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特集 膠原病とその周辺疾患にみられる血管病変—その病態と治療

多発性筋炎・皮膚筋炎*

原　まさ子**

Key Words：polymyositis, dermatomyositis, vasculopathy, cutaneous vasculitis, gastric ulcer, brain infarction

多発性筋炎(polymyositis；PM)は四肢近位筋，頸筋の対称性の筋力低下を主症状とする原因不明の炎症性筋疾患である．筋症状に加えて特徴的な皮膚症状を伴うものは皮膚筋炎(dermatomyositis；DM)という．原因は不明であるが，種々の自己抗体が認められ，自己免疫的機序の関与が想定されている．PMでは筋組織にリンパ球やマクロファージが浸潤し，細胞性免疫による筋細胞障害が主体と考えられている．DMにおいては筋組織内の血管周囲の炎症が著明で，血管壁に免疫グロブリンと補体の沈着が認められることから，免疫複合体による筋肉と皮膚の微少血管の炎症が病因と考えられている．このように筋炎においては①毛細血管の変化，②種々の細胞表面分子を発現して浸潤細胞と相互作用する筋細胞の変化，③筋細胞や浸潤細胞による種々のサイトカインの産生により筋細胞が障害されていると考えられている．発症には疾患感受性遺伝子と環境因子の関与が考えられている．

予後を左右する臓器障害として，間質性肺炎，心筋障害，血管炎がある．

血管病変

PM・DMの血管病変には皮膚の血管炎から消化管の潰瘍，穿孔などの重篤な臓器血管炎がある．これら血管病変は小児に多く，PMよりDMに多くみられる．成人では悪性腫瘍を合併した例に多く，血管炎を伴う症例ではとくに悪性腫瘍の検索が必要となる．Chwalinskaら[1]による20年間の50例の筋炎患者の集計では，血管炎症状はDMの39.4%，PMの17.6%にみられ，DMに多いとしている．Kohら[2]はシンガポールの成人PM/DM 75例の臨床をまとめ18.7%に皮膚の血管炎を認めている．DMと悪性腫瘍やほかの膠原病の合併例に多いと報告している．

これら血管病変は筋肉，皮膚，肺，眼，腎，睾丸，神経，消化管などの臓器に認める．消化管の潰瘍は粘膜下の梗塞による．壊死性微少血管障害では血管壁に免疫グロブリンと補体の沈着が認められ，免疫複合体によると考えられる．

皮膚の血管炎

皮膚血管炎の症状としては皮下結節，爪床梗塞，網様皮斑，指先梗塞，紫斑，皮膚潰瘍(図1)などがある．

血管病変として頻度の高いものに毛細血管障害がある．簡単にみることのできる部位として爪床と歯肉がある．爪床毛細血管顕微鏡検査は

* Polymyositis and dermatomyositis.

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疾患活動性のモニターとして簡単で有用である．

顕微鏡オイルあるいは水溶性のジェルを用い，拡大鏡で観察できる．

毛細血管脱落，分岐，拡張，出血，血管数の減少が観察される．

皮下脂肪織炎を四肢，躯幹に認めることがある．

有痛性の皮下結節，爪周囲の皮膚梗塞，指先潰瘍は小児のPM/DMには珍しくないが成人では少ない．

Feldmanら[3]は11年間のフォローアップで76人の成人PM/DMの7例(9.2%)に皮膚血管炎を認めている．その症状は皮下結節4例，爪周囲の皮膚梗塞3例，指先潰瘍2例であった．多くはDMで認めることが多く，PM 31例中では1例にすぎず，overlap 18例では認められなかった．血管炎を伴った症例の28.6%に悪性腫瘍を認めたのに対し，血管炎のない症例では5.8%に悪性腫瘍を伴っていた．皮下結節の病理所見では細静脈，毛細血管，細動脈へのリンパ球や形質細胞の浸潤と血管内皮の腫大がみられ，血栓の形成やフィブリノイド変性はみられなかった．脂肪織炎も認められるが，巨細胞や肉芽の形成は認められない．小～中型の血管は障害されないと報告している．

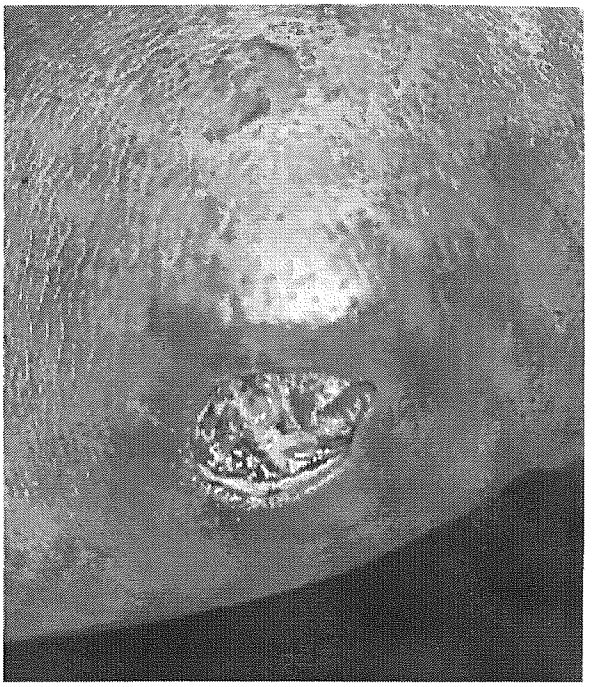
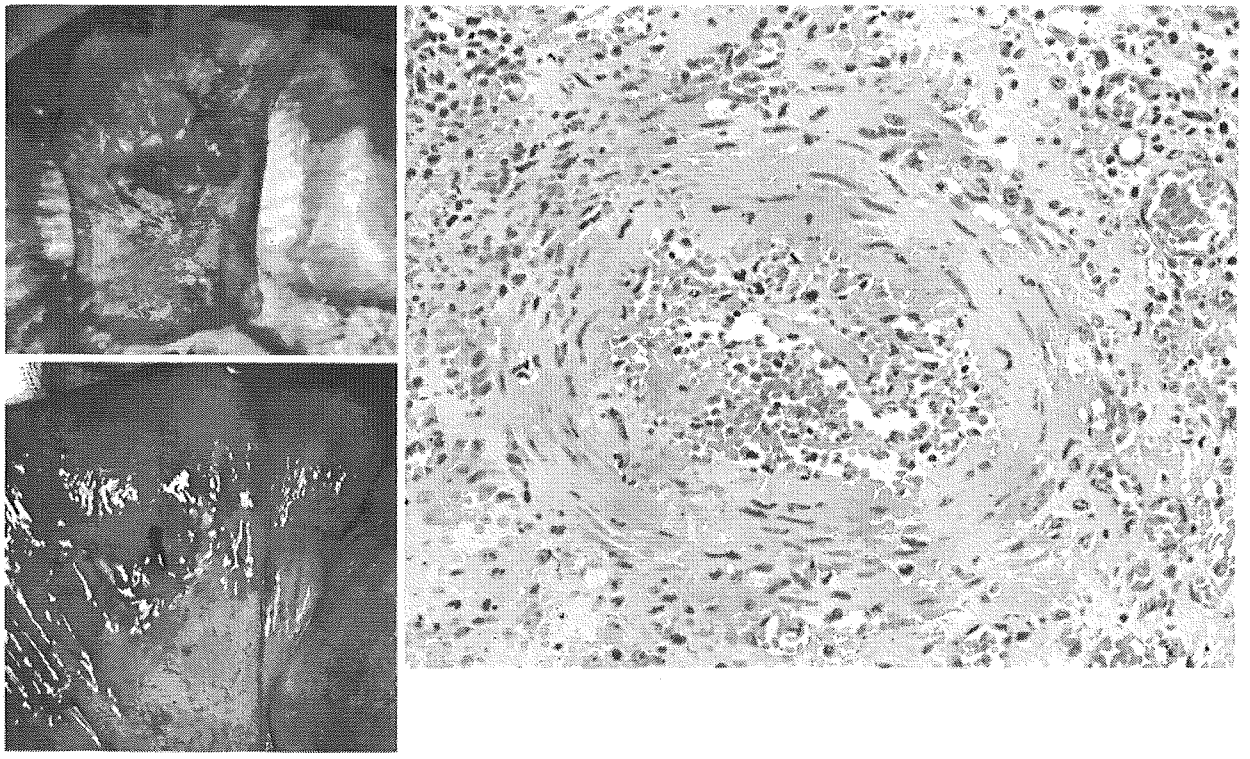


図1　皮膚筋炎の皮膚潰瘍

Hungerら[4]は23例のDM患者の皮膚生検で悪性腫瘍を合併した5例中4例に白血球破壊性血管炎の所見を得，悪性腫瘍の合併のない18例では3例に血管炎を認め，皮膚血管炎の存在は悪性



A　　B

図2　皮膚筋炎の消化管穿孔

A：空腸の穿孔，B：切除組織で血管壁の破壊と多数のリンパ球の浸潤が認められる．

腫瘍の予知因子となるとしている．

消化管の血管炎

血管病変は消化管のどの部位にも生じ，結果として潰瘍，穿孔，出血を起こす．小児ではとくに小腸の血管障害，虚血による壊死，穿孔が多く，診断の遅れが致死的となることがある．成人でも頻度は少ないが認められる．自験例を紹介する．

〔症例1〕54歳，女性[5]．

筋力低下，嚥下困難，ヘリオトロープ疹，ゴットロン疹にてDMと診断された．プレドニゾロン(PSL)60mg/日で軽快したが，PSL 17mg/日まで減量したところで再燃した．PSLを40mg/日で改善傾向を示し，37.5mg/日に減量した．その1週間後，激しい腹痛を訴え，消化管穿孔を疑い，開腹手術を行った．Tritz靱帯より80cm口側の空腸に直径5mmの穿孔を認め(図2-A)，同部位を5cm切除し左側腹部に腸瘻を形成した．切除組織では血管壁の破壊と多数のリンパ球の浸潤を認め血管炎の所見であった(図2-B)．血管炎に対し，ステロイドパルス療法を行ったが，消化管出血持続し死亡した．本邦の腸管穿孔を合併したDMの報告は数例で，いずれもステロイド減量時に筋症状や皮疹の増悪を認め，原病の活動期に起こっている．部位は1例の回腸を除き，すべて小腸であった．治療はPSLに加え，シクロホスファミドやアザチオプリン，メトトレキサート(MTX)などの免疫抑制薬を併用している．

ステロイド治療中は腹膜刺激症状が比較的軽度となるので，腸穿孔の診断には注意を要する．

そのほか

1．神　経

Matsuiら[6]はDMに多発神経炎を伴った症例を報告し，筋肉，皮膚，腓腹神経生検で血管炎の所見を得た．同部位にVEGFとVEGF-receptorの発現亢進を認め，血管炎への関連を考察している．

Reganら[7]は，脳梗塞を伴った47歳のDMの症例を報告している．MRIで前頭葉にT2で高信号域を，MR angioで右内頸動脈に2か所分節的な狭窄像を得，血管炎と診断した．右前頭葉の脳生検では小血管周囲への炎症細胞浸潤と大グリア細胞増多と小グリア細胞/マクロファージの反応を認めた．浸潤細胞は主にT細胞と少数の形質細胞であった．シクロホスファミド2mg/kg/日の連日内服治療により改善した．小児DMにおける中枢神経の血管炎の報告はあるが，成人DMにおける報告はReganらが初めてである．

小児例ではBankerら[8]が1966年，8例の小児DMの剖検例で，皮膚，筋肉のみならず脂肪，消化管，神経に血管炎の存在を報告している．Jimenezら[9]は脳皮質内毛細血管と心筋内毛細血管の壊死，二次性の血栓形成と出血により死亡した6歳の稀なDMの症例を報告している．

