◀ **Fig. 4** The frequencies of Vα24 iNKT cells in TILs and mononuclear cells from normal lung and lymph node tissues. **a**, The proportion of Vα24 iNKT cells ($V\alpha24^{+}V\beta11^{+}$ cells) in PBMCs on day 14, TILs, normal lung MNCs and lymph node MNCs in theα-GalCer-pulsed APC administration group were assessed by flow cytometry. The lymph node MNCs column depicts one representative MNC profile in the draining lymph nodes including hilar (#10, 11 and 12) and mediastinal (#1, 3, 4 and 7) nodes. **b**, The proportion of Vα24 iNKT cells in PBMCs, TILs and normal lung MNCs in the control group were assessed by flow cytometry. **c-d**, The comparison between the Vα24 iNKT cell contents in normal lung MNCs **c** and TILs **d** in the α-GalCer-pulsed APC treatment group and the control group. **e** The TIL/Normal Lung MNC ratio of Vα24 iNKT cell proportion. control, control group; Tx, α-GalCer-pulsed APC administration group; *p=0.0008

control group (mean percentage, 0.68 % and 0.165 %, Fig. 4d). The Vα24 iNKT cell ratio of TILs/normal lung in the α-GalCer-pulsed APC administration group was significantly higher than that of the control group (p=0.0008, Fig. 4e).

The number of IFN-γ-producing cells after restimulation with α-GalCer in vitro was concurrently monitored in PBMCs, TILs, normal lung MNCs and lymph node MNCs using an ELISPOT assay. An analysis of the PBMCs and resected specimen showed the highest value of α-GalCer-responsive IFN-γ-producing cell number in the TILs of the α-GalCer-pulsed APC treated group (Fig. 5a). The absolute number of α-GalCer-responsive IFN-γ-producing cells in the TILs was apparently high in cases 001 and 002, whereas a relatively low value was seen in cases 003 and 004. This observation was not detected with the use of control group specimens (Fig. 5b). Together with the results in Fig. 4, the administration of α-GalCer-pulsed APCs induced the mobilization of endogenous Vα24 iNKT cells into the primary site of the lung cancer and augmented the IFN-γ-producing ability of tumor infiltrating Vα24 iNKT cells.

In addition, the number of IFN-γ-producing cells in PBMCs was determined after restimulation with α-GalCer

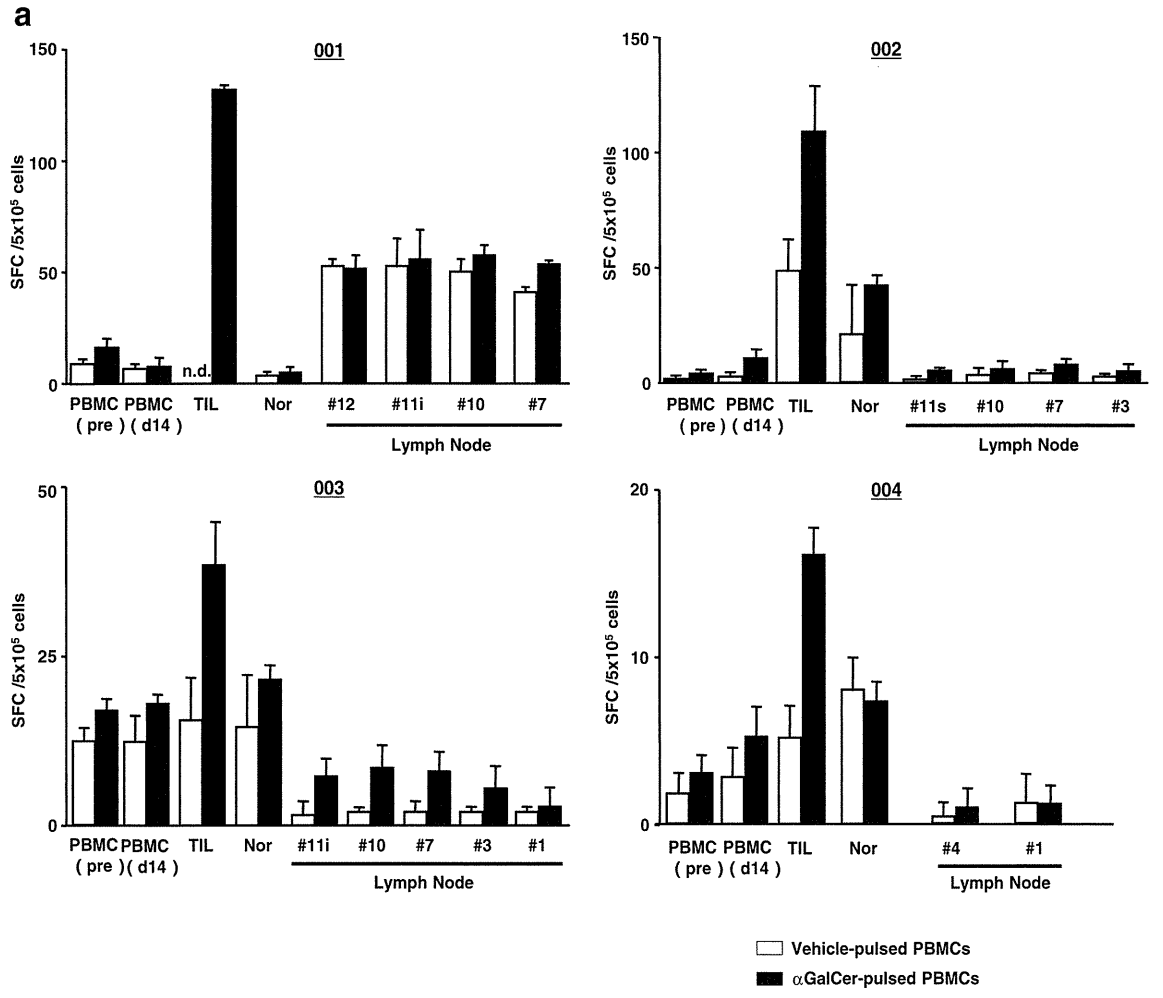

Fig. 5 Detection of α-GalCer-reactive IFN-γ-producing cells by enzyme-linked immunospot assay. **a** Cryopreserved PBMCs, TILs, normal lung MNCs and lymph node MNCs of the α-GalCer-pulsed APC treated group were thawed and cultured overnight with either α-GalCer or vehicle. The presence of IFN-γ-producing cells was quantified by an enzyme-linked immunospot assay. The resected draining lymph nodes including hilar (#10, 11 and 12) and mediastinal (#1, 3, 4 and 7) nodes are shown. Spot number of IFN-γ with standard deviation for triplicate culture of 4 cases are shown. **b** Spot-forming cell number in the control group. SFC, Spot Forming Cell; pre, pretreatment; d14, day 14; Nor, normal lung MNCs; n.d., not done; #11 s, lymph node #11 superior; #11i, lymph node #11 inferior

