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4. 急性期川崎病への抗サイトカイン療法（抗 TNF α製剤 Infliximab）

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key words　Kawasaki disease, coronary arterial aneurysm, TNF α, Infliximab (Remicade)

動　向

本来生物学的製剤とは，生体が持つ活性分子を標的として作成されたものであり，抗tumor necrosis factor（腫瘍壊死因子，TNF）α製剤はこれらを阻害する目的で遺伝子工学的手法を用いて作製されたものである．現在，我が国では①炎症性腸疾患（Crohn病），②リウマチ性関節炎（RA），③Behçet病の難治性網膜ぶどう膜炎，④乾癬，⑤強直性脊椎炎，⑥潰瘍性大腸炎で承認されている．

その中でInfliximab（Remicade）は，TNF αが病態の進展や重症度に深く関与しているリウマチ性疾患でその有用性が試みられ，欧米でも，小児期の若年性特発性関節炎（JIA），炎症性腸疾患で有用性が報告されており[1]，FDAでは2006年に小児Crohn病が6歳以上で承認されている．

A. 生物学的製剤

現在，我が国で承認されている抗TNF α作用を示す生物学的製剤は，Infliximab（レミケード，静注），Etanercept（エンブレル，皮下注），Adalimumab（ヒュミラ，皮下注）である．

1. Infliximabの構造

InfliximabはヒトTNF αをマウスに免疫して得られた，マウス由来特異的抗体であるV領域の部分（25%）と，ヒトIgG$_1$ κ鎖のC領域（75%）を結合して作られた“キメラ型モノクローナル抗体”である．1分子あたりマウスのタンパク質を約25%含むため，抗キメラ抗体（中和抗体HACA）が概ね40%で出現し，効果の減弱やアレルギー反応が生じることがある．そのため，RAではmethotrexate（MTX）と併用することで中和抗体の産生が抑制される．Infliximabは流血中のTNF αに結合して流血中からの除去を，またTNF産生細胞表面上のTNF αに結合して細胞破壊をきたす．投与経路は経静脈的であり，安定期の投与量は3～10mg/kg/8週間毎である．血中半減期は9.5日で，抗原性（ヒト抗キメラ抗体出現の可能性）がある．TNF βには結合せず，効果を有さない特徴がある．

2. 作用機序

Infliximabは，体内で特異的にTNF αと結合する．その様式は，①soluble TNF αの中和，②受容体結合TNF αの解離と，③TNF α産生細胞上の膜型TNF αとの結合の作用がある．

まず①では，TNF αとTNFレセプター（p55とp75）との結合を阻害する．更に②では受容体に結合したTNF αを解離させる作用に加え，③

ではTNFα産生細胞表面に発現されている膜型TNFαと結合し，補体とのCDCや抗体とのADCCを介してapoptosisを誘導し産生細胞を阻害する．TNFα産生細胞の障害により，細胞の活性化，IL-1，IL-6などのサイトカイン産生などを抑制し炎症を沈静化する．

3．使用方法

成人RAでは，3mg/kgを200～500mlの生理食塩水に混ぜ，2時間以上かけて点滴静注する．RAその他では，2回目をその2週間後，3回目をその4週間後，4回目以後は8週毎に投与する．急性期の川崎病では，急性疾患であること，またMTXやステロイドを併用しないことなどからも，1回の使用が適切であろう．小児では5mg/kgを1回投与量として使用する．小児Crohn病では3mg/kgまたは6mg/Kgが使用されている．

B．川崎病と抗TNFα製剤

急性期の川崎病では，様々な炎症性サイトカイン，ケモカインや接着因子の活性化が見られ，特にTNFαは，疾患自体の重症度と冠動脈瘤の発症頻度に有意な相関があり，その使用が検討されてきた．

2004年Weissらは，IVIG不応，methylprednisolone（IMP）不応の3歳児において45病日にInfliximabを用いてその有効性を報告した[2]．その後，2005年Burns ら[3]により，IVIG不応，IMP不応の川崎病17例へのInfliximabのpilot studyの成績が集計された．その結果，有効性・安全性とも短期的には満足できる結果であるが，副作用と長期予後を観察する必要があると結論された．その後も使用に関する報告が散見される[4-13]．著者らも血清中IL-6がInfliximab投与により低下すると報告した[4]．さらに，InfliximabはCRP，IL-6，可溶性TNFα受容体1などの炎症性マーカーは抑制するが，血管炎のマーカーであるVEGF，S100A12，MRP8/MRP14，DAMP Moleculeなどは難治例では低下させないことも報告されている[14]．

最近の英国からの報告によれば，2009年までに39例が報告され，年齢は1カ月から13歳で，実に22例を冠動脈瘤を合併している．その多くはIVIG不応，IMP不応例であった．使用量は5mg/kg，1回が中心であるが，2～3回の投与例や，6mg/kg，10mg/kgの投与例もある[10]

通常，繰返し投与はinfusion reactionのriskが高いのでMTX使用例以外は避けられるべきと考えられている．

全米の主要な27病院における2001年から2006年の治療報告では，IVIG不応に対して，Infliximabは4,811症例中1%で使用されており，2001年の0%から2006年の2.3%へ増加してきている[15]．

最近のreviewにおいても，IVIG不応例には，IVIG追加投与か3日間のIMPまたはInfliximab投与を，との推奨がある[16]．

実験的な川崎病類似血管炎においても，抗TNFα製剤Etanerceptの効果が示されていた[17]．

表1にこれまでに発表された使用報告例をまとめた[2-13]．

C．我が国での使用状況

我が国では，IVIG不応の難治例に対してoff-label治療薬として初めて使用されてから約4年が経過した[4]．日本川崎病学会が2006～2009年の間に計4回の使用実態全国調査を行ってきた．難治性でIVIG抵抗性の重症川崎病にたいする調査の結果，2009年度までには計97症例で使用されていた．そのうち概ね80%近くでは解熱などの有効性があるが，10%前後では解熱せず，追加のIVIG等が使用されていた．大きな副作用

表1　川崎病におけるInfliximab 使用例のReview[12)]

報告者 (年)	症例数 男/女	年　齢	用量（mg/kg/回） 投与日	冠動脈瘤（An）	その他
Weiss et al[2)] 2004	1 (1/0)	13歳	5mg×3回 45病日	両側巨大An	AN増悪なし その後2週目，6週目に再投与
Burns et al[3)] 2005	16 (10/6)	0.12～13.1歳	5mg×1（n＝15） 10mg×1（n＝1） 8～53病日	11/16例	13/16例で解熱 1例（An）53日後死亡
Saji et al[4)] 2006	1 (1/0)	13歳	5mg×1 21病日	巨大An	解熱
Stenbog et al[5)] 2006	2 (2/0)	11週，33週	6mg×3 38病日，69病日	なし	解熱，An退縮 その後，2週目，6週目に再投与
O'Connor et al[6)] 2007	1 (1/0)	17週	5mg×2回 8,11病日	両側巨大An	2回目投与で解熱
Oishi et al[7)] 2008	1 (1/0)	11カ月	5mg×1 27病日	両側An	解熱，一過性膨疹
Girish et al[8)] 2008	1 (1/0)	14歳	5mg×1 19病日	両側An	解熱
Burns et al[9)] 2008	12 (8/4) 不明4	0.7～3.1歳	5mg×1 7.5病日（6～15）	5/16例	1回IVIG不応例11/12例で解熱， 2回のIVIG不応例2/4例で解熱
Brogan et al[10)] 2009	1 (1/0)	18歳	6mg×1 34病日	両側An	解熱
佐地ら[11)] (使用実態調査) 2009	40 (22/18)	5例;＜1y, 14例;1-4y, 17例;＞4y 4例; 不明	5mg/kg 8～29病日	11/40例	再発熱: 6例（15%）， 10日未満の使用でAn; 1/16例， 11日以後の使用でAn10/20例
Song et al[12)] 2010	16	3例;＜1y, 8例; 1-4y, 5例; ＞4y 0.2～15歳	5～6.6mg・kg 1例は16と40病日 6～40病日	9/16例， 一過性拡大3例	1例で肝炎，4カ月後有石胆嚢炎
Shirley et al[13)] 2010	1 (1/0)	13歳	5mg/kg 11病日以後	なし	Methylpredonisoloneとの初回併用 (IVIG禁忌の症例)