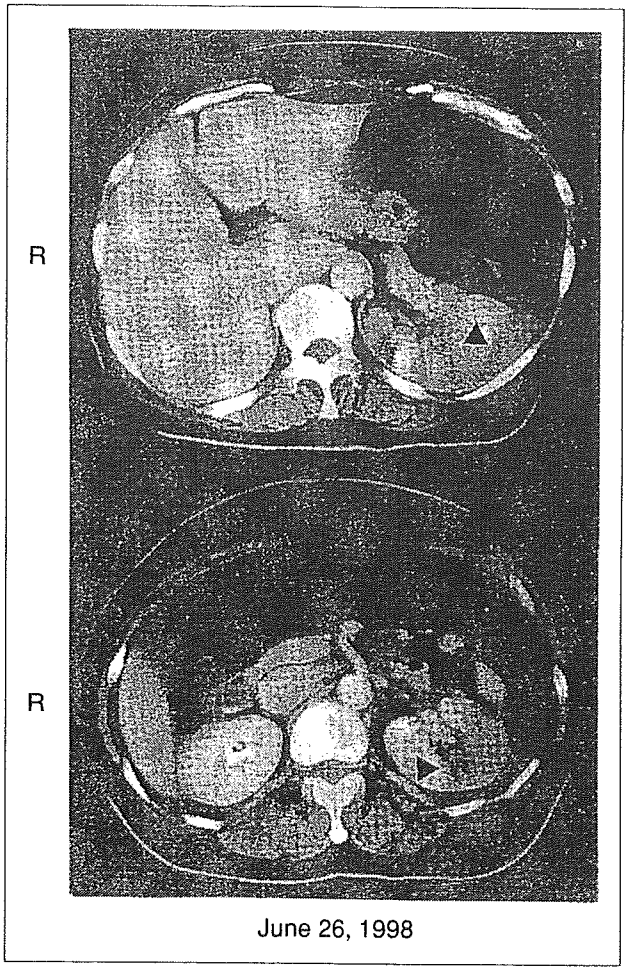


図3　脾梗塞・左腎梗塞(腹部造影CT)
脾臓ならびに左腎臓に多発するlow density areaを認める．

2．腎　臓

腎・脾梗塞を併発したDMの自験例を紹介する[10]．

〔症例2〕60歳，女性．

筋力低下とヘリオトロープ疹，ゴットロン徴候，間質性肺炎にてDMと診断された．PSL 60mg/日

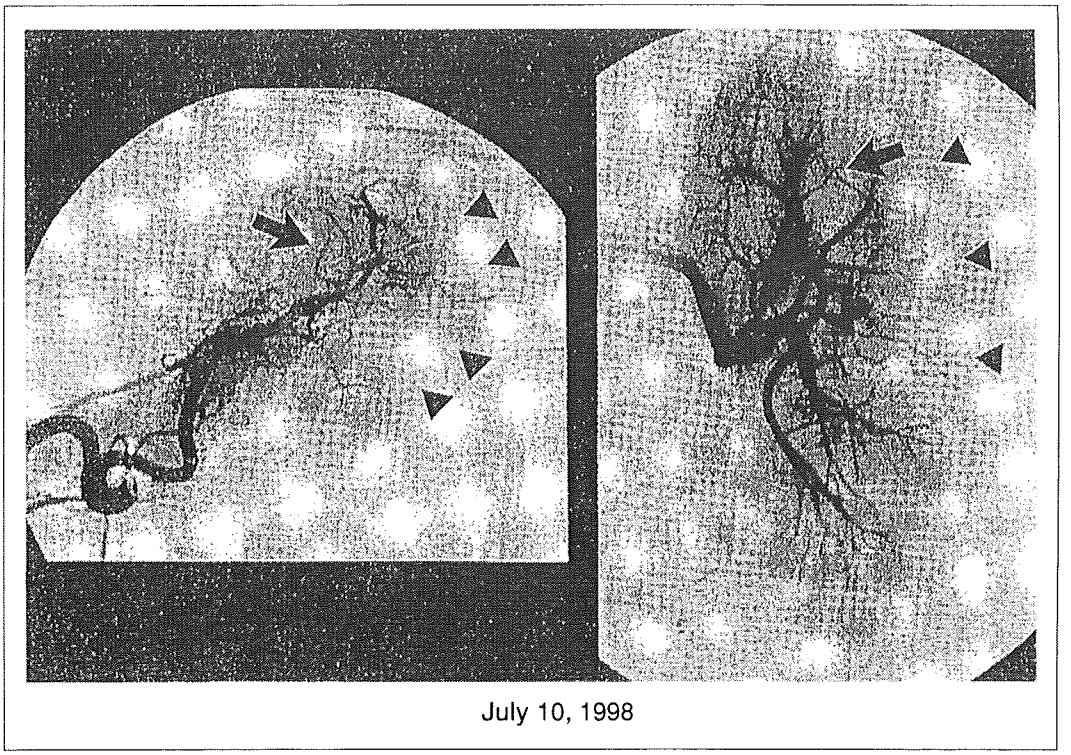


図4 血管造影
脾臓内末梢動脈の狭窄，途絶，不正像を認める．

において治療を開始し，筋原性酵素は徐々に低下した．1週間後突然激しい左上腹部痛が出現した．造影CTで脾臓と左腎臓に多発性にlow density areaを認め，脾梗塞・左腎梗塞と診断した(図3)．血管造影では，脾臓内に多発する欠損像があり，末梢動脈の狭窄，途絶，不正像を認めた．左腎臓でも多発する欠損像を認め，弓状動脈より末梢の動脈で狭窄，途絶，不正像を認めた(図4)．フィブリノーゲン，FDP，D-ダイマー，プラスミン・α2プラスミンインヒビターコンプレックス，トロンビンアンチトロンビン複合体，トロンボモジュリンが上昇していた．本例では梗塞の原因となる心疾患や高脂血症はなく，心腔内血栓もなかったこと，および梗塞部位が多発性であったこと，抗リン脂質抗体症候群は否定的であることより，DMに伴う血管炎と考えた．治療は血栓溶解のためt-PA製剤を1回投与し，ヘパリンを持続投与するとともに，シクロホスファミドパルス療法を行った．その後ヘパリンからワーファリンに切り替え，再度シクロホスファミドパルス療法を行い，改善している．

成人のDMに伴う血管炎による臓器梗塞の合併は報告されていない．小児のDMにおいても，血管炎による腸管潰瘍，穿孔などは知られているが，脾・腎梗塞の報告はない．本例では，治療前より凝固線溶系の亢進および血管内皮障害が認められており，それが臓器梗塞の発症に関与したと考えられる．

治　療

薬物療法の第一選択は抗炎症効果と免疫抑制効果を有するステロイド薬で，効果不十分，あるいは副作用が問題となるとき，免疫抑制薬が用いられる．

1．ステロイド療法

PSL換算40mg/日以上の大量投与が一般的で，嚥下困難，血管炎，間質性肺炎などで生命を脅かす臓器症状を有する症例にはメチルプレドニゾロン1g3日間点滴静注するステロイドパルス療法が試みられる．4週間以上のステロイド大量の内服，あるいはパルス療法でも改善が認められなければ，ステロイド抵抗性と考えてほかの免疫抑制薬の適応を考える．

2．免疫抑制薬

免疫抑制薬はステロイド薬と併用する．葉酸拮抗薬のMTXは，週1回5～7.5mgの経口投与を行

う．アザチオプリンやシクロホスファミドは連日50～100mg/日の内服投与が行われる．間質性肺炎，血管炎合併例には，シクロホスファミド500～700mgを3～4週に1回点滴静注するエンドキサンパルス療法が行われる．シクロスポリンは血中濃度が100～250mg/mlになるように投与する．

3．ガンマグロブリン大量静注療法

ステロイド薬や免疫抑制薬に反応しない症例に，ガンマグロブリン0.4g/kg/日を5日間連続点滴静注するガンマグロブリン大量静注療法の有効性が報告されている．皮膚血管炎に対しても有効性が評価されている．しかし，わが国では保険適応はなく，現在適応拡大の申請中である．効果はガンマグロブリンと免疫担当細胞や，リンパ球のFcを介した相互作用，あるいはリンパ球上の抗原レセプターを介した作用，抗イデオタイプ抗体活性や抗サイトカイン抗体活性に負うところが多いと考えられている．

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Autoantibodies to a 140-kd Polypeptide, CADM-140, in Japanese Patients With Clinically Amyopathic Dermatomyositis

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Objective. **To identify novel autoantibodies specific for dermatomyositis (DM), especially those specific for clinically amyopathic DM (C-ADM).**

Methods. **Autoantibodies were analyzed by immunoprecipitation in 298 serum samples from patients with various connective tissue diseases (CTDs) or idiopathic pulmonary fibrosis (IPF). Antigen specificity of the sera was further examined by immunoblotting and indirect immunofluorescence (IF). The disease specificity and clinical features associated with the antibody of interest were determined.**

Results. **Eight sera recognized a polypeptide of ~140 kd (CADM-140 autoantigen) by immunoprecipitation and immunoblotting. Immunoreactivity was detected in the cytoplasm, and indirect IF revealed a granular or reticular pattern. Anti–CADM-140 antibodies were detected in 8 of 42 patients with DM, but not in patients with other CTDs or IPF. Interestingly, all 8 patients with anti–CADM-140 antibodies had C-ADM. Among 42 patients with DM, those with anti–CADM-140 autoantibodies had significantly more rapidly progressive interstitial lung disease (ILD) when compared with patients without anti–CADM-140 autoantibodies (50% versus 6%; $P = 0.008$).**

Conclusion. **These results indicate that the presence of anti–CADM-140 autoantibodies may be a novel marker for C-ADM. Further attention should be directed to the detection of rapidly progressive ILD in those patients with anti–CADM-140 autoantibodies.**

Polymyositis (PM)/dermatomyositis (DM) is a chronic inflammatory disorder that culminates in injury to the skin and muscle and, sometimes, is associated with interstitial lung disease (ILD) and/or neoplasia (1,2). A number of autoantibodies are associated with myositis, including those specific for aminoacyl–transfer RNA synthetase (anti-ARS) (3), signal recognition particle (anti-SRP) (4), and Mi-2 (5). These autoantibodies have proven to be clinically useful in the diagnosis and classification of these diseases and are predictive of responses to treatment.

It has been known for some time that certain patients may have the typical skin manifestations of DM but no evidence of myositis, a condition known as amyopathic DM. Recently, Sontheimer proposed the existence of a unique subgroup of patients with DM who have the clinical cutaneous features of DM but no evidence of clinical myositis symptoms for at least 2 years after the onset of skin manifestations (referred to as clinically amyopathic DM [C-ADM]) (6). In other words, C-ADM includes patients with amyopathic DM and patients with hypomyopathic DM (patients with subclinical signs of myositis and DM skin manifestations). Some patients with C-ADM, especially those in Japan (7), have been noted to develop rapidly progressive ILD. This condition in many of these patients is resistant to treatment, and fatal outcomes have been observed.

Because of the severity of ILD accompanying C-ADM, a marker autoantibody would be useful for early diagnosis and treatment monitoring. Potential marker autoantibodies have been described by Targoff et al, who, in a preliminary study, described specificity for a 95-kd Se protein, as well as an unidentified 155-kd protein (8). However, a full survey of the autoantibodies

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associated with C-ADM has not been performed. In the present study, we examined the sera from 15 Japanese patients with C-ADM to identify additional autoantibodies associated with this disease.

PATIENTS AND METHODS

Patients and sera. Serum samples were obtained from 255 randomly selected Japanese adult patients with connective tissue diseases (CTDs) who were being followed up in clinics at Keio University in Tokyo and collaborating medical centers. These sera were obtained, prior to therapy, from a cohort of 61 patients with PM, 42 with DM (including 15 with C-ADM), 50 with rheumatoid arthritis, 46 with systemic lupus erythematosus, 27 with mixed CTD/overlap syndrome, 22 with systemic sclerosis, and 7 with Sjögren's syndrome. Sera from 43 patients with idiopathic pulmonary fibrosis (IPF) and 16 normal human sera were used as control sera. The diagnosis of C-ADM was based on diagnostic criteria proposed by Sontheimer (6), i.e., DM patients with no clinical muscle symptoms for more than 2 years after the onset of skin manifestations.

The patients were diagnosed as having ILD according to the results of chest radiography, chest computed tomography (CT), and pulmonary function testing, which included the percent predicted values for forced vital capacity and diffusing capacity for carbon monoxide. A subset of patients with rapidly progressive ILD was defined as those presenting with progressive dyspnea and progressive hypoxemia, and a worsening of interstitial change on the chest radiograph within 1 month from the onset of respiratory symptoms.

Immunoprecipitation. The immunoprecipitation assay was performed using extracts of the leukemia cell line, K562, as previously described (9). A total of 10 μl of patient serum was mixed with 2 mg of polypeptide A–Sepharose CL-4B (Pharmacia Biotech AB, Uppsala, Sweden) in 500 μl of immunoprecipitation buffer (10 m*M* Tris HCl, pH 8.0, 500 m*M* NaCl, 0.1% Nonidet P40) and incubated for 2 hours at 4°C, and then washed 3 times with immunoprecipitation buffer.

For polypeptide studies, antibody-coated Sepharose beads were mixed with 100 μl of ^{35}S-methionine–labeled K562 cell extracts derived from 2×10^5 cells, and rotated at 4°C for 2 hours. After 6 washes, the Sepharose beads were resuspended in sodium dodecyl sulfate (SDS) sample buffer and the polypeptides were fractionated by 6% SDS–polyacrylamide electrophoresis gels. Radiolabeled polypeptide components were analyzed by autoradiography.

For analysis of RNA, the antigen-bound Sepharose beads were incubated with 100 μl of K562 cell extracts (6×10^6 cell equivalents per sample) for 2 hours at 4°C. To extract bound RNA, 30 μl of 3.0*M* sodium acetate, 30 μl of 10% SDS, 2 μl of carrier yeast transfer RNA (10 mg/ml; Sigma, St. Louis, MO), and 300 μl of phenol:chloroform:isoamyl alcohol (50:50:1, containing 0.1% 8-hydroxyquinoline) were added. After ethanol precipitation, the RNA was resolved using a 7*M* urea–10% polyacrylamide gel, which was subsequently silver-stained (Bio-Rad, Hercules, CA).

Immunoblotting. Immunoblotting analysis was performed using K562 cell extracts in a modification of the procedure described by Towbin et al (10).

Immunodepletion. A 10-μl aliquot of the prototype serum of autoantibodies to the 140-kd polypeptide was mixed with 2 mg of Sepharose beads and incubated for 2 hours at 4°C, followed by 3 washes with immunoprecipitation buffer. Another serum that recognized the 140-kd polypeptide was added in a dose-dependent manner (0 μl, 10 μl, 25 μl, and 50 μl) and then incubated. After 3 washes, immunoprecipitation for polypeptide analysis was performed as described above.

Indirect immunofluorescence (IF). Indirect IF was performed using HEp-2 cells and fluorescein-labeled anti-human immunoglobulin (Inova Diagnostics, San Diego, CA).

Clinical studies. The patients whose sera immunoprecipitated a 140-kd polypeptide were examined for their clinical symptoms, clinical course, muscle enzyme levels (creatine kinase [CK] and aldolase), results on chest radiographic and CT scans, and findings of skin pathology. An assessment of muscle weakness was performed using a manual muscle test (11). Some patients were also examined by electromyogram and muscle magnetic resonance imaging (MRI), and by pathologic analysis of the muscle.

Statistical analysis. The 2 groups of DM patients with or without autoantibodies to the 140-kd polypeptide were compared. The results of comparisons between groups were analyzed using the chi-square test, with Yates' correction where appropriate.

RESULTS

Detection of anti–140-kd polypeptide antibodies in patients with C-ADM. We screened 298 patient sera and 16 normal human sera by immunoprecipitation. Sera from 8 (19%) of 42 patients with DM immunoprecipitated a polypeptide of ~140 kd from ^{35}S-methionine–labeled K562 cell extracts (Figure 1A, lanes 1–8). All 8 patients were diagnosed as having C-ADM, a subtype of DM. In the analysis of RNA specificity, these sera did not immunoprecipitate any nucleic acid band, except for 1 patient's serum, which precipitated hYRNA of SSA/Ro components.

The C-ADM sera that immunoprecipitated the 140-kd polypeptide were also used to immunoblot K562 cell extracts. These sera from C-ADM patients displayed a similar reaction on immunoblot, with a polypeptide band of similar molecular weight (results not shown).

For identification of novel autoantibodies recognizing the 140-kd molecule, the polypeptide immunoprecipitated by the prototype serum was compared with antigens of similar molecular weight recognized by other known autoantibodies (Figure 1B). The protein recognized by the prototype serum migrated slightly faster than the 140-kd protein recognized by anti-MJ antibody (Figure 1B, lane 2) and faster than that recognized by anti–RNA helicase A antibody (Figure 1B, lane 3), but more slowly than the 120-kd protein precipitated by

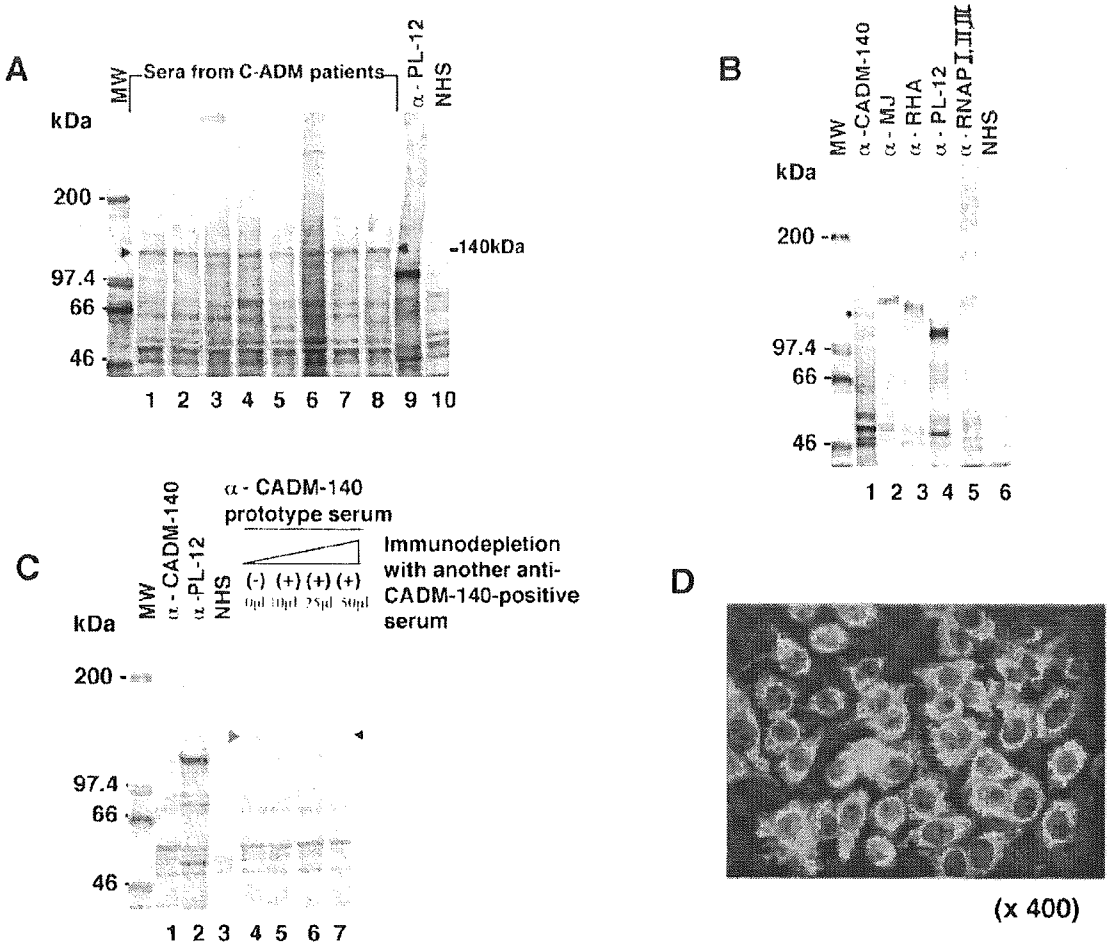

Figure 1. **A,** Immunoprecipitation of polypeptides with sera from patients with clinically amyopathic dermatomyositis (C-ADM), using ^{35}S-methionine–labeled K562 cell extracts. Lanes 1–8, Sera from C-ADM patients; lane 9, anti–PL-12 serum; lane 10, control normal human serum (NHS). A 140-kd protein was recognized by 8 sera from C-ADM patients (lanes 1–8). **B,** Immunoprecipitation of polypeptides by the prototype serum and by other known autoantibodies. Lane 1, The prototype anti–CADM-140 serum; lane 2, anti-MJ serum; lane 3, anti–RNA helicase A (RHA) serum; lane 4, anti–PL-12 (alanyl–transfer RNA synthetase) serum; lane 5, anti–RNA polymerase I, II, and III (RNAP I, II, and III) serum; lane 6, control NHS. Anti–CADM-140 serum immunoprecipitated an ~140-kd polypeptide that was easily distinguished from that of other known antibodies. **C,** Immunodepletion studies. Sera used for immunoprecipitation were as follows: lane 1, anti–CADM-140; lane 2, anti–PL-12; lane 3, control NHS; lanes 4–7, immunoprecipitation with anti–CADM-140 serum after absorption by another anti–CADM-140–positive serum in a dose-dependent manner. **Arrows** in **A** and **C** denote the 140-kd polypeptide. The sizes of the molecular weight markers are indicated to the left in **A–C. D,** Immunofluorescence pattern of HEp-2 cells stained with anti–CADM-140 serum. A granular or reticular cytoplasmic staining pattern on HEp-2 cells was observed. (Original magnification × 400.)

anti–PL-12 antibody (Figure 1B, lane 4). These results clearly indicate that the 140-kd polypeptide immunoprecipitated by the prototype serum was different from the proteins immunoprecipitated by these other known antibodies. We designated this new autoantibody specificity as anti–CADM-140.

The prototype serum depleted extracts of the 140-kd polypeptide in a dose-dependent manner (Figure 1C, lanes 4–7), and the polypeptide recognized by the prototype serum was no longer immunoprecipitated in these extracts (Figure 1C, lane 7). In contrast, the depletion of radiolabeled K562 cell extracts with the use of autoantibodies of different immunologic specificities did not affect the levels of the anti–CADM-140–specific antigen (results not shown). When sera positive for anti–CADM-140 antibodies were assessed in indirect IF studies, a granular or reticular cytoplasmic staining pattern was observed (Figure 1D).

Table 1. The frequencies of myositis-specific, myositis-associated, and anti–CADM-140 antibodies in patients with connective tissue diseases and IPF*

Autoantibodies	PM (n = 61)	DM (n = 42)		RA (n = 50)	SLE (n = 46)	MCTD/OL (n = 27)	Systemic sclerosis (n = 22)	Sjögren's syndrome (n = 7)	IPF (n = 43)
		Classic DM (n = 27)	C-ADM (n = 15)						
Myositis-specific									
Anti-ARS (anti-Jo-1)	10 (16)	6 (22)	0	0	0	0	0	0	0
Anti-ARS (non–anti-Jo-1)	10 (16)	2 (7)	0	0	0	1 (4)	0	0	4 (9)
Anti-SRP	5 (8)	0	0	0	0	0	0	0	0
Anti–Mi-2	0	2 (7)	0	0	0	0	0	0	0
Myositis-associated									
Anti-SSA/Ro	3 (5)	3 (11)	2 (14)	8 (16)	15 (33)	6 (22)	1 (5)	5 (71)	1 (2)
Anti–U1 RNP	2 (3)	2 (7)	0	1 (2)	18 (39)	23 (85)	2 (9)	0	0
Anti–CADM-140	0	0	8 (53)	0	0	0	0	0	0

* Values are the number (%) of patients. Anti-PM/Scl and other myositis-associated autoantibodies were not detected in any of the sera tested. PM = polymyositis; DM = dermatomyositis; C-ADM = clinically amyopathic dermatomyositis; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; MCTD/OL = mixed connective tissue disease/overlap syndrome; IPF = idiopathic pulmonary fibrosis; anti-ARS = anti–aminoacyl-transfer RNA synthetase; anti-SRP = anti–signal recognition particle.

Disease specificity of the anti–CADM-140 antibodies. The frequencies of myositis-specific antibodies, myositis-associated antibodies, and anti–CADM-140 antibodies are summarized in Table 1. Myositis-specific antibodies are found in most patients with myositis, whereas myositis-associated antibodies are frequently found in patients without myositis (12). Among the patients with CTDs or IPF, myositis-specific antibodies (33 with anti-ARS, 5 with anti-SRP, 2 with anti–Mi-2) and myositis-associated antibodies (44 with anti-SSA/Ro, 48 with anti–U1 RNP, none with anti-PM/Scl or other myositis-associated antibodies) were detected. Anti–CADM-140 autoantibodies were found in 19% of sera from patients with DM (especially in 53% with the C-ADM subtype), but were not detected in patients with other CTDs or IPF.

Clinical features of C-ADM patients with anti–CADM-140. Clinical findings were compared between DM patients (including those with C-ADM) with anti–CADM-140 autoantibodies and those without anti–CADM-140 autoantibodies (Table 2). There were no significant differences in the frequencies of skin symptoms. However, the frequency of rapidly progressive ILD was significantly increased in anti–CADM-140–positive patients compared with that in anti–CADM-140–negative patients (50% versus 6%; $P = 0.008$). No myositis-specific antibodies were found in patients with anti–CADM-140; nevertheless, there was no significant

Table 2. Comparison of clinical features in anti–CADM-140–positive versus anti–CADM-140–negative patients with dermatomyositis

Feature	Anti–CADM-140–positive (n = 8)	Anti–CADM-140–negative (n = 34)	P
Age at onset, mean ± SD years	44.5 ± 12.7	46.5 ± 15.7	NS
No. male/no. female	2/6	8/26	NS
Gottron's sign or papules	75	88	NS
Heliotrope rash	50	53	NS
Muscle weakness	0	76	0.02
Elevation of CK	25	74	0.03
Fever	25	50	NS
Raynaud's phenomenon	13	24	NS
Arthritis	50	71	NS
Rapidly progressive ILD	50	6	0.008
Malignancy	0	18	NS
MSAs	0	29	NS
MAAs	13	18	NS

* Except where indicated otherwise, values are the percent of patients. NS = not significant; CK = creatine kinase; ILD = interstitial lung disease; MSAs = myositis-specific autoantibodies; MAAs = myositis-associated autoantibodies.

difference in the frequency of these autoantibodies in comparison with the anti–CADM-140–negative group.

None of the 8 patients with anti–CADM-140–positive sera were treated with steroids or other immunosuppressive medications prior to being assessed for C-ADM. All of these patients had Gottron's sign or papules, or periorbital heliotrope erythema and skin biopsy specimens yielding results compatible with DM. None of these patients had muscle weakness. CK levels were in the normal range in 6 patients (75%) and slightly elevated in the remaining 2 patients. Of the 6 patients assessed for the muscle enzyme aldolase, levels were normal in 2 patients. Of the 2 patients who underwent muscle MRI, neither showed findings compatible with a diagnosis of myopathy. Four patients had a muscle biopsy, and 2 of the muscle specimens exhibited mild infiltration of inflammatory cells, but there was no evidence of necrosis of muscle fibers, variation in fiber size, regeneration, or phagocytosis. Of the 7 patients with ILD (88%), 4 developed rapidly progressive disease.

DISCUSSION

We have identified novel autoantibodies (anti–CADM-140 autoantibodies) to an ~140-kd polypeptide in patients with DM. Anti–CADM-140 antibodies were detected specifically in patients with DM, especially those with C-ADM. In addition, anti–CADM-140 antibodies were associated with rapidly progressive ILD.

It has been reported that amyopathic DM may be accompanied by rapidly progressive ILD, especially in Japanese patients and other Asian patients (7). In contrast, rapidly progressive ILD was shown to be rare in patients with amyopathic DM in a North American population (13). In our series, 5 of 15 patients with C-ADM (33%) (4 of whom had anti–CADM-140 antibodies) had rapidly progressive ILD during their clinical course. Rapidly progressive ILD was more frequent in our series compared with that reported previously in North American populations (13). Although the number of patients that we studied was very limited, it remains possible that racial differences are the reason for this discrepancy, because other clinical studies of Japanese patients also demonstrated findings similar to ours (7).

Furthermore, in a recent preliminary report, using immunoprecipitation and immunoblotting of HeLa cell extracts, Targoff et al documented the presence of antibodies to a 155-kd protein and/or Se protein in patients with C-ADM (8). Thirteen of 18 C-ADM sera possessed an anti–155-kd polypeptide antibody, and 6 also immunoprecipitated a 95-kd polypeptide (anti-Se antibody). In contrast, Oddis et al identified the anti-MJ antibody, which was also found to recognize a 140-kd polypeptide, in patients with juvenile DM (14,15). We have been able to conclude that anti–CADM-140 is distinctively different from anti-MJ, because the molecular weights of the immunoprecipitated polypeptides are different. Moreover, the clinical features of anti-MJ are quite different from those associated with anti–CADM-140. Anti-MJ is detected mainly in juvenile DM, has been observed in the US and Argentina, and is clinically characterized by severe DM with a chronic and polycyclic course, sometimes accompanied by vasculitis (14). In order to elucidate the racial differences in the frequency of these antibodies, the examination of a larger number of patients from several different populations is required.

Our results have thus demonstrated the presence of anti–CADM-140 autoantibodies in patients with C-ADM, and these were found to be associated with rapidly progressive ILD. Further studies of this novel autoantibody specificity may provide insight into the pathogenic mechanisms of C-ADM accompanied by rapidly progressive ILD.

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Elevated Levels of Soluble Fractalkine in Active Systemic Lupus Erythematosus

Potential Involvement in Neuropsychiatric Manifestations

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Objective. **To determine levels of the soluble form of the chemokine fractalkine (sFkn) and its receptor, CX_3CR1, in patients with systemic lupus erythematosus (SLE) with neuropsychiatric involvement (NPSLE) and in SLE patients without neuropsychiatric involvement, and to assess their relationship with disease activity and organ damage.**

Methods. **Levels of sFkn in serum and cerebrospinal fluid (CSF) were measured by enzyme-linked immunosorbent assay. Expression of Fkn and CX_3CR1 was quantified using real-time polymerase chain reaction. Surface expression of CX_3CR1 on peripheral blood mononuclear cells (PBMCs) was determined by flow cytometry. Disease activity and organ damage were assessed using the SLE Disease Activity Index (SLEDAI) and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index, respectively.**

Results. **Serum sFkn levels were significantly higher in patients with SLE than in patients with rheumatoid arthritis (RA) or healthy controls. In addition, significant correlations between serum sFkn levels and the SLEDAI, the SLICC/ACR Damage Index, anti–double-stranded DNA and anti-Sm antibody titers, immune complex levels (C1q), and serum complement levels (CH50) were observed. Expression of CX_3CR1 was significantly greater in PBMCs from patients with active SLE than in those from RA patients or healthy controls. Levels of sFkn were also significantly higher in CSF from untreated patients with newly diagnosed NPSLE than in SLE patients without neuropsychiatric involvement; treatment reduced both serum and CSF levels of sFkn in patients with SLE.**

Conclusion. **Soluble Fkn and CX_3CR1 may play key roles in the pathogenesis of SLE, including the neuropsychiatric involvement. Soluble Fkn is also a serologic marker of disease activity and organ damage in patients with SLE, and its measurement in CSF may be useful for the diagnosis of NPSLE and followup of patients with NPSLE.**

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multiorgan damage with infiltration and sequestration of various leukocyte subpopulations, and by the presence of autoantibodies (1). Its etiology is known to involve dysregulation of the immune system, leading to a functional imbalance of T cell subsets, production of a wide range of autoantibodies, and polyclonal B cell activation. In addition, the importance of dysregulation of cytokine expression has been noted (2).

A variety of diffuse and focal neuropsychiatric symptoms often occur in patients with SLE. The features of this condition may include seizures, stroke, depression, psychosis, and cognitive disorders (3). Although the pathogenesis of neuropsychiatric SLE (NPSLE) has not been completely elucidated, a variety of clinical,

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laboratory, and radiographic findings are reportedly abnormal in some, but not all, SLE patients with central nervous system (CNS) complications, and the direct and indirect effects of several inflammatory mediators have been emphasized as possible contributors (4).

The chemokine fractalkine (Fkn; CX_3CL1) is synthesized as a type 1 transmembrane protein by endothelial cells (5). The soluble form of Fkn (sFkn) reportedly exerts a chemotactic effect on monocytes, natural killer (NK) cells, and T lymphocytes and acts via its receptor, CX_3CR1, as an adhesion molecule that is able to promote the firm adhesion of a subset of leukocytes to endothelial cells under conditions of physiologic flow (6). Notably, prominent expression of both Fkn and CX_3CR1 has been observed in the CNS (7). Thus, Fkn appears to possess immunoregulatory properties that affect inflammatory/immune cell–endothelial cell interactions and inflammatory responses.

The aim of the present study was to determine serum and CSF levels of sFkn and CX_3CR1 in SLE patients (those with and those without neuropsychiatric involvement) and to assess the relationship of these levels with disease activity and organ damage.

PATIENTS AND METHODS

Patients and samples of serum and CSF. A total of 67 serum samples were obtained from 53 patients with SLE (50 women and 3 men; mean ± SEM age 35.8 ± 1.8 years). In 14 patients, serum samples were collected during both the active and inactive phases of disease. All patients previously or currently fulfilled the American College of Rheumatology (ACR) revised criteria for the classification of SLE (8). Serum samples were also obtained from 91 patients with rheumatoid arthritis (RA) (71 women and 20 men; mean ± SEM age 65.3 ± 1.3 years) who fulfilled the 1987 revised ACR (formerly, the American Rheumatism Association) criteria for a diagnosis of RA (9), and from 28 healthy volunteers (16 women and 12 men; mean ± SEM age 34.4 ± 2.7 years). CSF from the lumbar spine was collected for the purpose of diagnosing NPSLE. For ethical reasons, CSF samples were not collected from SLE patients without any neuropsychiatric involvement or from healthy volunteers.

The SLE Disease Activity Index (SLEDAI) (10) was used to estimate general disease activity, and the Systemic Lupus International Collaborating Clinics (SLICC)/ACR Damage Index (11) was used to estimate organ damage.

Because of the difficulty in confirming neurologic diagnoses and of assigning cause to SLE, we defined NPSLE as the presence of at least 1 clinical feature of neuropsychiatric syndromes (3) and at least 1 of the following: pathologic findings on brain magnetic resonance imaging, diffusely abnormal results of brain single-photon–emission computerized tomography, severely abnormal results on a neuropsychiatric test, an elevated CSF IgG index, or increased interleukin-6 (IL-6) activity in the CSF (12).

Serum levels of specific autoantibodies, complement hemolysis activity (CH50), and immune complex (C1q) as well as albumin and IgG levels in both serum and CSF were determined in the clinical laboratory at our hospital. All human experiments were carried out in accordance with protocols approved by the Human Subjects Research Committee at our institution, and informed consent was obtained from all patients and volunteers.

Soluble Fkn levels. Soluble Fkn was quantified using a double ligand enzyme-linked immunosorbent assay (ELISA) that was a modification of an assay described previously (13). Monoclonal murine anti-human Fkn (4 μg/ml; Genzyme/Techne, Cambridge, MA) and biotinylated polyclonal goat anti-Fkn (0.25 μg/ml; Genzyme/Techne) served as the primary and the secondary antibodies, respectively. This ELISA detects the chemokine domain of human Fkn, and the sensitivity limit is ~150 pg/ml.

Flow cytometry. Flow cytometric analyses of CX_3CR1 expression on peripheral blood mononuclear cells (PBMCs) were carried out as previously described (14). PBMCs were obtained from heparinized venous blood from patients with SLE, patients with RA, and healthy volunteers and then labeled with the indicated primary antibody (anti-CD3–fluorescein isothiocyanate [FITC], anti-CD4–phycoerythrin [PE], anti-CD8–PE, and anti-CD14 [monocyte]–FITC; BD PharMingen, San Diego, CA), or rabbit anti-CX_3CR1 antibody (ProSci, Poway, CA), and then with a secondary antibody (biotin-conjugated anti-rabbit IgG) and a tertiary reagent (CyChrome-conjugated streptavidin; BD PharMingen). The fluorescence intensity was measured on a 3-color FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

Isolation of total RNA, and real-time polymerase chain reaction (PCR). Total RNA extracted from PBMCs was reverse transcribed, and then real-time PCR was carried out in a LightCycler (Roche Diagnostics, Mannheim, Germany). To compare quantitative results between different samples, a dilution series of complementary DNA from unstimulated human umbilical vein endothelial cells and normal human PBMCs, which served as internal standards for Fkn and CX_3CR1, respectively, were loaded every time and assigned a value of 100 units. The primers used in the real-time PCR were as follows: for human CX_3CR1, 5′-AGCAGGCATGGAAGTGTTCT (sense) and 5′-GTTGTTTTGTGTGCATTGGG (antisense); for human Fkn, 5′-GCTGAGGAACCCATCCAT (sense) and 5′-GAGGCTCTGGTAGGTGAACA (antisense); for β-actin, which served as an internal control, 5′-CCCAAGGCCAACCGCGAGAAGAT (sense) and 5′-GTCCCGGCCAGCCAGGTCCAG (antisense).

Statistical analysis. Data are expressed as the mean ± SEM. Differences between groups were analyzed using the Mann-Whitney U test. Followup data were analyzed using Wilcoxon's test. The relationship between sFkn levels and the indicated parameters was evaluated using Spearman's rank correlation. P values less than 0.05 were considered significant.

RESULTS

Serum sFkn levels. We initially used ELISAs to assay the levels of sFkn in serum samples obtained from SLE patients with and those without neuropsychiatric

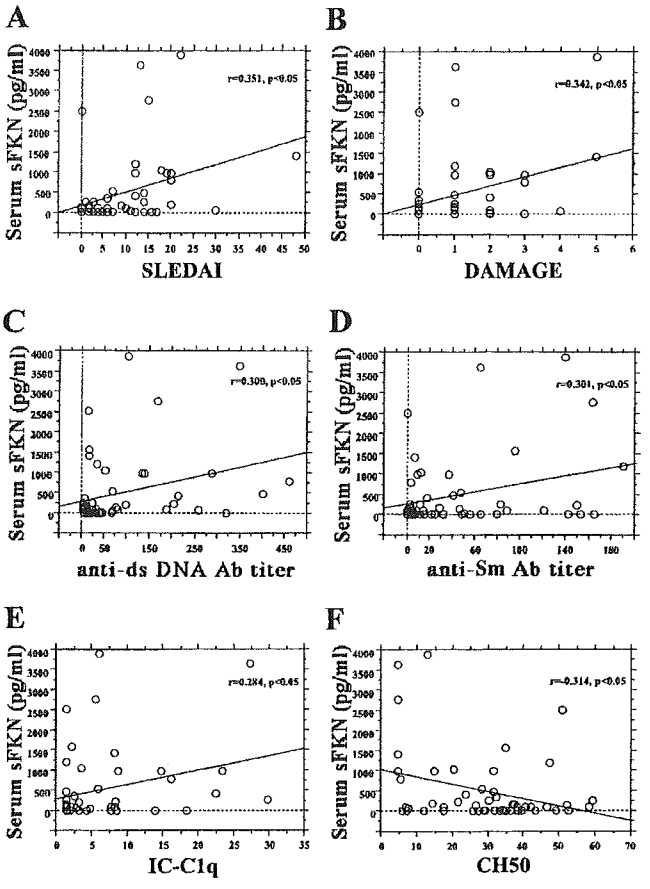

Figure 1. Correlation between serum levels of soluble fractalkine (sFkn) and various clinical parameters. The correlation between serum levels of sFkn (n = 67 samples) and the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (**A**), organ damage (Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index) (**B**), serum anti–double-stranded DNA (anti-dsDNA) antibody (Ab) titers (**C**), serum anti-Sm antibody titers (**D**), immune complex (IC-C1q) levels (**E**), and serum complement hemolysis activity (CH50) (**F**) in patients with SLE was examined. Serum levels of sFkn were assessed by enzyme-linked immunosorbent assay. Each point represents an individual SLE patient.

involvement (n = 67 samples), patients with RA (n = 91), and healthy controls (n = 28). Serum levels of sFkn were significantly higher in patients with SLE (mean ± SEM 452.7 ± 118.0 pg/ml) than in either patients with RA (mean ± SEM 225.2 ± 53.2 pg/ml; $P < 0.05$) or healthy controls (mean ± SEM 3.2 ± 3.2 pg/ml; $P < 0.01$). We then examined the relationship between serum levels of sFkn and disease activity, organ damage, and the indicated serologic parameters (Figure 1). We observed that serum levels of sFkn were correlated with both disease activity as measured by the SLEDAI (r = 0.351, $P < 0.05$) (Figure 1A) and organ damage as measured by the SLICC/ACR Damage Index (r = 0.342, $P < 0.05$) (Figure 1B) and were also positively correlated with anti–double-stranded DNA (anti-dsDNA) antibody titers (r = 0.300, $P < 0.05$), anti-Sm antibody titers (r = 0.301, $P < 0.05$), and immune complex C1q levels (r = 0.284, $P < 0.05$) (Figures 1C–E) and were negatively correlated with CH50 (r = −0.314, $P < 0.05$) (Figure 1F).

Expression of Fkn and CX_3CR1 messenger RNA (mRNA) and cell-surface expression of CX_3CR1. To better understand the dysregulation of Fkn/CX_3CR1 expression that occurs in SLE, we examined their expression profiles. CX_3CR1 mRNA was more strongly expressed in PBMCs from SLE patients than in those from patients with RA or healthy controls (Figure 2A). In contrast, Fkn expression in PBMCs from all 3 groups was markedly weak, and no significant difference between the groups was observed (results not shown). To examine in more detail the phenotype of cells expressing CX_3CR1, we used flow cytometry to analyze the protein expression of CX_3CR1 in peripheral blood–specific cell populations from SLE patients with active or inactive disease, patients with RA, and healthy controls (Figure 2B). Although both the intensity of CX_3CR1 expression on macrophages (results not shown) and the relative number of affected cells were slightly higher in patients with active SLE than in patients with inactive SLE or healthy controls, the expression of CX_3CR1 protein was most pronounced on CD4+,CD3+ T cells and CD8+,CD3+ T cells from a patient with untreated active SLE.

Neuropsychiatric manifestations and CSF levels of sFkn. Because Fkn has been detected in the nervous system (7), we hypothesized that it may also be involved in the pathogenesis of NPSLE. To test this hypothesis, we first assayed the sFkn levels in CSF from untreated patients with newly diagnosed active SLE, with or without neuropsychiatric involvement. As shown in Figure 3, levels of sFkn in CSF samples from all but 1 SLE patient without neuropsychiatric involvement (non-NPSLE) were relatively low (n = 6, mean ± SEM 186.3 ± 177.1 pg/ml) compared with those in patients with NPSLE (n = 6, mean ± SEM 842.7 ± 190.0 pg/ml). Notably, in contrast with the results observed in CSF, no significant difference in serum sFkn levels was observed between untreated patients with newly diagnosed NPSLE (n = 6, mean ± SEM 467.4 ± 24.0 pg/ml) and SLE patients without overt neuropsychiatric involvement (n = 6, mean ± SEM 400.3 ± 182.0 pg/ml). In addition, there were no significant differences in any serologic para-

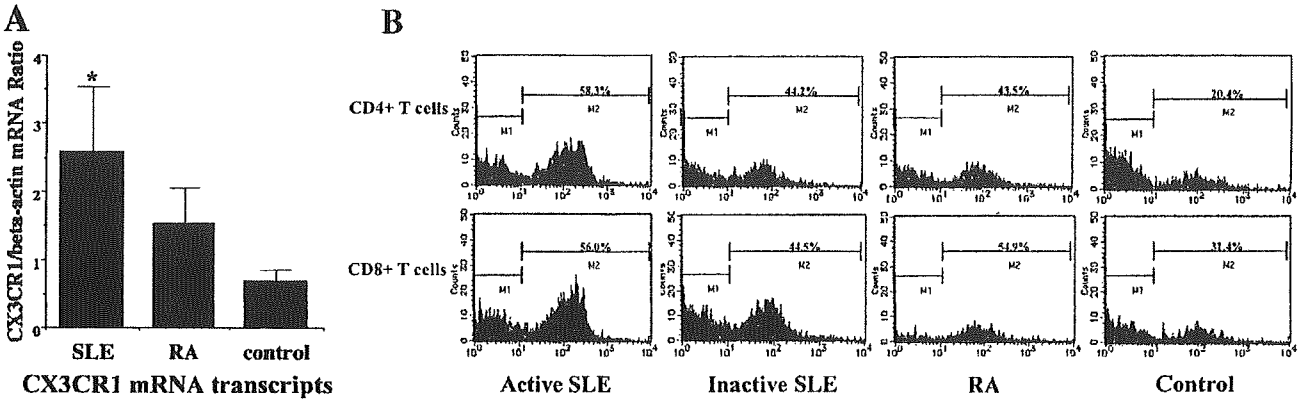

Figure 2. CX_3CR1 expression in peripheral blood mononuclear cells (PBMCs). **A,** Total RNA was isolated from PBMCs obtained from 21 patients with systemic lupus erythematosus (SLE), 30 patients with rheumatoid arthritis (RA), and 10 healthy controls, after which the cDNA was reverse transcribed, and real-time polymerase chain reaction was carried out. Levels of CX_3CR1 mRNA are expressed as the mean and SEM units. * = $P <$ 0.05 versus RA and control. **B,** PBMCs obtained from untreated patients with newly diagnosed SLE (active), treated patients with inactive SLE, patients with RA, and healthy controls were labeled with anti-CD3+, anti-CD4+, anti-CD8+, or anti-CX_3CR1 antibody. CX_3CR1 expression on gated cells (CD4+,CD3+ T cells; CD8+,CD3+ T cells) was assayed by 3-color flow cytometry. Samples obtained from patients with SLE were followed up. M1 = background intensity of isotype-matched control staining. M2 = percent of CX_3CR1-positive cells. Histograms are representative of 3 independent experiments.

meters between patients with NPSLE and SLE patients without neuropsychiatric involvement. Moreover, the IL-6 concentration was shown to be elevated in the CSF of some patients with NPSLE (12), but we found no significant correlation between CSF levels of sFkn and IL-6 activity in the CSF (P = 0.32).

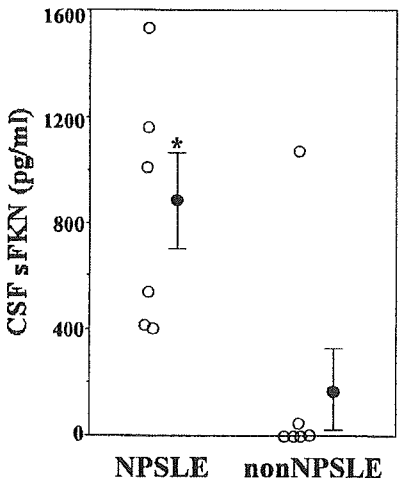

Figure 3. Levels of soluble fractalkine (sFkn) in cerebrospinal fluid (CSF). Samples of CSF were obtained from 6 untreated patients with newly diagnosed neuropsychiatric systemic lupus erythematosus (NPSLE) and 6 SLE patients without neuropsychiatric involvement (non-NPSLE; of these 6 patients who did not fulfill our criteria for NPSLE, 4 described having mild headache, and 2 had mild mood disorder). Soluble Fkn levels were determined by enzyme-linked immunosorbent assay. Each point represents an individual patient. Bars show the mean ± SEM. * = $P < 0.05$ versus non-NPSLE.

Because of the small number of samples examined, we were unable to determine the statistical significance of differences in CSF sFkn levels among patients with any particular neuropsychiatric manifestation. However, when neuropsychiatric manifestations were classified as either diffuse CNS disease (n = 2), which included psychosis, mood disorder, cognitive dysfunction, and acute states of confusion, or as focal CNS disease (n = 4), which included cerebrovascular disease, demyelinating syndrome, headache, aseptic meningitis, seizures, or myelopathy (3), sFkn levels tended to be higher in CSF from patients with focal disease (mean ± SEM 1,029.0 ± 234.1 pg/ml versus 470.0 ± 69.0 pg/ml in patients with diffuse disease).

Followup studies of the effect of treatment on CSF and serum sFkn levels. Figure 4 summarizes the results of followup studies of serum levels of sFkn in 14 patients with SLE (with or without neuropsychiatric manifestations) before and 2–3 months after treatment with glucocorticoids and other immunosuppressive drugs (12 patients received glucocorticoids alone, and 2 patients received glucocorticoids plus cyclophosphamide or cyclosporin A). Notably, serum sFkn levels in patients with active SLE were significantly diminished following successful treatment and clinical improvement (mean 559.4 pg/ml in patients with active disease versus 102.1 pg/ml in patients inactive disease). Although the mean reduction in the CSF

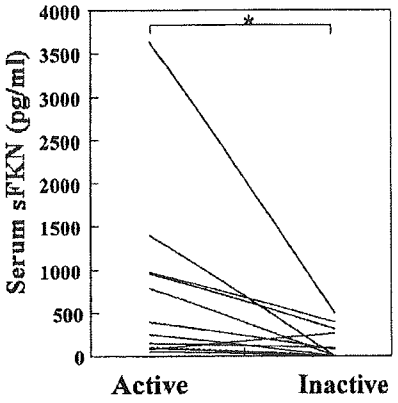

Figure 4. Followup measurements of soluble fractalkine (sFkn) levels in serum from patients with systemic lupus erythematosus (SLE), with or without neuropsychiatric involvement. Paired samples of serum were obtained from 14 patients with SLE (with or without neuropsychiatric involvement) at the time of active disease (newly diagnosed, untreated) and after treatment (inactive disease). Each line represents an individual patient. * = $P < 0.05$.

of 4 patients with NPSLE was quite pronounced (from 877.3 pg/ml to 155.3 pg/ml), it did not reach statistical significance.

DISCUSSION

In the present study, we showed that serum sFkn levels were significantly higher in patients with SLE than in patients with RA or healthy controls and were positively correlated with disease activity, organ damage, anti-dsDNA and anti-Sm antibody titers, and immune complex levels and were negatively correlated with CH50 activity. In addition to the increased expression of sFkn itself, increased expression of its receptor, CX_3CR1, was also detected, especially on CD4+ and CD8+ T cells from patients with active SLE. Finally, levels of sFkn in the CSF were elevated in patients with NPSLE, and both serum and CSF levels of sFkn were reduced by successful treatment with glucocorticoids and other immunosuppressive drugs.

This study is the first to demonstrate increases in sFkn levels in the peripheral blood and CNS of patients with active SLE and patients with NPSLE, respectively. Recent evidence indicates that receptor expression determines the spectrum of action of chemokines in Th1 and Th2 cells. Indeed, Fraticelli et al recently reported that CX_3CR1 was preferentially expressed in Th1 cells, and that Th1 cells, but not Th2 cells, respond to Fkn (15). Furthermore, Fkn also acts via CX_3CR1 as an adhesion molecule and as a chemoattractant, recruiting monocytes, NK cells, and T lymphocytes to endothelial cells. Thus, Fkn likely plays multiple roles in the development of SLE, via Th1 cell–endothelial cell interactions.

Intracranial increases in a variety of cytokines, including IL-6, have been observed in patients with NPSLE (12). This suggests that these various proinflammatory and antiinflammatory cytokines all play specific roles during the progression of NPSLE. In the present study, however, we observed no significant correlation between the levels of sFkn and IL-6 in the CSF of patients with NPSLE, which may indicate that the expression of Fkn and IL-6 is differentially regulated by these 2 mediators during the evolution of the neuropsychiatric manifestations in patients with SLE. Furthermore, we observed that patients with focal neuropsychiatric manifestations had higher CSF levels of sFkn than did those with diffuse disease. These findings are not consistent with the results reported by Erichsen et al (16), who found that sFkn levels in the CSF of human immunodeficiency virus type 1 (HIV-1)–infected patients with cognitive impairment (diffuse disease) were significantly higher than those in HIV-1–infected patients without cognitive impairment. It would be interesting to know whether this difference reflects a difference in the underlying mechanism of the pathogenesis of NPSLE and HIV-induced encephalopathy, and the extent to which Fkn participates in those processes.

In healthy individuals, surface expression of CX_3CR1 has been demonstrated in NK cells, monocytes, and effector T cells (17). CX_3CR1 is also expressed on CD4+ and CD8+ T cells in patients with RA (18). Consistent with those findings, we observed increased expression of CX_3CR1 mainly on CD4+ and CD8+ T cells in patients with active SLE. Moreover, T cell expression of CX_3CR1 was significantly reduced by treatment that diminished disease activity. Although there have been few studies of the expression and regulation of CX_3CR1 under pathologic conditions, it is noteworthy that CX_3CR1 expression on immune cells parallels the sFkn levels, suggesting that CX_3CR1 mediates activation of recruited inflammatory cells, especially CD4+ and CD8+ T cells, during active SLE.

In conclusion, sFkn and CX_3CR1 may play important roles in the pathogenesis of SLE, including the neuropsychiatric involvement. Soluble Fkn is also a serologic marker of disease activity and organ damage in patients with SLE, and its measurement in CSF may be useful for the diagnosis of NPSLE and the followup of patients with NPSLE.

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