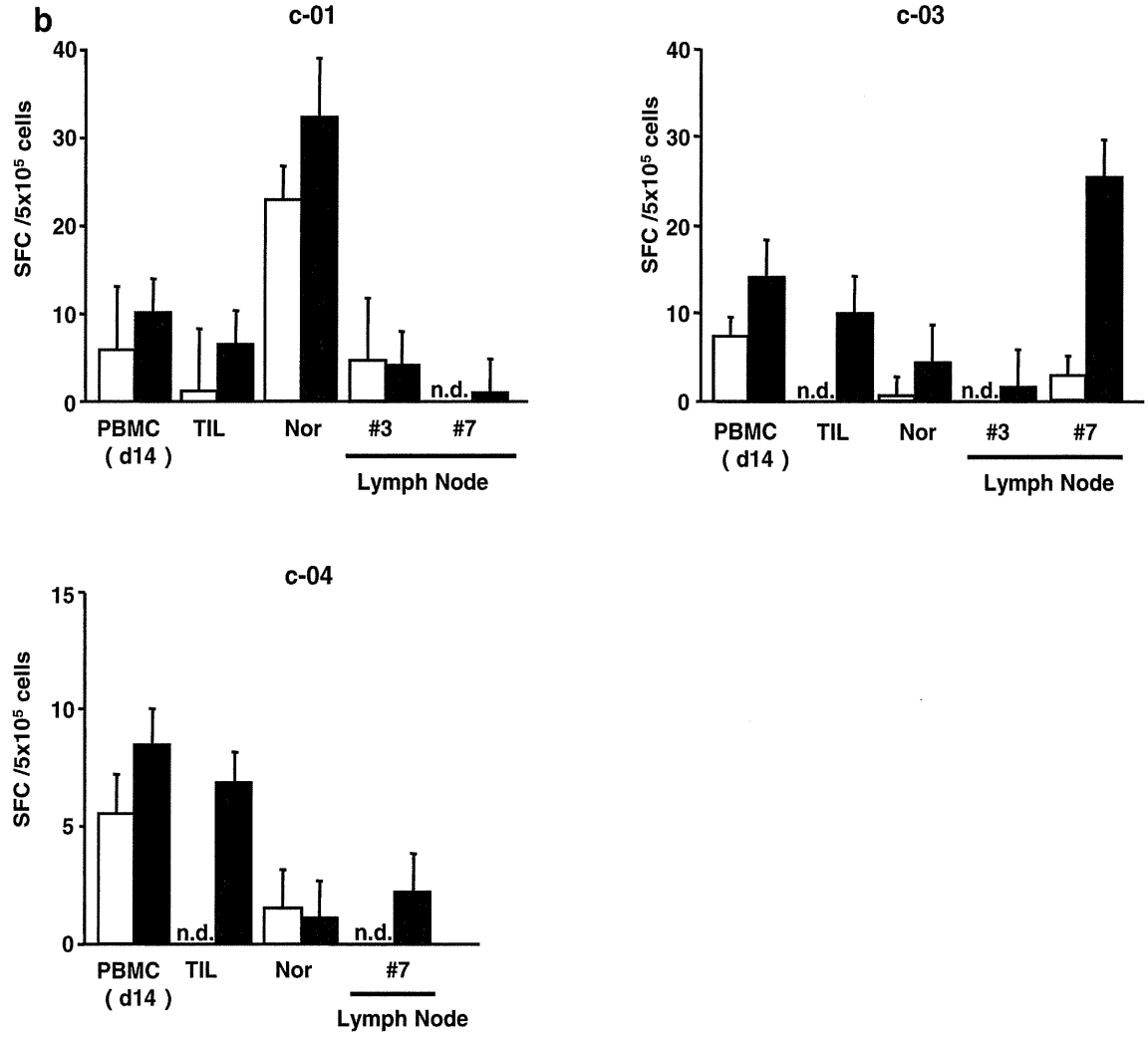

Fig. 5 (continued)

in vitro. In Fig. 5a, the number of IFN-γ producing cells in PMBC increased 14 days after treatment in case 002 and 004, indicating that global NKT cell activation in these patients.

The mRNA Expression Level of Vα24 Inkt Cell Receptor and CD1d

Quantitative RT-PCR was performed to further confirm the increase in the number of Vα24 iNKT cells. The mRNA from the primary tumor, normal lung tissue and lymph node samples was obtained from the α-GalCer-pulsed APC treated patients and the relative expression level of Vα24 invariant TCR and the constant region of TCR (Cα) mRNA were evaluated. The mRNA for Vα24 TCR was highly expressed in the tumor (Fig. 6a). The Vα24-Jα18 invariant TCR mRNA could not be detected in the normal lung tissue since the lung parenchyma included mainly alveolar epithelial cells and only a low copy number of Cα mRNA was detected. The relative gene expression of CD1d was also evaluated by quantitative RT-PCR. CD1d expression was ascertained both in normal lung tissue and tumor tissue in all 4 cases and the expression level appeared to be higher in normal lung tissue (Fig. 6b).

Discussion

The major aim of this study was to investigate the Vα24 iNKT cell-specific immune responses in the primary tumor site after the intravenous injection of α-GalCer-pulsed APCs in patients with advanced NSCLC. The development of these immunotherapeutic approaches with the administration of α-GalCer-pulsed APCs requires a thorough knowledge of local immune responses. Vα24 iNKT cells accumulate in lung cancer lesions [5], as observed in this report (Fig. 4e). A significant increase in the tumor infiltrating Vα24 iNKT cell population was detected after the administration of α-GalCer-pulsed APCs in comparison to the non-injected control group. This observation is quite reasonable for the iNKT cell-targeted therapy aimed at activation of Vα24 iNKT cells in the tumor located site in vivo. The activation status of CTLs, rather than just the existence

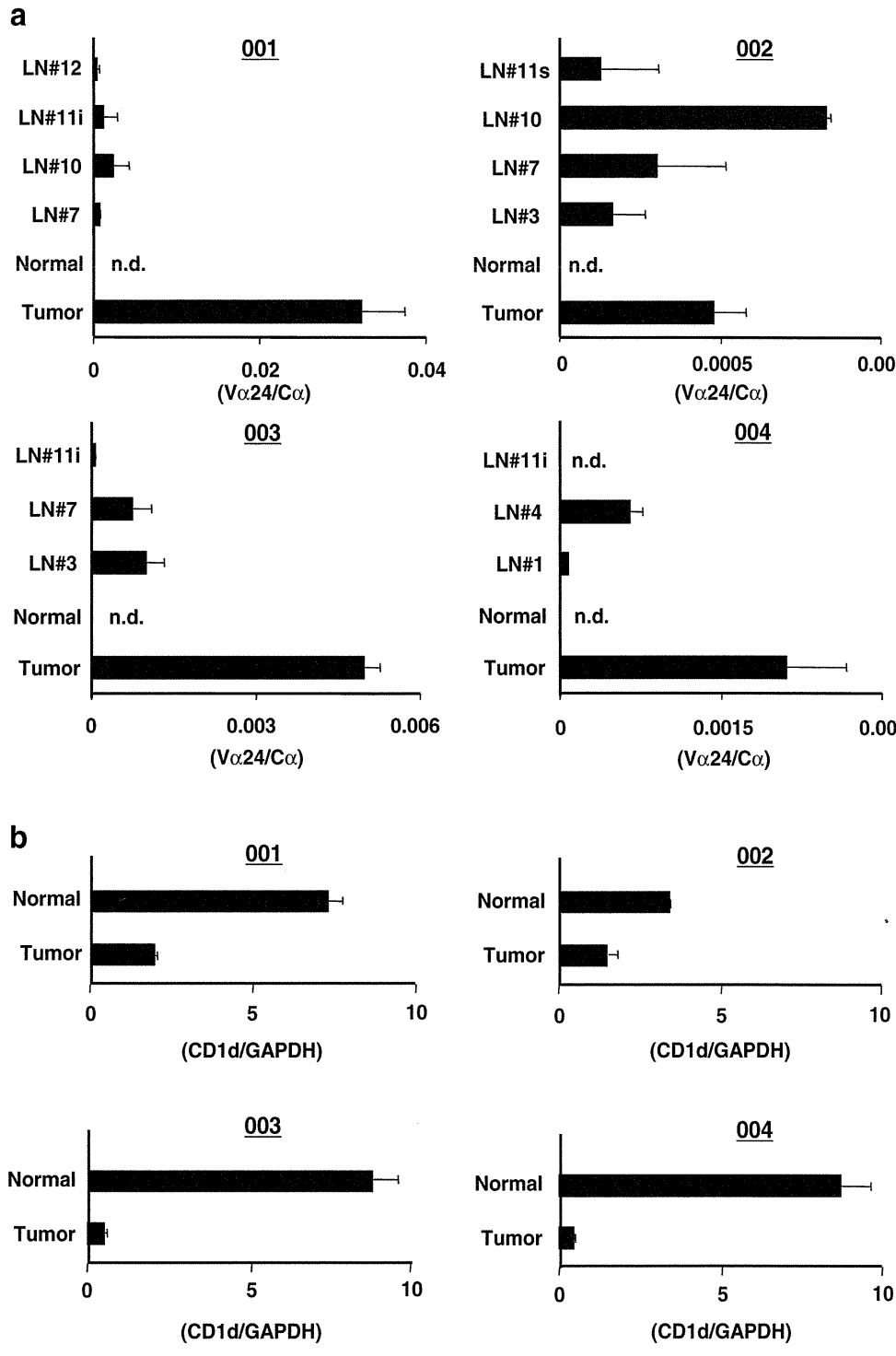

Fig. 6 The relative mRNA expression of the Vα24$^+$ TCR in tumor, normal lungs and lymph nodes. Cancer tissue, non-cancerous lung tissue and lymph nodes were obtained from α-GalCer-pulsed APC treated patients. **a** The expression level of Vα24 TCR mRNA in each sample was analyzed by quantitative RT-PCR. Each mRNA was quantified by the standard curve method and copy numbers of Vα24 TCR were normalized by the copy number of the constant region of the TCR α chain (Cα mRNA. **b** The expression level of CD1d mRNA in each sample was analyzed by quantitative RT-PCR. Each mRNA was quantified by the standard curve method and copy numbers of CD1d were normalized by the copy number of the GAPDH mRNA. LN, lymph node; Normal, normal lung tissue; Tumor, tumor tissue; n.d., not detected; #11 s, lymph node #11 superior; #11i, lymph node #11 inferior

of CTLs, has great prognostic significance [16–18]. Therefore, IFN-γ-producing cells were also monitored in PBMCs, TILs and MNCs from normal lung tissue and lymph nodes using an ELISPOT assay. After starting the protocol, standard preoperative chemo-radiotherapy was introduced to treat locally advanced NSCLC, and such a change of treatment strategy hindered the entry of patients into this protocol. In spite of the limited number of patients analyzed, the results obtained by ELISPOT assay indicated that tumor infiltrating mononuclear cells had augmented IFN-γ producing capacity, which may have a positive impact on the tumor microenvironment.

Tumor infiltrating lymphocytes are found in a variety of cancers and they are thought to be a result of a host immune response directed against tumor cells. Several reports have shown that the presence of large numbers of tumor-infiltrating CD8$^+$ T cells are associated with a favorable prognosis in esophageal carcinoma [18, 19], colorectal cancer [20, 21], ovarian cancer [22], and pancreatic carcinoma [23], while the infiltration of CD4$^+$ T cells that posses regulatory function,

such as Foxp3$^+$ regulatory T cells (Treg) are associated with the poor prognosis of ovarian cancer [24, 25]. The balance between CD8$^+$ CTLs and Tregs in tumors is critical for disease progression and survival [24, 26, 27]. These diverse results indicate that the functional roles of TILs are complicated and uncertain and the effects of TILs might vary with the type and stage of cancers. In addition to CD8$^+$ cytotoxic T cells, tumor infiltrating Vα24 iNKT cells have been reported to be a positive prognostic factor for colorectal carcinoma [28]. The current results indicated that the injection of α-GalCer-pulsed APCs could induce the accumulation of Vα24 iNKT cells in TILs, which would therefore lead to a good prognosis after a complete surgical resection.

Although a complete surgical resection is regarded as the optimal treatment for NSCLC, only around 25 % of NSCLC are suitable for potentially curative resection. Despite optimal surgical management, the 5-year survival rate of resected NSCLC ranges between 85.9 % for pathological stage Ia and 41 % for pathological stage IIIa [29]. Approximately 50 % or more of patients with NSCLC who undergo surgery experience relapse due to the existence of microscopic lesions that could not be detected by preoperative screening. Recently, adjuvant chemotherapy given after surgery has been shown to improve survival [30–32]. A meta-analysis suggested that cisplatin-based adjuvant chemotherapy could yield an absolute overall survival advantage of 5 % at 5 years [33]. At the same time, chemotherapeutic agents often show severe toxic effects and it was reported that in patients with early-stage disease have deleterious effects on long-term survival. This emphasizes the importance of development of less-invasive preoperative or postoperative therapy to suppress the growth of micrometastases. Therefore, immune cells for tumor surveillance, such as NK and iNKT cells, which possess anti-tumor activity, should be beneficial and post-surgical adjuvant immunotherapy by the use of these cells may be favorable since the residual tumor is quite small after a complete resection.

Conclusions

α-GalCer-pulsed APC administration successfully induced the dramatic infiltration and activation of Vα24 iNKT cells in the tumor lesion. This report is the first clinical trial of Vα24 iNKT cell targeted immunotherapy that shows a functional Vα24 iNKT cell accumulation in the tumor microenvironment. These results encourage the further development of immunotherapy aimed at the activation of endogenous Vα24 iNKT cells in the lung.

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Conflict of interest The authors declare that they have no conflict of interest.

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第11回CRCと臨床試験のあり方を考える会議2011 in 岡山／2011年9月25日・岡山コンベンションセンター

●パネルディスカッション3／
これからの時代を担うIRBの機能と責務－中央IRB（共同IRBを含む）の普及に向けて－／講演4

中央IRB等への移行過程で生じた課題とその解決に向けた取り組み

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これからの時代を担うIRBの機能と責務－中央IRB（共同IRBを含む）の普及に向けて－／講演4

中央IRB等への移行過程で生じた課題とその解決に向けた取り組み

花岡英紀[1)]　青柳玲子[1)]　松本和彦[2)]　吉澤弘久[3)]　小池竜司[4)]
大学病院臨床試験アライアンス推進室および中央IRB検討ワーキンググループ

大学病院臨床試験アライアンスではいわゆる中央IRBのあり方について検討を行うとともに，千葉大学，信州大学，新潟大学による中央IRBの審議を2010年7月より試験的に一試験について開始した。本稿では大学病院臨床試験アライアンスで実施している中央IRB検討ワーキンググループ（以下WG）およびその上部組織である推進室での議論，さらに欧州の中央IRBのあり方をふまえたIRBのあるべき姿と，実際の取り組みについて述べるものである。また，この内容については，筆者らの私見にもとづいており，各大学でのコンセンサスを得られた段階に至っていない。

はじめに

近年，治験の活性化の中で，中央IRBに向けた取り組みの必要性が謳われている。これは，多くの場合，治験を開始するにあたり，それぞれのIRBへの申請に関して多くの時間と労力を必要とするため，その業務に当たる費用が高額となり，治験依頼者への負担が増しているという事実がある。また，一方で，経験の乏しいIRBにおいて複雑な治験の審議をすることについて問題視する事実もある。このような状況のなか，GCPでは2度の改訂を経ているにもかかわらず，いまだ中央IRBの活動は活発ではない。平成23年6月には日本医師会治験促進センターによる治験等適正化作業班による提言がなされ，これを受ける形でさらなるGCPの改訂がなされた。作業班では，中央IRBの設置とIRB事務局と治験事務局の外部委託による効率化も検討された。

大学病院臨床試験アライアンスは，国際共同試験をわが国で主導することを目的として関東信越7大学において先進的な取り組みを治験において実施してきた。そのなかで中央IRBについては，はたして実施にあたりどのような課題があるか具体的に検討が必要との結論に至り，推進室の下部組織として信州大学を担当校とし各大学の担当者からなるWGを構成し議論を重ねてきた。

WGの検討内容について

そもそも中央IRBの設置の前提としていくつかの疑問がある。治験を実施する各施設個別の状況を知らない中央IRBにおいて，①迅速性と効率性と，科学性と倫理性は共に成り立つにはどのようにしたらいいのか，②被験者を保護するという原則はどのようにしたら確保されるのか，という2課題はWGの検討以前においてそもそも重要な問題であり，IRBの審議の根幹を成すものである。中央IRBで十分な議論ができる体制を維持すること，さらにそこでは，その施設固有の問題点を被験者保護という立場から話し合える状況にする必要があると考えている。

一方で，③審査手続きの共通化，④中央IRBの設置場所，⑤具体的な審議の方法，という3課題については，技術的な側面が強い課題であり，解決可能な課題とした。

欧米の中央IRBのあり方について

海外には中央IRBがすでに多く設置され，活用さ

[1)] 千葉大学　[2)] 信州大学　[3)] 新潟大学　[4)] 東京医科歯科大学

英国	3機関の承認が必要 1.NHS National Health Service 行政区ごとの local Research Ethical Committee (REC) 2.Main REC 3.MHRA (Medicines and healthcare products Regulatory Agency)
フランス	被験者保護委員会 CCP 被験者保護に関する事項 臨床試験のプロトコルデザインに関する事項 施設に関する事項 医療製品保険安全局 AFSSAPS 試験薬等の品質と安全性に関する事項 被験者の安全性に関する事項（適格基準、投与量、被験者のモニタリング，対照薬）
米国	All committees are registered with the Office for Human Research Protections (OHRP). FDA, pharmaceutical companies, contract research organizations, as well as AAHRPP perform regular inspections of our processes. An FDA inspection in July 2009 resulted in no findings. CERTIFICATION: Staff and board members have earned certifications, such as Certified IRB Professional (CIP), Certified Clinical Research Professional (CCRP) and Certified IRB Manager (CIM).

図1　欧米の中央IRBに関する大学病院臨床試験アライアンスによる訪問調査

IRB審議資料

IRB開催日	千葉大	新潟大	信州大
2010/7/20	新規実施の適否	新規実施の適否 （治験参加カード）	新規実施の適否 （治験参加カード）
2010/9/21	実施計画書 2.0 版 同意説明文書	実施計画書 2.0 版	実施計画書 2.0 版
2010/10/18		同意説明文書	
2010/11/15	実施計画書 2.1 版 同意説明文書 監査手順書 2.0 版	実施計画書 2.1 版 同意説明文書 監査手順書 2.0 版	実施計画書 2.1 版 同意説明文書 監査手順書 2.0 版
2011/2/21	院内の重篤有害事象	—	—
2011/3/22	継続審査，安全性情報	継続審査，安全性情報	継続審査，安全性情報
2011/4/18	実施計画書 2.2 版 治験薬概要書 2.0 版 同意説明文書	実施計画書 2.2 版 治験薬概要書 2.0 版	実施計画書 2.2 版 治験薬概要書 2.0 版
2011/5/16	同意説明文書	同意説明文書	同意説明文書
2011/9/20	実施計画書 2.3 版 同意説明文書 緊急逸脱報告書	実施計画書 2.3 版 同意説明文書	実施計画書 2.3 版 同意説明文書

図3　3大学から千葉大学中央IRBへ提出され審議された内容

れている国々があるが，その設置の基準や役割はさまざまである。海外の状況（⑥）を検討するためにわれわれは実際に規制当局や医療機関を訪問し，調査を行った（**図1**）。このなかで，施設固有の問題点を審議するローカルIRBと計画書の審議をする中央IRBがそれぞれ業務を分担し審議を行っていることを知った。さらに，規制当局との連携も重要な点であった。また，米国ではコマーシャルIRBが数多く設置されているが，それぞれのIRBではその質の高さと独立性を示すための取り組み（認定の取得，業務内容の公開，FDAの調査を受けた時期の公開）を行っていることが明らかになったが，一方で施設固有の問題点を審議することについては難しい状況であることが判明した。

医師主導治験の中央IRBについて

2010年7月千葉大学医学部附属病院治験審査委員

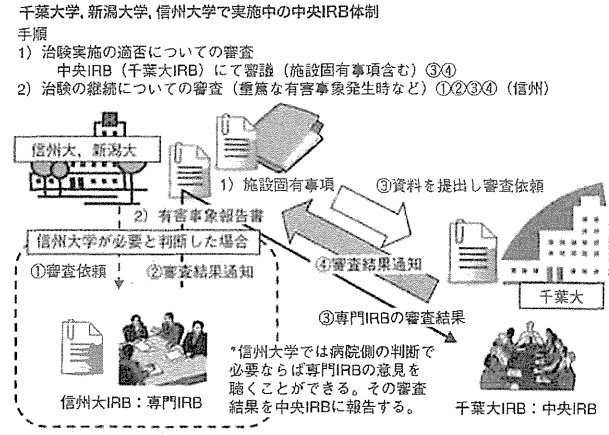

図2　WGで検討された中央IRBのフロー

会では，信州大学医学部附属病院長および新潟大学医歯薬総合病院長から千葉大学医学部附属病院長への医師主導治験の審議依頼を受け審議を開始した。これは国立大学病院としては初めての取り組みである。この審議を実施するにあたり3大学ではWGで検討された中央IRBの審議のフロー（**図2**）をもとにそれぞれ手順書の改訂（⑦）契約書の作成とこれに伴う審査費用の算定（⑧）を行った。ここでは申請元の大学のIRBを「施設で発生した重篤有害事象を審議する専門IRB」と位置づけている。(⑨)3大学から中央IRB事務局へ提出される書類は内容がすべて同一でないことより中央IRBで承認された内容や時期が異なっている（**図3**）。このことは治験事務局と中央IRB事務局との独立性を意味し，推進する立場とこれを監督する立場のそれぞれの事務局の独立性が確保された状況となっている。(⑩) 医師会の作業班の提案する各大学の治験事務局を廃止し中央に委託することについては，作業の効率性に加え，技術的に千葉大学の事務局が各大学の業務を引き受けることは困難との結論に至った。

ま　と　め

およそ10の課題について検討を行いながら，具体的に中央IRBを運用するに至った。規制当局との連携については今までまったく議論をされていな内容である。つまり，その本質において単なる効率化ではなく真の意味での中央IRBの役割を考えることが重要であり，欧米での取り組みを十分考慮のうえ，進めて行く必要がある。大学病院臨床試験アライアンスではわが国を代表するグループとして欧米からも信頼される中央IRB体制の構築を実施する。

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ORIGINAL ARTICLE

Multiple angiogenetic factors are upregulated in POEMS syndrome

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Abstract Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome is a multisystem disorder associated with plasma cell dyscrasia. Elevated serum levels of vascular endothelial growth factor (VEGF), which strongly promotes neovascularization and vasopermeability, are considered to be responsible for the characteristic symptoms such as angiomata, pleural effusion/ascites, edema, and organomegaly in the disorder. To study whether other angiogenetic factors are upregulated in POEMS syndrome, we measured serum levels of basic fibroblast growth factor and hepatocyte growth factor (HGF), as well as VEGF, in 17 patients with POEMS syndrome. All these factors were significantly upregulated in the POEMS syndrome patients. After the treatment with anti-VEGF antibody, the levels of HGF did not change, suggesting that elevation of HGF levels is not secondary to VEGF overproduction. These results suggest that different angiogenetic factors might contribute to the pathogenesis of POEMS syndrome, and this fact might contribute to the insufficient clinical effects obtained by suppression of VEGF alone.

Keywords POEMS syndrome · Vascular endothelial growth factor (VEGF) · Basic fibroblast growth factor (bFGF) · Hepatocyte growth factor (HGF) · Bevacizumab

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Introduction

Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome is a rare cause of multisystem disorder associated with plasma cell dyscrasia [1, 2]. Pathogenesis of POEMS syndrome is not fully understood, but overproduction of vascular endothelial growth factor (VEGF), which strongly promotes neovascularization and vasopermeability [3], is considered to be responsible for the characteristic symptoms such as angiomata, pleural effusion/ascites, edema, and organomegaly [4].

VEGF is potentially secreted by monoclonal plasma cells [5] and therefore could be a target of treatment for POEMS syndrome. Previous case series have shown that in appropriate candidates, high-dose chemotherapy with autologous peripheral blood stem-cell transplantation (auto-PBSCT) is an effective treatment for POEMS syndrome [6, 7]. This treatment leads to obvious improvement in peripheral neuropathy and other symptoms with a significant decrease in serum VEGF levels.

However, the effects of treatment with anti-VEGF monoclonal antibody, bevacizumab, were controversial. Some reports showed that POEMS patients treated with bevacizumab do not

benefit from this agent despite a dramatic decrease in serum VEGF levels [8, 9], and a case report presented that a patient with POEMS syndrome experienced relapse after successful treatment with auto-PBSCT without increasing levels of VEGF [10]. These findings raise the possibility that angiogenetic factors other than VEGF might contribute to the pathogenesis of POEMS syndrome. We therefore simultaneously measured serum levels of three angiogenetic factors, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), as well as VEGF, in patients with POEMS syndrome.

Patients and methods

Patients

Serum samples were collected from 17 POEMS syndrome patients (12 men; median, age 58 years; range, 34–73 years) before treatment. Their condition fulfilled diagnostic criteria [2]. Serum samples from 13 healthy subjects (nine men; median age, 56 years; range, 42–64 years) served as normal controls.

Serum samples were also obtained from another three patients with POEMS syndrome before and after treatment with bevacizumab. Before bevacizumab administration, all of them had received conventional melphalan chemotherapy or thalidomide treatment but had no or insufficient responses with high serum VEGF levels. All subjects gave informed consent to the procedures, which were approved by the Ethics Committee of Chiba University School of Medicine.

Measurements of the serum angiogenetic factor

After the blood samples were collected, they were allowed to clot at room temperature for about 2 h and were then centrifuged at 3,000×*g* for 5 min. Sera were stored in aliquots at −80 °C. Enzyme-linked immunosorbent assays (ELISAs) for the serum levels of VEGF, HGF, and bFGF (Quantikine HS, R&D Systems, Minneapolis, MN, USA) were performed according to the manufacturer's instructions.

Serum levels of VEGF, bFGF, and HGF in the POEMS syndrome and normal control groups were compared by the Mann–Whitney *U* test. Correlations between serum levels of VEGF and bFGF or HGF were analyzed by the Spearman's rank-correlation coefficient.

Results

Results of ELISA are shown in Fig. 1. Serum levels of VEGF were increased in all patients with POEMS syndrome (mean, 4,688 pg/ml); there was no overlap between POEMS patients and normal subjects (mean, 219 pg/ml). Compared with normal controls, POEMS patients had significantly higher levels of serum bFGF (mean, 21.2 versus 4.51 pg/ml) and HGF (mean, 1,508 versus 671 pg/ml).

In the patients with POEMS syndrome, there was no correlation between the serum levels of VEGF and bFGF (r=−0.00245, P=0.10) (Fig. 2a). This could suggest an independent production of bFGF and VEGF. In contrast, there was a significant correlation between the serum levels of VEGF and HGF (r=0.659, P=0.004) (Fig. 2b). In three patients who received treatment with bevacizumab, serum levels of VEGF and HGF were increased before the therapy but, after treatment, only VEGF levels decreased while HGF levels did not (Table 1). This result suggests that HGF may be secreted independently from VEGF.

Table 1 shows changes in serum levels of VEGF, bFGF, and HGF in three patients before and after bevacizumab

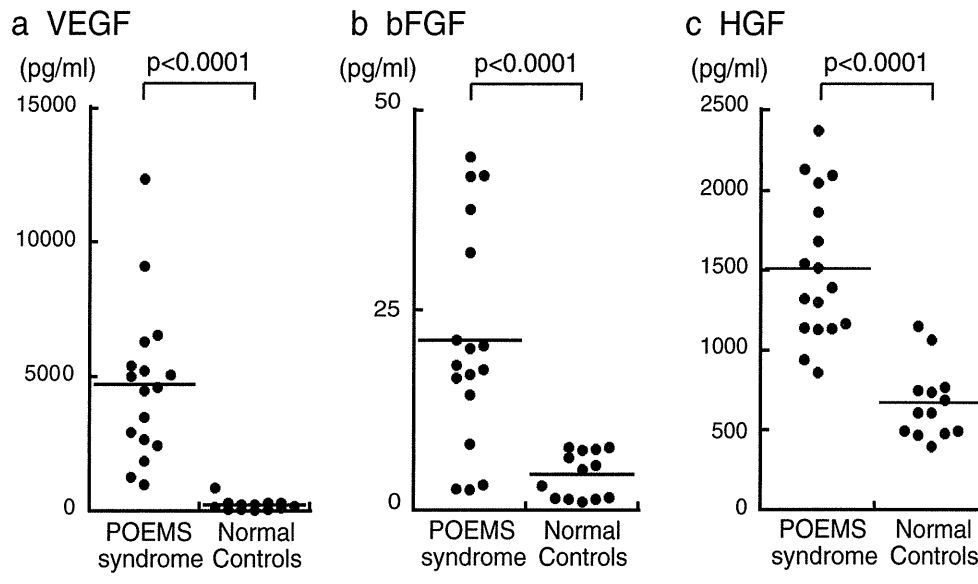

Fig. 1 Serum levels of vascular endothelial growth factor (*VEGF*; **a**), basic fibroblast growth factor (*bFGF*; **b**), and hepatocyte growth factor (*HGF*; **c**) from 17 patients with POEMS syndrome before treatment and 13 normal controls measured by ELISA were presented. All of them significantly increased in POEMS syndrome than in normal subjects (the Mann–Whitney *U* test). The *lines* indicated the mean concentration in each group

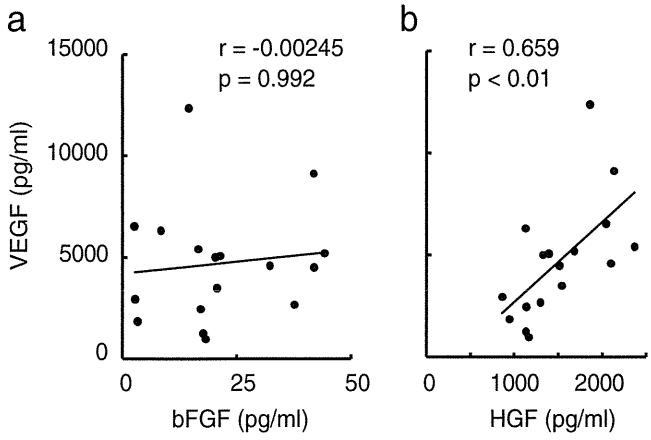

Fig. 2 Correlation between the serum levels of VEGF and other angiogenetic factors in POEMS syndrome. *Vertical lines* indicated serum levels of VEGF, and *horizontal lines* indicated **a** serum levels of bFGF and **b** HGF. Correlations were analyzed by the Spearman's rank-correlation coefficient, and only HGF levels had correlation with VEGF levels

treatment. Serum VEGF levels dramatically decreased after the treatment, but serum levels of bFGF and HGF did not change significantly. Serum bFGF levels were already low before therapy, possibly due to previous treatment with melphalan or thalidomide. Patients treated with bevacizumab did not show any rapid clinical improvement after its injection. Moreover, the modifications of VEGF, HGF, and bFGF by the therapy were the same among three patients, and we could not find the relationship with the response to the therapy.

Discussions

Among the growth factors which involve the angiogenetic process, bFGF is located in the upstream of the pathway and regulates production of VEGF and HGF [11]. Our study shows that bFGF levels are increased in POEMS syndrome and do not correlate with VEGF levels, suggesting that upregulation of bFGF is not induced by elevation of VEGF levels. Regarding changes in vascular permeability, bFGF has opposite effects to those by VEGF; bFGF enhances the function of blood–nerve barrier by increasing the expression of claudin-5 [12]. Therefore, the possibility that bFGF was increased as the feedback of elevated VEGF levels cannot be excluded.

Table 1 Serum levels of angiogenic factors before and after anti-VEGF monoclonal antibody therapy

Patient		VEGF (pg/ml)	bFGF (pg/ml)	HGF (pg/ml)
1	Before therapy	1,970	6.55	1,334
	8 weeks	198	5.50	1,255
2	Before therapy	1,640	1.33	1,980
	4 weeks	46	0.64	2,552
	6 weeks	71	0.34	2,215
3	Before therapy	4,670	0.78	1,557
	1 week	317	1.26	1,840
	4 weeks	220	3.56	5,345

VEGF vascular endothelial growth factor, *bFGF* basic fibroblast growth factor, *HGF* hepatocyte growth factor

In the three patients treated with anti-VEGF therapy, serum bFGF levels were not so high before therapy, possibly by previous treatment with melphalan or thalidomide. This raises the possibility that factors other than bFGF were more critical. This should be further examined in future studies.

In regard to HGF, it was known to highly contribute to the vascular permeability with disruption of the tight junctions [13, 14], and this could contribute to the pathogenesis of POEMS syndrome. This might explain the controversial effects of bevacizumab that affects VEGF alone.

In this study, it was suggested that HGF was secreted independently to VEGF, and there was a correlation between the serum levels of VEGF and HGF in POEMS syndrome. From this aspect, it was possible that VEGF and HGF were secreted from the same cells or some same molecules provoked the production of these factors simultaneously. VEGF levels were reduced when the patients received monoclonal plasma cell targeted therapy such as resection of the sclerotic bone lesions [5] or auto-PBSCT [7]. This showed that monoclonal plasma cells had some associations with production of VEGF. But the production site remains unresolved, and previous studies suggested that VEGF was secreted from monoclonal plasma cells [5], platelets [15], or bone marrow stromal cells [16]. There was a possibility that monoclonal plasma cells might produce some molecules which would trigger secretion of VEGF and HGF, and to identify these molecules might reveal the pathogenesis of POEMS syndrome and lead the new molecular targeting therapy for the disorder. Analyses of multiple angiogenetic factors could provide new insights into the pathophysiology of this mysterious syndrome and into the choice of optimal treatments.

The other possibility is that angiogenesis could play a less important role than initially thought. Serum levels of multiple inflammatory cytokines such as TNF-α, interleukin (IL)-6 [17], and IL-12 [18] are also increased in POEMS syndrome, and these cytokines might also contribute to the pathophysiology of POEMS syndrome.

This study included relatively few subjects, and understanding of the disease needs further study with large scale in the future.

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Conflict of interest The authors declare that they have no conflict of interest.

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Markedly upregulated serum interleukin-12 as a novel biomarker in POEMS syndrome

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ABSTRACT

Objective: To systematically study abnormalities in cytokine profiles in polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome, which has been increasingly recognized as a cause of demyelinating neuropathy associated with plasma cell dyscrasia and elevated serum level of vascular endothelial growth factor (VEGF).

Methods: In this case-control study, we measured serum levels of 27 cytokines in patients with POEMS syndrome using a multiplex suspension array system, and compared them with those of controls. In 10 patients, serial changes after treatment were analyzed.

Results: Interleukin (IL)-12 as well as VEGF levels were markedly increased ($p < 0.0001$) in all the patients ($n = 23$). Ten kinds of other proinflammatory cytokines such as IL-6 and tumor necrosis factor-α were also significantly increased in the POEMS syndrome group, but in some patients the serum levels of such cytokines remained within the normal ranges. After treatments, the IL-12 as well as VEGF levels significantly decreased with clinical improvements ($p > 0.01$ and $p > 0.05$, respectively).

Conclusions: Our findings suggest that serum IL-12 is a biomarker of the disease activity in POEMS syndrome. The overproduction of IL-12, as well as VEGF, is likely to play an important role in the pathogenesis of the disorder, and could contribute to the peripheral nerve demyelination in POEMS syndrome. *Neurology*® 2012;79:575-582

GLOSSARY

CIDP = chronic inflammatory demyelinating polyneuropathy; **FGF** = fibroblast growth factor; **G-CSF** = granulocyte colony-stimulating factor; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **HCT** = high-dose chemotherapy; **IFN** = interferon; **IL** = interleukin; **IL-1ra** = interleukin-1 receptor antagonist; **IP** = induced protein; **MCP** = monocyte chemotactic protein; **MIP** = macrophage inflammatory protein; **NK** = natural killer; **NKT** = natural killer T; **PBSCT** = peripheral blood stem cell transplantation; **PDGF** = platelet-derived growth factor; **POEMS** = polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes; **RANTES** = regulated upon activation, normal T cell expressed and secreted; **TNF** = tumor necrosis factor; **VEGF** = vascular endothelial growth factor.

Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome is a rare cause of demyelinating polyneuropathy with multiorgan involvement. The pathogenesis of POEMS syndrome is not fully elucidated, but overproduction of vascular endothelial growth factor (VEGF) has been considered responsible for the characteristic symptoms.[1,2] POEMS syndrome is known as an important example illustrating the remote effect of a malignancy on neural and non-neural tissues not due to direct invasion of malignant cells or an antibody-targeted attack but due to abnormal overproduction of cytokines resulting in a proliferative effect on vessels and functional alterations.[3]

To date, the clinical importance of the serum level of VEGF as a biomarker of the diagnosis and disease activity in POEMS syndrome has been well established,[1,2,4–6] whereas the increased

Supplemental data at www.neurology.org

Supplemental Data

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Go to Neurology.org for full disclosures. Disclosures deemed relevant by the authors, if any, are provided at the end of this article.

serum levels of other cytokines such as interleukin (IL)–6 and tumor necrosis factor (TNF)–α[7,8] have also been reported.

However, it has been considered that elevated VEGF alone does not cause demyelinating polyneuropathy,[9,10] because pathologic examination showed no peripheral nerve demyelination in the VEGF overexpression mouse model.[9] In addition, it is still controversial whether anti-VEGF monoclonal antibody is effective in the treatment of POEMS syndrome.[11–14] Therefore, we hypothesized that other serum cytokines or molecules would also contribute to the pathophysiology of POEMS syndrome. To investigate changes in the cytokine network and their contribution to the pathophysiology in POEMS syndrome, we systematically investigated multiple serum cytokine levels in 25 patients with POEMS syndrome and also measured their changes after treatment.

METHODS We used a case-control design. We compared serum levels of 27 cytokine/chemokines of untreated and relapsed patients with POEMS with those of normal and disease controls. To investigate clinical significance of the change of serum cytokine levels, we analyzed the changes of serum levels of 27 cytokine/chemokines before and after treatments in patients with POEMS syndrome.

Subjects. We recruited patients with POEMS syndrome who met following conditions: 1) patients who visited Chiba University Hospital from April 2000 to July 2009, 2) fulfilled the diagnostic criteria of "definite POEMS syndrome" defined in the previous review,[6] and 3) whose sera at untreated state or at the time of relapse or whose paired sera before and after treatment were stored in adequate condition. The number of cases determined the sample size.

Of those, patients at the untreated state were defined as "untreated group," and patients at the time of a relapse were defined as "relapsed group." In the analysis of the changes of serum cytokine levels before and after treatments, "before treatment" and "after treatment" groups were defined. The details of each subgroup are described in the Methods in appendix e-1 on the *Neurology*® Web site at www.neurology.org.

In the present study, sera of the "after treatment" group were chosen to bring the mean treatment periods at analysis close to 3 months. This is because a previous report has shown that improvements of symptoms began within about 3 months both in patients with POEMS treated with high-dose chemotherapy (HCT) with peripheral blood stem cell transplantation (PBSCT)[5] and thalidomide therapy (Methods in appendix e-1).[4]

We aimed to select 1 normal or disease control for every case. As disease control, patients with chronic inflammatory demyelinating polyneuropathy (CIDP), typical immune-mediated demyelinating polyneuropathy, were recruited. The controls were matched to cases by age, but not matched by sex. As a result, 19 age-matched normal subjects and 10 patients with CIDP were included in this study.

Ethical approval was granted by the Ethics Committee of the Chiba University School of Medicine, Chiba, Japan. All subjects gave informed consent for their participation.

Cytokine assay. Serum concentrations of 27 cytokines/chemokines were measured using the Bio-Plex human 27-Plex cytokine panels and a Bio-Plex cytokine reagent kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. The 27-cytokine panel consisted of IL-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor (FGF)–2, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)–γ, 10 kDa IFN-γ-induced protein (IP-10), monocyte chemotactic protein–1 (MCP-1), macrophage inflammatory protein (MIP)–1α, MIP-1β, platelet-derived growth factor (PDGF)–BB, regulated upon activation, normal T cell expressed and secreted (RANTES), TNF-α, and VEGF. As described above, serum concentration of IL-12 (p70), composed of 2 disulfide-linked subunits designated p35 and p40, was measured in this study.

We defined the upper and lower limits of normal of each cytokine as mean ±3 SD of normal control values.

The sera were stored at −70°C until cytokine analysis was performed. The study was approved by the Ethics Committee of the Chiba University School of Medicine.

Statistical analysis. The differences in cytokine levels between the untreated POEMS group, relapsed POEMS group, normal subjects group, and CIDP group were compared by nonparametric multiple comparison tests (Steel tests) with the Bonferroni correction to determine which pairs differed significantly. The differences in cytokine levels pretreatment and posttreatment were compared by nonparametric paired Wilcoxon tests.

RESULTS Participants. From April 2000 to July 2009, 30 patients with POEMS syndrome visited Chiba University Hospital. Of them, 25 patients met the recruiting criteria and were included in this study, and their sera were utilized. Of these, 16 serum samples of untreated group, 7 samples in 6 patients of relapsed group, and 10 paired samples before and after treatments were obtained, respectively. Among the treated 10 patients, 4 were treated with HCT with PBSCT, and the remaining 6 were treated with thalidomide (table e-1), and their mean treatment periods at analysis were 92.9 ± 8.0 days (±SEM). Of those 10 patients, 4 were already treated partially by steroid at the start of HCT with PBSCT or thalidomide therapy.

Clinical manifestations of patients with POEMS syndrome. The detailed clinical data are shown in table e-1. The mean ages of untreated, relapsed, and treated patients groups were 57.1 (range 34–78), 62.4 (range 41–84), and 61.2 (range 43–84), respectively. In the relapsed group, high incidence of pleural effusions was noted.

Abnormal cytokine network in POEMS syndrome: Markedly increased serum IL-12 as well as VEGF. Serum levels of the following 27 cytokines were measured using the Bio-Plex Cytokine Assay System (Bio-Rad Laboratories, Hercules, CA). Among the

Table 1 **Summary of serum cytokine levels**[a]

	Control (n = 19)		POEMS syndrome (n = 23)			CIDP (n = 10)		
	Mean	(SD)	Mean	(SD)	p Value (vs control)	Mean	(SD)	p Value (vs control)
Increased in POEMS syndrome								
VEGF	154	(94)	2,588	(1,180)	<0.000005	230	(214)	NS
IL-12 (p70)	10.7	(5.6)	78.7	(32.1)	<0.000005	12.9	(9.4)	NS
IL-10	0.06	(0.21)	1.67	(1.35)	<0.00001	0.26	(0.21)	NS
IL-13	4.2	(1.1)	9.6	(2.8)	<0.00001	3.8	(1.2)	NS
IL-6	4.5	(1.9)	207	(683)	<0.00005	74	(217)	NS
IL-7	4.9	(1.2)	12.4	(5.7)	<0.0001	7.6	(3.1)	NS
IL-17	ND	—	8.5	(11.4)	<0.005	8.8	(15.0)	NS[b]
TNF-α	1.5	(4.5)	73.6	(103)	<0.005	82.5	(142)	NS
IL-8	12.9	(2.6)	461	(932)	<0.01	356	(926)	NS
IL-1β	0.73	(0.57)	14.5	(44.6)	<0.05	3.6	(8.2)	NS
MIP-1α	0.69	(3.0)	125	(268)	<0.05	21.9	(51.9)	NS
GM-CSF	9.5	(12.6)	26.0	(18.9)	<0.05	12.3	(11.0)	NS
Decreased in POEMS syndrome								
RANTES	10,740	(2,728)	5,135	(1,398)	<0.000005	9,090	(3,954)	NS
Eotaxin	391	(190)	191	(81)	<0.005	339	(104)	NS
Unvaried between POEMS syndrome and control								
IL-1ra	41.7	(16.9)	67.5	(38.9)	NS[b]	79.1	(55.1)	NS
IFN-γ	68.7	(34.6)	93.0	(33.9)	NS[b]	69.5	(20.6)	NS
IL-5	1.4	(0.5)	2.4	(2.7)	NS[b]	1.4	(0.6)	NS
IL-4	1.9	(0.6)	2.3	(0.8)	NS	2.1	(0.6)	NS
IL-9	53.9	(162)	33.2	(76.5)	NS	8.8	(4.4)	NS
MCP-1	25.5	(11.6)	53.9	(48.6)	NS	31.6	(18.0)	NS
G-CSF	1.5	(1.0)	2.2	(1.5)	NS	1.4	(1.4)	NS
MIP-1β	148	(44)	593	(621)	NS	603	(779)	NS
FGF basic	ND	—	2.4	(8.7)	NS	ND	—	NS
PDGF-BB	13,870	(2,915)	14,280	(2,638)	NS	1,393	(440)	NS
IP-10	2,549	(1,726)	2,590	(1,164)	NS	3,442	(3,882)	NS
Undetected cytokines both in POEMS syndrome and control								
IL-2	ND	—	ND	—	—	ND	—	—
IL-15	ND	—	ND	—	—	ND	—	—

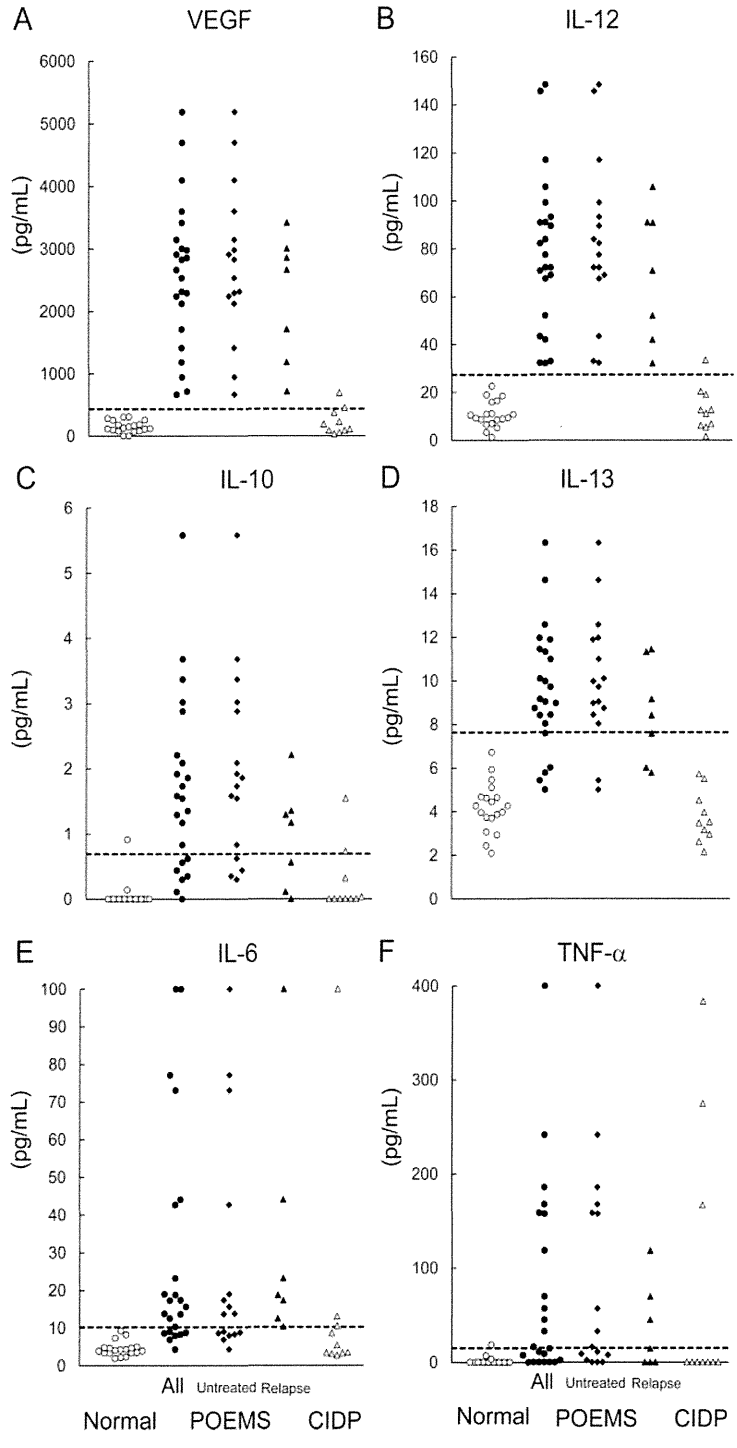
Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy; FGF = fibroblast growth factor; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; IP = induced protein; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; ND = not detected; NS = not significant; PDGF = platelet-derived growth factor; POEMS = polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes; RANTES = regulated upon activation, normal T cell expressed and secreted; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

[a] Unit: pg/mL.

[b] Significant before Bonferroni correction.

27 cytokines, IL-2 and IL-15 were not detected in any samples. Table 1 shows the profiles of the remaining 25 cytokines detected in POEMS syndrome, CIDP, and normal controls. Fourteen of the 25 cytokines exhibited significant differences between the POEMS group (untreated and relapsed patients group; n = 23) and the normal group. The serum levels of the following 12 cytokines were sig-

Figure 1 **Serum cytokine levels in patients with polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome, normal subjects, and patients with chronic inflammatory demyelinating polyneuropathy (CIDP)**



Serum levels of (A) vascular endothelial growth factor, (B) interleukin (IL)-12, (C) IL-10, (D) IL-13, (E) IL-6, and (F) tumor necrosis factor (TNF)-α. Open circle: normal subjects; closed circle: all POEMS syndrome patients group; closed diamond: untreated POEMS syndrome group; closed triangle: relapsed POEMS syndrome group; open diamond: CIDP group. Dashed lines indicate the upper or lower limits of normal (mean ±3 SD in the normal group) serum cytokine levels.

nificantly increased in the POEMS group: IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, GM-CSF, MIP-1α, VEGF, and TNF-α (table 1). In contrast, eotaxin and RANTES levels (the range of RANTES: 4,013–7,962 pg/mL) were significantly decreased in the POEMS group.

Out of the 12 cytokines, the levels of which were significantly increased in POEMS syndrome, the values of serum VEGF (range 665–5,186 pg/mL) and IL-12 (range 32.3–148.4 pg/mL) were markedly increased in all patients, and they were all above the upper normal limits of normal (VEGF 436 pg/mL; IL-12 27.5 pg/mL) (figure 1, A and B). The levels of the other cytokines such as IL-6 (range 4.3–1,183 pg/mL) or TNF-α (range <1.7–400 pg/mL) in POEMS syndrome were significantly increased as a group, but in some patients the serum levels of such cytokines remained within the normal ranges (IL-6 10.2 pg/mL; TNF-α 15.0 pg/mL) (figure 1, C–F).

The cytokine profiles in the relapsed POEMS group were similar to those in the untreated POEMS group (figure 1, table e-2). These findings indicate that both IL-12 and VEGF levels were invariably increased in the initial progressive phase and during relapse of the disorder.

Changes in cytokine profiles after treatments: Rapid reduction of serum IL-12 and VEGF levels. The serum levels of the 25 cytokines in 10 patients with POEMS were compared before and 3 months after treatment (table 2). After the treatments, the VEGF and IL-12 levels were significantly decreased, whereas the IP-10, MCP-1, and RANTES levels were significantly increased after treatment (table 2, figure 2, A–D). Although not significant, the IL-6 and TNF-α levels were higher after treatment than their levels before treatment.

The changes in the serum IL-12 level and the individual clinical courses by treatments are presented in table 3. It shows that the decrement of serum IL-12 level by treatment would be associated with the improvement of clinical symptoms, especially in the HCT with PBSCT group. Two showed adverse increase of serum VEGF level after treatment. One was patient 9 (in table 3) and he also showed an increase in serum IL-12 level after treatment. He showed gradual improvement in neuropathic symptoms/signs, and his clinical recovery was seen after 6 months of initiation of chemotherapy. Another was patient 23, whose serum VEGF level was already normalized at the initiation of treatment because of preceding steroid treatment for several months. The highest serum IL-12 and VEGF levels after treatment were observed in patient 25 in table 3, whose clinical response to thalidomide therapy was poor. Those results suggested that the degrees of the decrements of

Table 2 **Summary of the effect of treatments on serum cytokine levels**[a]

	POEMS (n = 10)				
	Before		After		
	Mean	(SD)	Mean	(SD)	*p* Value (vs before)
Decreased after treatment					
IL-12 (p70)	60.9	(29.9)	30.1	(21.5)	<0.01
VEGF	1,996	(1,367)	785	(774)	<0.05
Increased after treatment					
IP-10	2,183	(1,541)	7,413	(5,139)	<0.01
RANTES	5,017	(1,071)	6,228	(1,029)	<0.05
MCP-1	48.9	(55.1)	114	(106)	<0.05
No change by treatment					
IL-1β	1.1	(0.6)	107	(239)	NS
IL-1ra	52.0	(21.1)	127	(120)	NS
IL-4	2.1	(0.5)	2.3	(0.8)	NS
IL-5	1.5	(0.5)	1.5	(0.5)	NS
IL-6	12.8	(11.6)	933	(1,693)	NS
IL-7	9.5	(4.4)	7.0	(3.6)	NS
IL-8	53.0	(81.0)	1,045	(1,561)	NS
IL-9	89.2	(169)	34.0	(52.1)	NS
IL-10	1.3	(1.1)	1.9	(1.8)	NS
IL-13	8.2	(2.6)	6.1	(2.4)	NS
IL-17	1.0	(1.6)	11.4	(16.5)	NS
TNF-α	75.8	(155)	346	(865)	NS
G-CSF	1.8	(0.7)	4.3	(5.6)	NS
GM-CSF	15.8	(9.7)	24.1	(23.8)	NS
IFN-γ	76.2	(25.1)	94.1	(51.2)	NS
MIP-1α	32.4	(70.6)	1,92	(2,454)	NS
MIP-1β	255	(355)	817	(841)	NS
PDGF-BB	14,700	(1,430)	11,400	(4,753)	NS
Eotaxin	241	(157)	349	(151)	NS

Abbreviations: G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; IP = induced protein; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; NS = not significant; PDGF = platelet-derived growth factor; POEMS = polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes; RANTES = regulated upon activation, normal T cell expressed and secreted; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

[a] Unit: pg/mL. FGF basic was not detected in any sample tested.

serum IL-12 as well as VEGF were associated with the clinical responses in individual patients.

DISCUSSION This study shows the occurrence of the cytokine storm in the POEMS syndrome. Among them, we found a marked increase in serum IL-12 as well as VEGF in patients with POEMS syndrome at untreated state or at the time of relapse. The findings of this study suggest that IL-12 could be a causative cytokine for the pathogenesis of demyelinating polyneuropathy and other systemic symptoms in POEMS syndrome, and that IL-12 can be effectively used as a biomarker of the disease activity associated with POEMS syndrome.

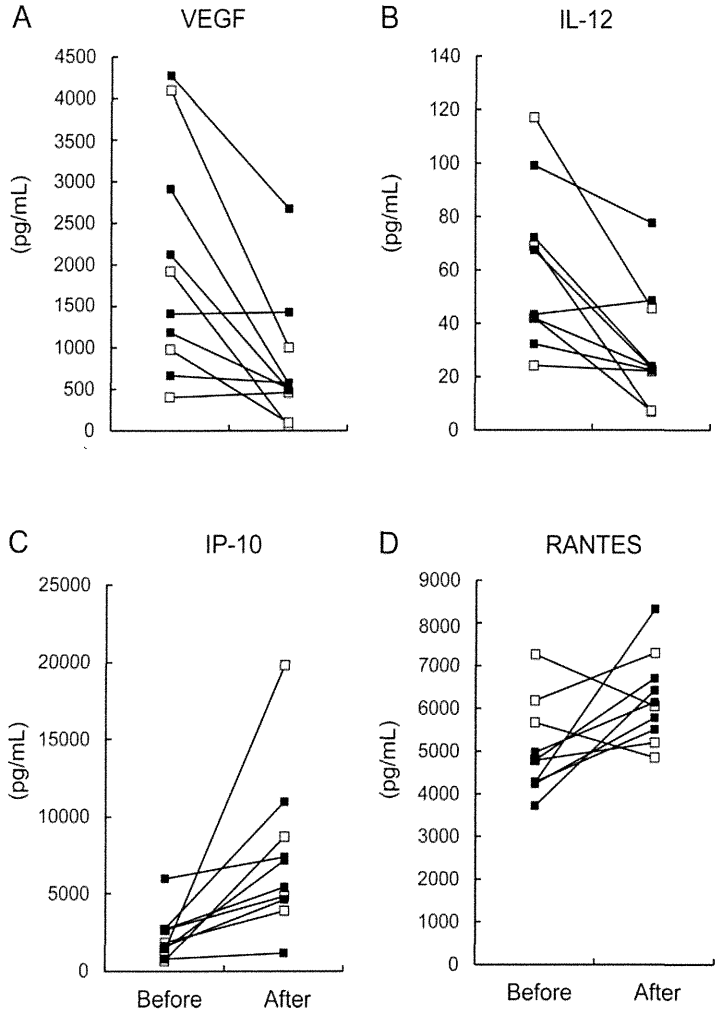
The serum levels of a number of the cytokines measured of patients with POEMS syndrome were significantly higher than normal, as well as abnormally high serum VEGF level in this study. Although it is difficult to discern which changes are primary or secondary, considering abnormally high serum VEGF level in all the patients[1] and rapid clinical responses to therapies,[1,2,15] we suppose that clinically significant changes of cytokines in POEMS syndrome would fulfill the following conditions: 1) greatly high (or low) level of the cytokine (over or below mean ±3 SD of normal subjects) in all the untreated POEMS syndrome patients, 2) similar change (greatly high or low) in the relapsed patients with POEMS syndrome, and 3) rapid change (decrease or increase) in response to the therapy. In this study, in addition to VEGF, IL-12 fulfilled all the above conditions. Furthermore, the changes in the serum IL-12 level by treatments, as the same as VEGF, seemed to correspond well with the individual clinical courses. These results suggest that both IL-12 and VEGF might play an important role in the pathogenesis of POEMS syndrome.

IL-12 is one of the proinflammatory cytokines, which is composed of 2 disulfide-linked subunits designated p35 and p40. IL-12 is produced by monocytes, macrophages, dendritic cells, neutrophils, and B cells. IL-12 plays important roles in both innate resistance and acquired immunity, because it induces interferon-γ production from natural killer (NK) and natural killer T (NKT) cells in the early phases of the immune response, and it is involved in the differentiation of Th1 cells, important in the anti-infective and antitumor responses.[16,17] To date, a mild to moderate increase of IL-12 has been seen in some diseases such as psoriasis or rheumatoid arthritis.[18–20]

At present, the precise molecular mechanisms involved in overproduction of both VEGF and IL-12 in POEMS syndrome are unclear. Abe et al.[21] reported that immunoglobulin light chain M protein found in POEMS syndrome is restricted to the Vλ-1 subfamily and markedly to very limited number of germlines. We think that overproductions of both VEGF and IL-12 would be possibly associated with unclarified mechanisms of monoclonal proliferation of plasma cells in POEMS syndrome, and also that it is possible that certain specific M proteins would trigger the overproduction of both VEGF and IL-12.

The results of this study provided us further new important insights into the pathophysiology of POEMS syndrome. Peripheral nerve demyelination is one of the important pathologic hall-

Figure 2 **Changes in serum cytokine levels in patients with polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) after treatment**



Serum levels of (A) vascular endothelial growth factor (VEGF) and (B) interleukin (IL)-12 significantly decreased after treatment, whereas (C) induced protein (IP)-10 and (D) regulated upon activation, normal T cell expressed and secreted (RANTES) levels increased. Open squares: values for patients treated with high-dose chemotherapy with autologous peripheral blood stem cell transplantation; closed squares: values for patients treated with thalidomide.

marks of POEMS syndrome.[22] To date, several proinflammatory cytokines are believed to contribute to nerve demyelination,[23] and the pathologic role of IL-12 in autoimmune demyelinating polyneuropathy has also been reported both experimentally and clinically.[20,24,25] Experimentally, injection of IL-12 into healthy rat peripheral nerves directly caused prominent demyelination,[24] and the critical role of IL-12 has been known in experimental autoimmune neuritis.[25] Clinically, mild and transient increase of serum IL-12 levels were reported in the acute phase of acute inflammatory demyelinating polyneuropathy.[20] Based on those circumstances, we considered that the sustained increase in serum IL-12 may contribute to the pathogenesis of demyelinating polyneuropathy in this syndrome, although this study did not show direct histopathologic evidence. We also hypothesize that in the molecular pathogenesis of the demyelinating polyneuropathy in POEMS syndrome, abnormally high serum levels of both VEGF and IL-12 would be necessary, where VEGF would disrupt the blood–nerve barrier, which in turn would allow IL-12 to access the nerve parenchyma, resulting in demyelination.

From a clinical viewpoint, the finding of an abnormally increased IL-12 level and rapid response to therapy in patients with POEMS, as shown in this study, has immense therapeutic implications. In particular, we have shown that IL-12 would be an important candidate for molecular targets in treatment of POEMS syndrome. The anti-IL-12 antibody has been used in several immunologic diseases.[26,27] Likewise, the anti-IL-12 antibody could be used as the molecular-targeted drug in the treatment of POEMS syndrome. While anti-IL-12 antibody alone would not be effective, as shown in anti-VEGF antibody monotherapy,[12,13] the combination of anti-VEGF and anti-IL-12 antibodies might prove to be a promising new treatment modality for POEMS syndrome.

Conversely, this study revealed the significant decrease of serum RANTES in POEMS syndrome and its significant recovery during the treatment. RANTES is known as one of the CC-chemokines, and it is associated with the activation and migration of eosinophils.[28] To date, relatively high incidence of drug-induced skin eruption has been reported in patients with POEMS syndrome treated with thalidomide.[4] The significant increase of RANTES during the chemotherapy in POEMS syndrome would be associated with such high incidence of drug-induced skin eruption. Therefore, RANTES would also be one of the factors that should be monitored during chemotherapy in POEMS syndrome.

This study has some limitations. One major point is the number of subjects: relatively few subjects were included in this study. In the analysis of the effects of treatments on cytokine levels, only 4 patients treated with HCT with PBSCT and 6 patients treated with thalidomide were involved. