.

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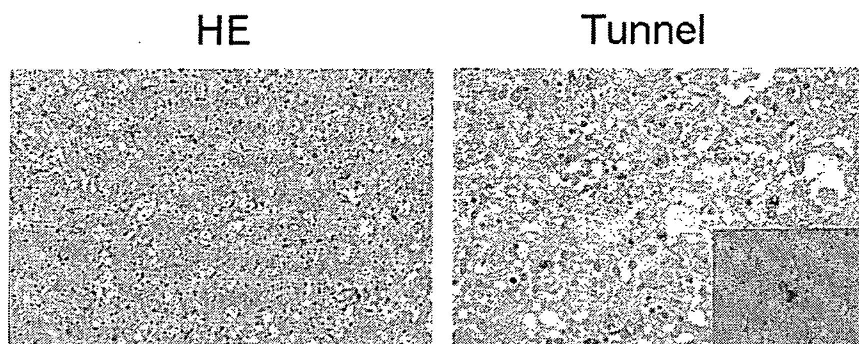

Fig. 3. TUNNEL staining of the mass lesion in acute NB (biopsy). Original magnification: ×25 (×50 in lower right).

2.2. Light microscopy

Brain tissues were fixed in formalin and embedded in paraffin. Sections were subjected to a variety of stain procedures, including hematoxylin and eosin, Klüver-Barrera, TUNNEL, and immunohistological stainings for B cells (L26, CD20), T cells (UCHL1, CD45RO), and monocytes (KP-1, CD68).

3. Results

3.1. Histopathology in patients with active NB (Patient 1 and Patient 2)

Histopathology of the mass lesion in Patient 1 (acute NB) revealed infiltration of mononuclear cells around small vessels, consisting of mononuclear cells and very few polymorph nuclear leukocytes in the brain parenchyma (Fig. 1). Immunohistological examination revealed that the infiltrating mononuclear cells consisted mostly of CD45RO+ T lymphocytes and CD68+ monocytes with very few CD20+ B lymphocytes (Fig. 2). Of interest, TUNNEL staining disclosed that most neurons were undergoing apoptosis in the inflammatory lesions. Some neurons undergoing apoptosis are binucleated (Fig. 3).

In Patient 2, who died of chronic progressive NB during treatment with high doses of steroid, similar histopathological changes were noted, although the degree of mononuclear cell infiltration was modest. Infiltration of mononuclear cells around small vessels was observed in pons, cerebellum, medulla, internal capsule, and midbrain (Fig. 4). The mononuclear cells consisted mostly of CD45RO+ T lymphocytes and CD68+ monocytes/macrophages with very few CD20+ B lymphocytes (Fig. 5). Of note, there were so called foam-cells [8] surrounding small vessels, which consisted of CD68+ cells. There were also scattered foci of neurons undergoing apoptosis with binucleation (Fig. 6). In addition, scattered foci of demyelination and gliosis were observed in hippocampus, pons and internal capsule.

3.2. Histopathology in a patient with clinically inactive NB (Patient 3)

The most prominent feature was atrophy of basal pons with formation of cystic or moth-eaten lesions (Fig. 7). The lesions in pons consisted of isomorphic gliosis, suggesting that the lesions were formed gradually by repeated minor attacks. In addition, there were viable neurons that were negative for TUNNEL staining within the lesions with isomorphic gliosis (Fig. 8), obviating the possibility that the pontine lesions might

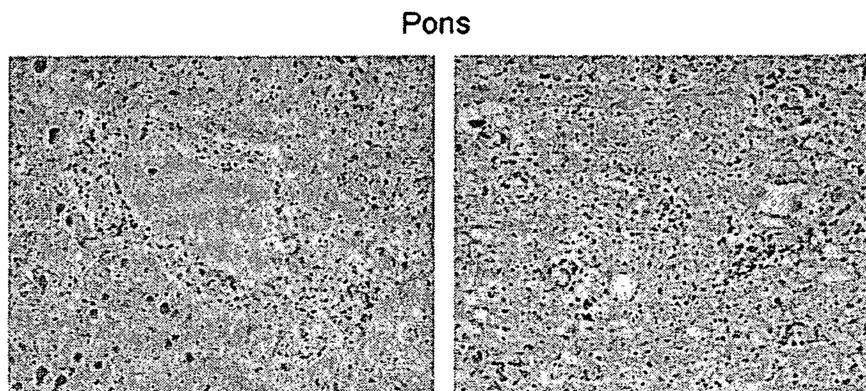

Fig. 4. Histopathology of the pontine lesion in chronic progressive NB (autopsy). H&E staining. Original magnification: ×25.

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