は経験されておらず，比較的安全に使用されていた．使用時期が10日以前であれば，冠動脈瘤を形成する頻度が極めて低いとする結果がまとめられている[11)]．

最近のFDA database によれば，120,000例以上の副作用報告の中で，妊娠中に抗TNF α製剤を投与された女性から出産した小児においては，先天性異常特にVACTERLの異常が41人の小児で報告されていた[18)]．

その他の疾患への応用として，特に血管炎症候群では，巨細胞性血管炎，高安動脈炎[19)]，ANCA関連血管炎，Churg-Strauss症候群，皮膚型血管炎，Wegner肉芽腫でも有用性が報告されている．最近では，Stevens-Johnson症候群への治療薬としても報告されている[20)]．

D. 副作用

抗TNF α製剤の5,000例に及ぶ我が国の成人RAにおける市販後調査では，投与後6カ月まで

の副作用発現率は28%，重篤な副作用6.2%で，細菌性肺炎2.2%（108例），ニューモシスチス肺炎0.4%（22例），敗血症0.2%（10例），結核0.3%（14例），重篤な投与時反応（infusion reaction）は0.5%（24例）であった．JIAではかえって3mg/kgの方が6mg/kgよりも副作用が多かったと報告されている[21]．しかし，生物学的製剤の使用における小児の安全性の研究成果は報告が少なく，症例ごとの適応とRisk/Benefitの判断が優先されている．

1．心不全増悪

InfliximabもEtanerceptも，心不全を増悪することが知られている．NYHA III～IVの心不全で収縮率の低下している状態では増悪する．従って急性期川崎病では，潜在的な心筋炎，心機能低下，心嚢液貯留，房室弁逆流等が存在するため，BNPの高い病初期は使用を控えるべきであろう[22]．

2．悪性腫瘍

汎用されているJIAにおいては，Etanerceptを使用した1,200例中5例で悪性腫瘍が報告されている．その内訳はHodgkinやNon-Hodgkin リンパ腫，甲状腺癌，卵黄嚢癌，子宮頸部dysplasia，である．しかし，全例他の免疫抑制剤が併用されており，うち2例はAdalibmab，Infliximab も併用されていた．しかし原因として否定できず，使用に関しては充分な説明と同意が必要であると述べている[23]．またFDAの副作用報告システムの集計によれば，抗TNFα製剤の使用後に小児で48例の悪性腫瘍の報告があり11例が死亡している．半数は悪性リンパ腫である．原因薬剤の検討では，31例はInfliximab投与後であり15例がEtanercept後，2例がAdalibmab後である．全体の88%までは免疫抑制剤（azathioprine，MTX）を併用されていた．一般頻度よりInfliximab使用時の悪性腫瘍の発症頻度は高いので，基礎疾患を考慮し注意を要する[24]．

3．投与時反応（infusion reaction）

Infliximabは特にキメラ型抗体のため，アナフィラキシー反応が起こり易い．点滴開始後，発熱，発疹，掻痒感，頭痛等に注意し，頻回にバイタルサインを確認する．投与中ないし投与後2時間以内は，重篤なアナフィラキシー様症状である呼吸困難，気管支痙攣，血圧上昇，血管浮腫，チアノーゼ，低酸素，発熱，蕁麻疹等に注意する[25]．

予防のためのacetoaminophen やcetirizine（抗ヒスタミン薬）の前投与は，infusion reactionの出現（8.3%）を予防できなかったとの報告がある[22]．長期投与の結果では，JIA 163例（Infliximab 68例，Etanercept 95例：平均年齢17歳）の6年間の観察において，平均22.9カ月の使用期間中71回の副作用があり，その62.9%はInfliximab使用例であり，26例では使用を中止している．しかし一方では，Infliximabは安全で耐忍性が高く副作用も少ないとの報告もある[26]．JIAでは，1年間の投与後に3mg/kg投与群：3.3%，6mg/kg投与群：7%でみられている[27]．またその多くは中和抗体HACAが出現していた．小児期Crohn病でも7.1～12.1%で認められている[28]．

また遅発性過敏症として，投与後3日以上（または24時間以後～3週間以内）経過後に遅発性過敏症（筋肉痛，発疹，発熱，多関節痛，掻痒，手・顔面の浮腫，嚥下障害，蕁麻疹，咽頭痛，頭痛等）が出現することが報告されている[29]．最近の抗TNFα製剤の小児期の使用に対する注意勧告を表2に示す．

4．感染症増悪

特に，BCGを接種していない可能性のある，乳幼児には要注意である．結核特異抗原によるリ

表2　抗TNF α製剤の小児領域での副作用と禁忌[1,21-29]

重篤な副作用	使用禁忌
・投与部位の過剰反応	絶対禁忌
・Infusion reaction	・活動性感染症
・水痘感染	・反復性感染症と慢性感染症の既往
・潜伏期にある感染症（Tbcなど）	・既感染・未治療Tbc
・神経脱髄疾患（MSなど）	・多発性硬化症，視神経炎
・精神神経系副作用（疲労感，頭痛，めまい，抑うつ，不安，疼痛増幅症候群 pain amplification syndrome）	・Anakinra（抗IL-1受容体拮抗薬）の併用
	・活動性か最近（過去10年）の悪性腫瘍（皮膚腫瘍以外）
・悪性腫瘍	比較的禁忌
・免疫原性	・妊娠，授乳
	・HIV, HBV, HCV 感染症

ンパ球刺激試験であるクォンティフェロン（QFT-TBゴールド）は，BCG接種，抗酸菌感染の影響を受けないが，過去の感染の影響を受ける．小児期には偽陰性を示すことが多いが，意味のない検査ではない．注意深い問診，家族内感染の有無，BCG接種の有無，胸部X線，胸部CT，等が重要となる．また生ワクチン接種後は3～6カ月は猶予が必要であろう．

むすび

急性期川崎病では，Infliximabは単回投与であり再投与はなく，活動性感染症の合併は比較的少ないため重篤な合併症の頻度は更に低いと思われる．しかし，好発年齢が1歳前後の感染症好初時期であり，BCG接種の直後や未接種の症例，また生ワクチンの接種時期でもあるため慎重な適応決定と長期の監察が必要である．また冠動脈瘤発生の頻度，程度，予後に与える影響は未確認である．巨大冠動脈瘤に伴うその後の虚血性心疾患のriskと，Infliximab使用に伴う副作用の可能性を熟慮し，利益が危険性を上回った時に初めて使用を決断するべきであろう．

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PEDIATRIC RHEUMATOLOGY

RESEARCH Open Access

Clinical characteristics of aseptic meningitis induced by intravenous immunoglobulin in patients with Kawasaki disease

Yasushi Kemmotsu*, Tomotaka Nakayama, Hiroyuki Matsuura and Tsutomu Saji

Abstract

Background: Aseptic meningitis is a serious adverse reaction to intravenous immunoglobulin (IVIG) therapy. We studied the clinical characteristics of patients with acute Kawasaki disease (KD) who developed IVIG-induced aseptic meningitis.

Methods: A retrospective analysis of the medical records of patients with KD who developed aseptic meningitis after IVIG treatment was performed.

Results: During the 10-year period from 2000 through 2009, among a total of 384 patients with Kawasaki disease, 4 (3 females and 1 male; age range, 19-120 months) developed aseptic meningitis after IVIG. All 4 developed aseptic meningitis within 48 hours (range, 25-40 hours) of initiation of IVIG. The analyses of cerebrospinal fluid (CSF) revealed elevated white blood cell counts (22-1,248/μL) in all 4 patients; a predominance of polynuclear cells (65%-89%) was noted in 3. The CSF protein level was elevated in only 1 patient (59 mg/dL), and the glucose levels were normal in all 4 patients. Two patients were treated with intravenous methylprednisolone; the other 2 children were observed carefully without any special therapy. All patients recovered without neurological complications.

Conclusions: In our patients with Kawasaki disease, aseptic meningitis induced by IVIG occurred within 48 hours after initiation of IVIG, resolved within a few days, and resulted in no neurological complications, even in patients who did not receive medical treatment.

Keywords: Kawasaki disease, intravenous immunoglobulin, aseptic meningitis

Background

Intravenous immunoglobulin (IVIG) is a blood product that is widely used in the treatment of a number of medical conditions, including immunodeficiency disorders, inflammatory diseases, and autoimmune diseases.

Kawasaki disease (KD) is a self-limited systemic vasculitis syndrome of childhood that was first reported by Tomisaku Kawasaki in 1967 [1]. Patients typically develop a fever, bulbar conjunctival injection, changes in the oropharyngeal mucosa and peripheral extremities, cervical lymphadenopathy, and a polymorphous rash. Coronary aneurysm and myocardial infarction are the most serious complications of this disease. In Japan, there are approximately 10,000 incident cases per year [2]. The etiology of the disease is not well understood, but high-dose IVIG is known to prevent the coronary complications [3,4].

There have been a number of reports regarding IVIG-induced adverse reactions, including mild reactions such as tachycardia, headache, facial flushing, nausea, diarrhea, and rash, as well as serious adverse reactions such as anaphylaxis, acute renal failure, and thromboembolic events [5]. Aseptic meningitis is a neurologic adverse event that can be caused by IVIG. Although there have been case reports describing IVIG-induced aseptic meningitis, few studies have described the characteristics of a group of such patients. In this study, we describe the clinical and laboratory characteristics of IVIG-induced aseptic meningitis in 4 patients with KD.

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Patients and methods

Patients

To investigate the clinical characteristics of IVIG-induced meningitis in KD patients, we retrospectively reviewed the medical records of patients who were admitted to our university hospital during the 10-year period from 2000 through 2009. All patients met the Japanese criteria for typical KD on admission. They were treated with oral aspirin and 1 or 2 g/kg of IVIG, the latter of which was administered over 12 or 24 hours, respectively. The IVIG products were freeze-dried sulfonated (Kenketsu Venilon®-I, Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) and freeze-dried, polyethylene glycol (PEG) -treated (Kenketsu Glovenin®-I, Nihon Pharmaceutical Co, Ltd, Tokyo, Japan) human normal immunoglobulin. Testing of the CSF was done soon after the diagnosis of suspected IVIG-induced meningitis, and a diagnosis of meningitis was made on the basis of clinical symptoms such as fever and headache, meningeal irritation signs, and CSF pleocytosis. A final diagnosis of aseptic meningitis was made by negative bacterial culture results.

Results

Characteristics of the study population and IVIG products

A total of 384 patients with KD were admitted to our hospital; 4 developed aseptic meningitis after IVIG. Table 1 shows the background characteristics of these 4 patients. Three were females older than 5 years. The other patient was a 1-year-old male. Their serum C-reactive protein (CRP) levels and white blood cell counts before IVIG treatment were 3.3-5.5 mg/dL and 6,500-27,100/μL, respectively. Sulfonated immunoglobulin was given to 2 patients, and a polyethylene glycol-treated product was given to the other 2 patients. Two patients were treated with 1 g/kg IVIG, and the other 2 received 2 g/kg IVIG. There were no adverse reactions during the IVIG administration in any of the patients.

Clinical course and laboratory findings

All 4 patients responded well to initial IVIG: their fevers ceased and the clinical symptoms of KD improved. Table 2 shows the clinical course of the patients. Aseptic meningitis developed within 48 hours (range, 25-40 hours) after initiation of IVIG. All 4 patients developed a sudden, severe fever. Their recorded highest body temperatures were 38.0, 38.7, 38.8, and 39.1°C. The 3 females complained of headache, and the 1-year-old male was irritable and vomited frequently. On physical examination, there were typical signs of meningeal irritation, including neck rigidity, Kernig's sign, and Brudzinski's sign. Table 3 shows the CSF findings of the 4 patients. The initial pressure was recorded in 1 patient and was mildly elevated (24 cm H_2O). The analyses of the CSF revealed elevated white blood cell counts (22-1,248/μL) in all 4 patients, 3 of whom were neutrophil-predominant (65%-89%). The CSF protein level was elevated in only 1 patient (59 mg/dL), and the glucose levels were normal in all 4 patients (51-77 mg/dL). The CSF chloride and lactate dehydrogenase (LDH) levels were measured in 3 patients and were normal (123-128 mEq/L and 33-40 U/L, respectively). In addition, the results of CSF bacterial culture were negative in all patients. There was no worsening of inflammatory markers, ie, serum CRP and peripheral white blood cell counts, at the onset of meningitis (mean ± SD CRP: 4.3 ± 4.1 mg/dL, WBC: 9,300 ± 7,700/μL), as compared with the levels at admission (mean ± SD CRP: 5.9 ± 2.0 mg/dL, WBC: 14,800 ± 9,000/μL). Two patients were treated with a single dose of 15 mg/kg of intravenous methylprednisolone; the other 2 patients recovered without medical treatment. Fever and signs of meningeal irritation disappeared in 1 or 2 days, and no patient developed any neurological complications such as seizures or disturbances in consciousness. There was no recurrence of KD in any of the patients, and all four patients were discharged without coronary artery aneurysms.

Discussion

Aseptic meningitis after IVIG was first reported in 1988 [6]. Since then, there have been similar case reports of IVIG-induced meningitis in patients with medical conditions such as idiopathic thrombocytopenic purpura

Table 1 Background characteristics of the patients

Age	Sex	KD criteria	CRP(mg/dL)/WBC(/μL) on admission	IVIG product and dose	Day on IVIG
1 y	male	5/6	5.5/6,500	PEG-treated 2 g/kg	8
6 y	female	5/6	7.1/15,600	Sulfonated 1 g/kg	5
7 y	female	6/6	7.8/27,100	Sulfonated 2 g/kg	5
10 y	female	6/6	3.3/9,900	PEG-treated 1 g/kg	4

KD = Kawasaki disease; CRP = C-reactive protein; WBC = white blood cell; IVIG = intravenous immunoglobulin; PEG = polyethylene glycol.

Table 2 The clinical course of the patients

Patient	Time from start of IVIG to onset, hrs	Treatment	Time to recovery
1 y male	33	15 mg/kg mPSL	1 day
6 y female	40	15 mg/kg mPSL	2 days
7 y female	25	None	2 days
10 y female	31	None	1 day

IVIG = intravenous immunoglobulin; mPSL = methylprednisolone.

(ITP), myasthenia gravis, and inflammatory demyelinating neuropathy [7-9]. There has previously been only 1 case report describing this complication in a patient with KD [10].

The rate of aseptic meningitis after IVIG was 1% (4 of 384) in this study, but the frequency varies widely, from 0% to 11%, in reports of patients with different underlying diseases [11,12]. It was also reported that the development of aseptic meningitis was not correlated with the patient age or the type of underlying neuromuscular disease [12].

Hamrock reported that most patients who developed aseptic meningitis received 2 g/kg of IVIG, and that meningitis did not occur in any of their patients receiving a standard replacement dose of IVIG for a congenital immunodeficiency [5]. All of our patients received high-dose IVIG at a dose of 1 or 2 g/kg. Our patients almost equally received sulfonated IVIG or PEG-treated IVIG, and 2 patients in each group (total 4) developed meningitis, thus indicating that there are no apparent differences in the effects of sulfaonated or PEG-treated IVIG with regard to the development of meningitis. In this study, patients were exposed to either sulfonated IVIG or PEG-treated IVIG, but not to products manufactured by other processes such as cold ethanol Cohn fractionation/ultrafiltration, ion exchange, or low-PH treatment. The inability to further explore the possible etiological factors related to specific IVIG brand or manufacturing lots may be a limitation of this study. There were no obvious differences of clinical and laboratory data, including the severity of KD on admission, day of initiating IVIG, or changes of inflammatory markers after IVIG between patients who developed meningitis and those who did not.

In the present study, aseptic meningitis developed within 25 to 40 hours after initiation of IVIG. In previous case reports, most patients also developed meningitis within 48 hours of beginning IVIG. Although all of our patients developed a fever and typical meningeal irritation signs, it may be possible that milder cases of aseptic meningitis could be misdiagnosed as IVIG-refractory KD, since the onset of fever after completion of IVIG therapy is often interpreted as recrudescence of KD. It is important to consider the possibility of IVIG-induced meningitis with careful physical examinations to avoid unnecessary therapies, such as additional IVIG, steroids, and infliximab.

CSF examinations revealed neutrophilic pleocytosis in 3 of our 4 patients, slight elevation of the protein level in 1 patient, and normal glucose levels in all 4 patients. These findings were similar to those of previous reports. The analysis of the CSF in patients with aseptic meningitis usually shows pleocytosis with neutrophil predominance, normal or slightly elevated protein, and normal glucose levels. It may therefore be difficult to differentiate IVIG-induced meningitis from viral meningitis by the CSF findings, as it has been reported that the CSF protein levels are normal to mildly elevated, glucose levels are normal to slightly depressed, and neutrophil predominance is also seen in pediatric patients with viral meningitis [13,14].

All of our patients recovered without developing any neurological complications. Two were treated with intravenous methylprednisolone, and the other 2 were monitored without medical treatment. Jayabose et al. reported that children with ITP who were given prednisone had a lower risk of neurological complications after IVIG [15]. However, it has also been reported that such symptoms are self-limiting, and that there is no specific therapy that shortens the duration of symptoms. Thus, it may be advisable to carefully observe such patients and avoid systemic therapy [5]. In our study, there were no obvious differences in the clinical courses between patients treated with intravenous methylprednisolone and those who received no medical treatment,

Table 3 Cerebrospinal fluid findings

Patient	Cells (/μL)	Glucose (mg/dL)	Protein (mg/dL)	LDH (U/L)
1 y male	1,248 (P 89%)	51	59	39
6 y female	120 (P 13%)	54	23	33
7 y female	648 (P 83%)	77	30	40
10 y female	21 (P 65%)	52	37	NT

LDH = lactate dehydrogenase; P = polynuclear cells; NT = Not tested.

which suggests that systemic steroid administration is not beneficial for IVIG-induced meningitis.

The mechanisms underlying IVIG-induced meningitis are not clear. One possible cause is an allergic hypersensitivity reaction caused by direct entry of the IVIG preparation into the CSF compartment. This is supported by the fact that CSF eosinophilia has been observed in some patients [11]. In our study, one patient exhibited peripheral eosinophilia (11% of the total 5,800/μL white blood cells) but CSF eosinophilia was not observed in any of our patients. None of our patients developed exanthema after IVIG. Although our patients received no pre-treatment, it may be useful to give antihistamines prior to IVIG if allergic reaction is one of the mechanisms responsible for IVIG-induced meningitis. Recently, it was reported that there were increased levels of CSF monocyte chemoattractant protein-1 (MCP-1) in ITP patients with IVIG-induced meningitis, which suggests a role for monocytes in the inflammation of the meninges [16]. On the other hand, Jarius et al. reported that aseptic meningitis was frequently associated with neutrophillic pleocytosis in the CSF and *in vivo* activation of TNF-α-primed neutrophils by atypical antineutrophil cytoplasmic antibodies in IVIG might contribute to aseptic meningitis [17]. In our present study, the CSF cytokines or chemokines were not measured.

Meningitis is also a known complication of KD. Dengler et al reported that one-third of patients with KD who underwent a lumbar puncture had CSF pleocytosis with mononuclear cell predominance [18], which is in contrast to the polynuclear cell predominance observed in IVIG-induced meningitis. Meningitis as a complication of KD usually occurs early in the course of the disease and improves after KD treatment, which is mainly IVIG therapy [19]. Table 4 shows a comparison between IVIG- and KD-induced meningitis. It is not difficult to differentiate IVIG-induced meningitis from aseptic meningitis complicating KD, as both the time of onset and CSF findings differ.

Conclusions

In conclusion, IVIG-induced meningitis developed within 48 hours of initiating IVIG and resolved in a few days, without neurological complications, and systemic steroid administration was not beneficial in our patients. Further investigations of the pathophysiology of IVIG-induced meningitis, including a detailed analysis of the underlying mechanisms, are needed.

Table 4 A comparison between IVIG- and KD-induced meningitis

	Meningitis due to IVIG	Meningitis due to KD
Appearance	Within 48 hrs after IVIG	Early in the stage, before IVIG
Clinical findings	Typical meningeal signs	Can lack meningeal signs
CSF findings	Polynuclear cell predominance	Mononuclear cell predominance
Effective therapy	No special therapy	Therapy for KD

IVIG = intravenous immunoglobulin; KD = Kawasaki disease; CSF = Cerebrospinal fluid.

Authors' contributions
YK contributed by taking care of the patients. All authors contributed to the analysis and interpretation of the data. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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CLINICAL REPORT

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Severe Pulmonary Emphysema in a Girl With Interstitial Deletion of 2q24.2q24.3 Including *ITGB6*

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Owing to the large size of chromosome 2, partial monosomy of the long arm of this chromosome gives rise to many specific phenotypes. We report on a 2-month-old girl with an interstitial deletion of 2q24.2q24.3, which was confirmed by microarray-based comparative genomic hybridization analysis. The patient showed delayed growth and mental retardation, early myoclonic seizures, and characteristic dysmorphic features including thick arched eyebrows, upslanting palpebral fissures, long eyelashes, depressed nasal bridge, short nose, long philtrum, small mouth, micrognathia, and low set ears. Her early myoclonic seizures were likely due to haploinsufficiency of *SCN1A* and *SCN2A*, which are included in the deletion region. When she experienced acute bronchopneumonia, she showed severe pulmonary emphysema. The deletion region of 2q24.2 includes the integrin β6 gene (*ITGB6*), which may prevent acute lung injury and pulmonary emphysema. Many previously reported patients with deletions of 2q24.2 showed poor outcomes because of respiratory failure. These observations suggest the possibility of a strong relationship between haploinsufficiency of *ITGB6* and pulmonary dysfunction.

Key words: chromosome 2; 2q24.2; integrin β6 (*ITGB6*); myoclonic seizure; pulmonary emphysema

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INTRODUCTION

Recent advances in molecular analyses, including multiplex ligation-dependent probe amplification and microarray-based comparative genomic hybridization (aCGH), have allowed the precise mapping of the boundaries of chromosomal aberrations in patients [Stankiewicz and Lupski, 2006]. Owing to the large size of chromosome 2, many regions may contribute to the specific phenotypes associated with partial monosomy of the long arm of this chromosome, that is, the terminal deletion of 2q37 and the interstitial deletions of 2q31, 2q24, and 2q14. Patients with a terminal deletion of 2q37 show developmental delay, severe behavioral disturbance, growth/pubertal retardation, and dysmorphic facial features [Conrad et al., 1995; Falk and Casas, 2007; Kitsiou-Tzeli et al., 2007]. The 2q31.2q32.3 deletion syndrome is an established syndrome with growth and developmental retardation, microcephaly, and distinctive craniofacial anomalies [Mencarelli et al., 2007; Prontera et al., 2009]. The locus for split-hand/foot malformation with long-bone deficiency is suspected to be located in the 2q14.2 region [Babbs et al., 2007; David et al., 2009]. It is well known that there is a cluster of ion channel genes in the 2q24 region. Among them, *SCN1A* on 2q24.3, which encodes voltage-gated sodium channel 1 (alpha subunit), is a critical gene responsible for severe myoclonic epilepsy in infancy (SMEI) [Wallace et al., 2003], and there are many reports of cryptic chromosomal deletions involving *SCN1A* [Pereira et al., 2004; Madia et al., 2006; Pereira et al., 2006; Suls et al., 2006; Davidsson et al., 2008; Wang et al., 2008]. However, only a few patients have been reported to have a deletion of 2q24.2 [Fryns et al., 1977; McConnell et al., 1980; Shabtai et al., 1982; Moller et al., 1984; Bernar et al., 1985; Takahashi et al., 1985; Wamsler et al., 1991; Woods et al., 1993; Boles et al., 1995; Chinen et al., 1996; Nixon et al., 1997; McMilin et al., 1998; Slavotinek et al., 1999; Maas et al., 2000; Pereira et al., 2004; Langer et al., 2006; Madia et al., 2006; Suls et al., 2006; Pescucci et al., 2007; Davidsson et al., 2008; Grosso et al., 2008; Wang et al., 2008].

Recently, we encountered a child with a 2q24.2q24.3 deletion who showed congenital anomalies, early myoclonic seizures, and

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severe pulmonary emphysema caused by viral infection. We report on the patient and discuss the genotype–phenotype correlation.

CLINICAL REPORT

A female infant was born spontaneously at 40 weeks and 4 days of gestation following an uncomplicated pregnancy. She was proportionally small for this gestation time, with birth weight of 2,230 g (<10 centile), length of 47 cm (<25th centile), and head circumference of 32 cm (<25th centile). Her family history was unremarkable. The patient was the second child of healthy parents (both at 24 years old), and her 7-year-old brother was in good health.

When she was hospitalized in our institution for acute bronchitis at her age of 2 months, she displayed retarded growth with weight of 3,835 g (<3 centile) and length of 53.6 cm (<10 centile), as well as continuous myoclonic jerks, generalized hypotonia and dysmorphic findings including thick arched eyebrows, upslanting palpebral fissures, long eyelashes, flat nasal bridge, short nose, long philtrum, small mouth, micrognathia, and low-set ears (Fig. 1A,B). Conventional chromosome analysis identified interstitial deletion of chromosome 2q with the karyotype of 46,XX,del(2)(q24q31). Echocardiography showed no signs of cardiac anomalies or pulmonary hypertension. Blood screening tests for inborn errors of metabolism, and neurophysiological examinations did not reveal any abnormalities.

At her age of 3 months, she had a number of episodes of afebrile generalized tonic-clonic seizures. Although sodium valproate decreased the frequency of seizure attacks, continuous myoclonic jerks were intractable.

At the age of 5 months, she was hospitalized for respiratory syncytial virus infection, which was confirmed by PCR-based analysis. She showed pyrexia and respiratory distress due to continuous coughing and was intubated for hypoxia and hypercapnia. She was transferred to an intensive care unit, where she was supported with invasive mechanical ventilation for acute hypoxic respiratory failure because of bronchopneumonia. Chest radiography revealed diffuse haziness of both lungs (Fig. 2A). On the 7th and 26th days after hospitalization, computed tomography scans of the chest revealed severe pulmonary emphysema, bullae, and pulmonary collapse (Fig. 2B,C). Pulmonary emphysema improved with pressure-limited ventilation. On the 80th day after hospitalization, she was extubated, and a repeat chest X-ray revealed normal lung expansion (data not shown).

METHODS

To investigate the precise chromosomal deletion region of the proposita's chromosome, we performed aCGH analysis using Human Genome CGH Microarray 44A (Agilent Technologies, Palo Alto, CA) with genomic template DNA extracted from peripheral blood according to the manufacturer's instruction. Chromosomal aberrations were visualized by CGH Analytics version 4.40 (Agilent Technologies). Metaphase or prometaphase chromosomes were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes for two-color fluorescence in situ hybridization (FISH) analysis using bacterial artificial chromosome (BAC) clones as probes as described [Shimojima et al., 2009a]. BAC clones were selected from an in silico library

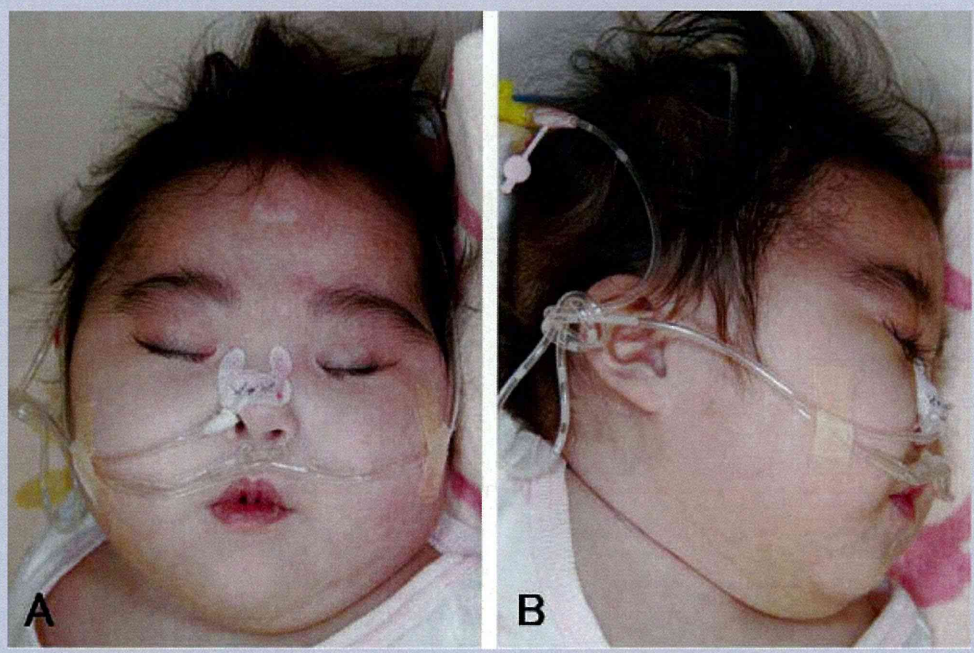

FIG. 1. Facial features of the proposita at 7 months of age. A: Frontal view of the proposita showing thick arched eyebrows, upslanting palpebral fissures, depressed nasal bridge, short nose, long philtrum, small mouth, and micrognathia. B: Side view of the proposita showing long eyelashes, low set ears, and micrognathia. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

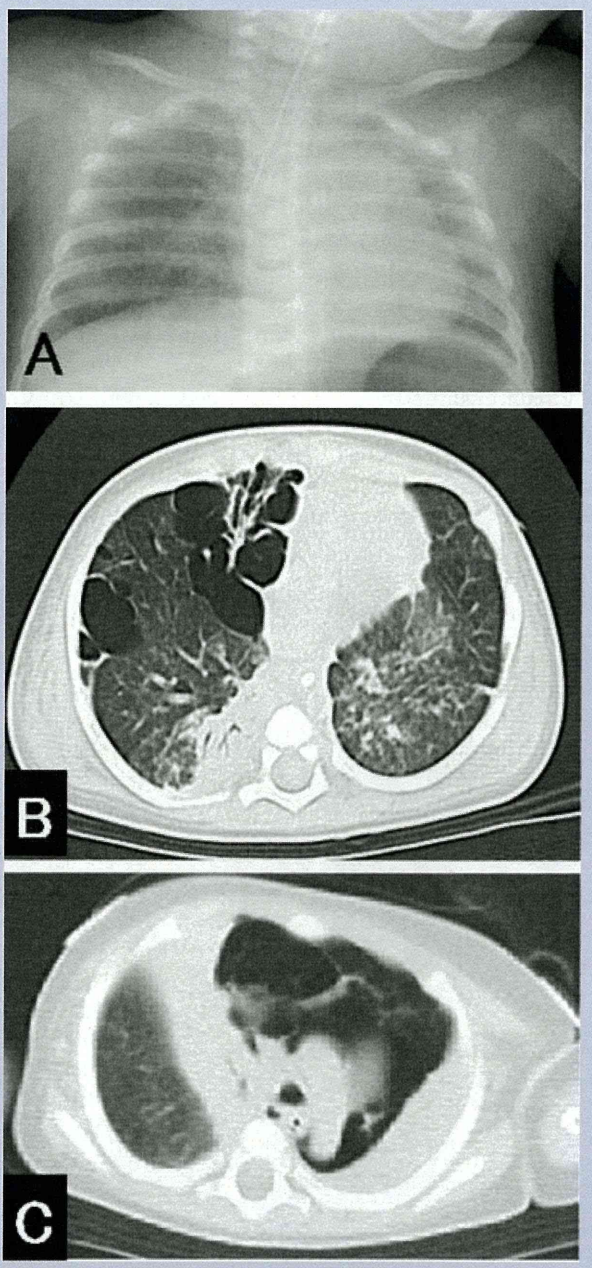

FIG. 2. Radiological examination of the proposita. A: Chest X-ray taken upon hospital admission at 5 months of age showing bilateral infiltration. B,C: Computed tomography scans of the chest showing extremely large pulmonary bullae at the 7th and 26th days after hospitalization, respectively.

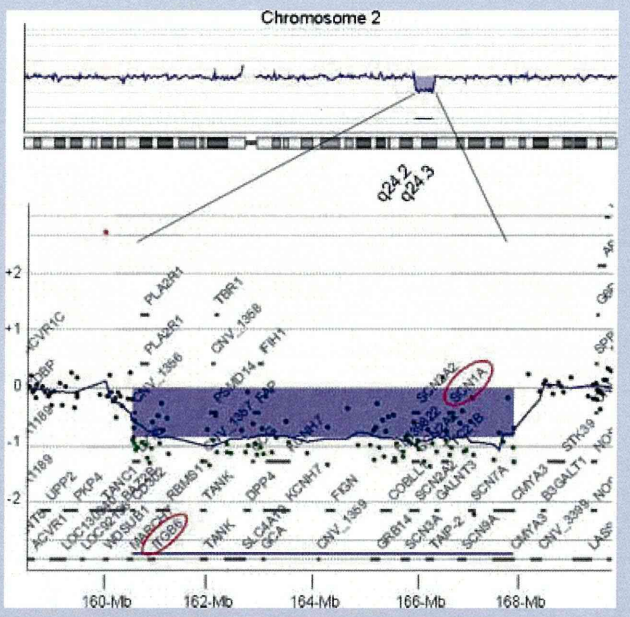

FIG. 3. Results of aCGH analysis. Chromosome view (top) indicates a deletion of 2q24.2q24.3 determined by the CGH analytics by applying the Aberration Detection Method 2 algorithm with a threshold of 6.0. The range of the aberration was expanded on gene view (bottom). The blue-shaded area and the blue line in each view indicate the copy number aberration region and the moving average of the $\log_2$ ratio of intensity on the y axis, respectively. Red circles emphasize the locations of *ITGB6* and *SCN1A*. Dots indicate the positions of the probes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(UCSC Human Genome Browser, March 2006) (Table I). Microsatellite marker D2S2330 was analyzed using the ABI Prism Linkage Mapping Set and GeneMapper (Applied Biosystems, Foster City, CA) [Shimojima et al., 2009b].

RESULTS AND DISCUSSION

A 7.5-Mb deletion of 2q24.2q24.3 was identified in this patient by aCGH analysis (Fig. 3). Two-color FISH analysis confirmed the deletion in the patient (Fig. 4), and parental chromosome analysis showed normal chromosomes indicating de novo occurrence of the deletion in the patient (data not shown). Microsatellite marker analysis revealed that the patient shared a common allele only with her mother, which indicated that the paternally derived allele was

TABLE I. Summary of the FISH Analyses

Clone name	Chromosome band	Position[a] Nucleotide Start	Position[a] End	Result	Included genes
RP11-664N22	2p25.3	44,074	230,847	Not deleted	*SH3YL1*
RP11-690N19	2q24.2	160,587,760	160,779,118	Deleted	*ITGB6*
RP11-763J13	2q24.2	160,629,947	160,830,554	Deleted	*ITGB6*
RP11-214A4	2q24.2	162,846,383	162,941,799	Deleted	*IFIH1, GCA, KCNH7*

[a]Chromosome locations and nucleotide positions are from UCSC coordinates, March 2006 assembly.

TABLE II. The Result of the Microsatellite Marker Analysis With D2S2330

Father	Patient	Mother
171/171	—/173	167/173

The numbers indicate bp length.

deleted in the patient (Table II). The final karyotype was 46,XX,del(2)(q24.2q24.3).arr 2q24.2q24.3(160,332,614-167, 824,700) × 1 dn. The chromosomal deletion region of this patient is common to those reported by Langer et al. [2006] and Maas et al. [2000], and her dysmorphic facial features were similar to those seen in the previous reports, in particular to that by Maas et al. [2000] (Table III).

According to the UCSC genome browser, at least 34 genes are included in the deletion region of this patient, in which there is a cluster of sodium channel genes, including *SCN3A*, *SCN2A*, *SCN1A*, *SCN9A*, and *SCN7A*. *SCN9A* was reported as the responsible gene for congenital inability to experience pain, but which was transmitted as autosomal recessive traits [Cox et al., 2006]. *SCN7A* is suspected to have some functions in central nervous system, but there is no report of mutation in human diseases [Watanabe et al., 2000]. Although only one missense mutation of *SCN3A* has been identified in a patient with cryptic pediatric partial epilepsy, we do not know any clinical effect of *SCN3A* haploinsufficiency [Holland et al., 2008]. *SCN1A* and *SCN2A* are responsible for SMEI [Wallace et al., 2003] and benign

TABLE III. Previously Reported Patients With Chromosomal Deletions Around 2q24.2

References	Subject no.	Deletion region	Outcome	Locus of *ITGB6* (2q24.2)
Fryns et al. [1977]		2q21q24	Died at 2 m by recurrent bronchopneumonias	Ambiguous
McConnell et al. [1980]		2q22q31	Died at 15 min	Included
Shabtai et al. [1982]		2q23q31	Died at 20 y by respiratory infection	Included
Moller et al. [1984]		2q24.2q31	NA	Ambiguous
Takahashi et al. [1985]	Familial	2q23.3q24.2	NA	Ambiguous
Bernar et al. [1985]		2q24.2q24.3	Died at 1 y 4 m by respiratory infection	Ambiguous
Wamsler et al. [1991]		2q24q31	NA	Ambiguous
Woods et al. [1993]		2q22q24.2	NA	Ambiguous
Boles et al. [1995]		2q24.2q31.1	NA	Ambiguous
Chinen et al. [1996]		2q24.2q31	Died at 2 m by heart failure	Ambiguous
Nixon et al. [1997]		2q24.3q32.1	NA	Excluded
McMilin et al. [1998]	Patient 1	2q22.3q23.3	NA	Excluded
	Patient 2	2q23.3q24.2	NA	Ambiguous
	Patient 3	2q23.3q24.2	NA	Ambiguous
	Patient 4	2q24.2q31	NA	Ambiguous
Slavotinek et al. [1999]	First child	2q31.1q31.3	NA	Excluded
	Second patient	2q24.3q31.3	NA	Excluded
Maas et al. [2000]		2q23q24.3	Died at 2 and 1/4 years of age by RSV infection	Included
Pereira et al. [2004]		2q24.3q24.3	NA	Excluded
Langer et al. [2006]		2q24.1q31.1	Died at 3 y 2 m by apnea	Included
Madia et al. [2006]	Three patients	2q24.3q31.1	NA	Excluded
Suls et al. [2006]	EP514	2q24.3q24.3	NA	Excluded
	EP530	2q24.3q24.3	NA	Excluded
	EPD64	2q24.3q31.1	NA	Excluded
Pescucci et al. [2007]		2q24.3q31.1	NA	Excluded
Grosso et al. [2008]	Patient 11	2q23q31	NA	Included
	Patient 12	2q24.3q31.1	NA	Excluded
	Patient 13	2q37qter	NA	Excluded
Davidsson et al. [2008]		2q24.3q31.1	NA	Excluded
Wang et al. [2008]	Seven patients	2q24.3q24.3	NA	Excluded
The present patient		2q24.2q24.3	Severe pulmonary emphysema at 2 m	Included

"Ambiguous" means that we could not determine whether the reported patients have deletion of *ITGB6* or not. Gray columns emphasize the patients who had died and the patients whose deletions includes *ITGB6* region.
m, months; y, years; RSV, respiratory syncytial virus.
NA, not available.

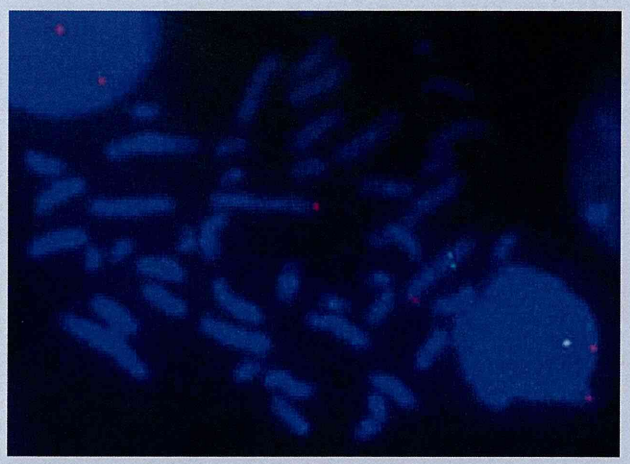

FIG. 4. FISH analysis verified the deletion of 2q24.2. RP11-664N22 on 2p25.3 was labeled by SpectrumRed and two copies of chromosome 2 were identified. A single signal for RP11-690N19 on 2q24.2 covering *ITGB6* was labeled by SpectrumGreen (arrowhead), which indicates the deletion of this region on the unlabeled chromosome 2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

familial neonatal–infantile convulsions [Berkovic et al., 2004], respectively, and both of which are segregated as autosomal dominant traits. Especially, most *SCN1A* hemizygous mutations were loss of function mutations derived from nonsense or frameshift mutations [Wallace et al., 2003]. Therefore, haploinsufficiency of both *SCN1A* and *SCN2A* is likely to be responsible for the intractable seizures in this patient [Pereira et al., 2004].

The integrin β6 gene (*ITGB6*) is included in the proximal deletion region of 2q24.2. Integrins are heterodimeric transmembrane receptors that were identified based on their ability to bind and mediate cell adhesion to distinct components of the extracellular matrix during processes that include cell survival, proliferation, and migration [Fernandez-Ruiz and Sanchez-Madrid, 1994]. The epithelial-restricted integrin αvβ6 binds to and activates the latent transforming growth factor-β, and *ITGB6* null mice show a marked induction of macrophage metalloelastase 12, which has been implicated in the chronic lung disease emphysema [Morris et al., 2003]. Thus, haploinsufficiency of *ITGB6* might be related to extremely severe pulmonary emphysema during acute bronchopneumonia in our patient.

Table III summarizes data obtained from previously reported patients with chromosomal deletions around the 2q24.2 locus. Among them, seven patients were reported to have died, of whom four died due to respiratory infections. Intriguingly, four patients had confirmed chromosomal deletions including the region of *ITGB6*, and the other three possibly had the same deletions which are ambiguous because of the conventional karyotyping. The present patient had a chromosomal deletion including *ITGB6* with severe pulmonary involvement. Therefore, we suggest that there is a relationship between haploinsufficiency of *ITGB6* and pulmonary involvement. Ongoing care of this patient and others, who have chromosomal deletions of 2q24.2, should focus on preventing severe pulmonary disease, especially when patients incur respiratory infections.

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Successful Treatment with Infliximab for Inflammatory Colitis in a Patient with X-linked Anhidrotic Ectodermal Dysplasia with Immunodeficiency

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Abstract X-linked anhidrotic ectodermal dysplasia with immunodeficiency (X-EDA-ID) is caused by hypomorphic mutations in the gene encoding nuclear factor-κB essential modulator protein (NEMO). Patients are susceptibile to diverse pathogens due to insufficient cytokine and frequently show severe chronic colitis. An 11-year-old boy with X-EDA-ID was hospitalized with autoimmune symptoms and severe chronic colitis which had been refractory to immunosuppressive drugs. Since tumor necrosis factor (TNF) α is responsible for the pathogenesis of NEMO colitis according to intestinal NEMO and additional TNFR1 knockout mice studies, and high levels of TNFα-producing mononuclear cells were detected in the patient due to the unexpected gene reversion mosaicism of NEMO, an anti-TNFα monoclonal antibody was administered to ameliorate his abdominal symptoms. Repeated administrations improved his colonoscopic findings as well as his dry skin along with a reduction of TNFα-expressing T cells. These findings suggest TNF blockade therapy is of value for refractory NEMO colitis with gene reversion.

Keywords NEMO colitis · infliximab · gene reversion

Introduction

X-linked anhidrotic ectodermal dysplasia with immunodeficiency (X-EDA-ID) is a rare inherited disease caused by hypomorphic mutations in the gene encoding nuclear factor-κB

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(NF-κB) essential modulator (NEMO), which is the regulatory subunit of IκB kinase [1–3]. Mutations of NEMO can cause an impaired capacity to activate NF-κB, resulting in defects in ectodermal differentiation and innate and adaptive immunity [4, 5]. Affected patients generally show multiple developmental anomalies in ectodermal tissues such as sparse hair, hypodontia with conical teeth, and anhidrosis or hypohidrosis due to lack of sweat glands. These patients also suffer from severe life-threatening infections in various sites caused by Gram-positive or Gram-negative bacteria or mycobacteria. Immunological abnormalities are characterized by defects in the production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation, hypogammaglobulinemia, specific antibody deficiency, and natural killer cell dysfunction. Hematopoietic stem cell transplantation for X-EDA-ID has been employed as a curative treatment [6–10], but has sometimes resulted in engraftment failure.

NEMO colitis, which is inflammatory colitis associated with mutated NEMO protein [11], is found in one fifth of all X-EDA-ID patients [12] and is usually reported as inflammatory bowel disease (IBD), atypical colitis, or Behcet's disease [6, 11, 13]. The onset of inflammatory colitis occurs early in childhood and often causes failure to thrive [2, 5–7, 9, 11–13]. The age of onset of colitis in X-EDA-ID is earlier than that of Crohn's disease, ulcerative colitis, or chronic granulomatous disease [14]. Histological examination reveals active colitis with abundant neutrophilic infiltration, and the colitis usually improves with corticosteroids but not with antimicrobial agents [6, 11]. Susceptibility to colitis remains after hematopoietic stem cell transplantation [6, 9].

Recently, Nenci et al. demonstrated that mice lacking NEMO in intestinal epithelial cells developed spontaneous severe colitis [15]. However, an additional lack of tumor necrosis factor (TNF) receptor-1 in these mice inhibited intestinal inflammation. These interesting findings suggest that TNFα plays a role in the progression of NEMO colitis and that TNF blockade therapy would be a promising treatment.

We describe here an X-EDA-ID boy suffering from severe intractable colitis who improved dramatically following treatment with a chimeric anti-TNFα monoclonal antibody, infliximab. Infliximab administration reduced all symptoms relating to inflammatory colitis, not only frequent diarrhea and severe abdominal pain, but also inflammatory findings by colonoscopy. These effects have lasted for more than 2 years with regular administrations of infliximab.

Methods

Cell Preparation and Culture

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood from our X-EDA-ID patient and his mother using Ficoll-Paque gradient centrifugation. PBMCs were suspended in RPMI 1640 medium (Sigma-Aldrich, USA) and non-adherent cells were used to obtain stimulated T cells. Adherent cells were cultured for 10 days with 500 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) (Peprotech, USA) to induce monocyte proliferation. T cells were stimulated for 48 h with 1-μg/mL phytohemaggulutinin (PHA) (Seikagaku Kogyo, Japan) and then for 8 days with 10-U/mL recombinant human interleukin (IL)-2 (Genzyme Techne, USA).

Cytokine Production Assay

PBMCs from our patient and healthy volunteers were incubated with LPS (1 μg/mL) (Sigma-Aldrich) at a concentration of 1×10^6 cells/mL at 37°C for 24 h. The concentration of TNFα in supernatant was measured using human BD OptEIA enzyme-linked immunosorbent assay kits (Becton-Dickinson, USA).

Mutation Analysis and Reversion Analysis

Genomic DNA from our patient and his mother was extracted from PBMCs, stimulated T cells, and stimulated monocytes using Puregene DNA purification kit (Gentra/Qiagen, USA); total RNA was extracted using TRIzol, according to the manufacturer's instructions (Invitrogen, USA). Complementary DNA (cDNA) was synthesized from total RNA with TaKaRa RNA PCR™ Kit (AMV) (Takara, Japan). Polymerase chain reaction (PCR) of genomic DNA and cDNA was performed using TaKaRa LA Taq (TaKaRa) with primers to amplify between exon 2 and exon 4 in the *IKBKG* gene. PCR primers were as follows: c1F, 5′-GCGCTCCTGAGACCCTCCAG-3′; c2R, 5′-GAGGAGAAGGAGTTCCTCAT-3′; G3F, 5′-CCCAGCTCCCCTCCACTGTC-3′; G4R, 5′-AACCCTG GAAGGGGTCTCCGGAG-3′. Genomic DNA was denatured at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s, and elongation at 68°C for 2 min 30 s, and a final extension for 7 min at 72°C using G3F and G4R primers. cDNA was denatured at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing and elongation at 68°C for 1 min, and a final extension for 5 min at 68°C using c1F and c2R primers. After gel electrophoresis and visualization, targeted bands were extracted and sequenced using ABI Big-Dye Terminator (Applied Biosystems, USA).

To analyze the reversion of mutation, we used our X-EDA-ID patient's PBMCs and stimulated cells. Mononuclear cells sorted with FACSVANTAGE (Becton-Dickinson) were used only at analysis after 12 months of infliximab treatment. PCR products were subcloned using a TOPO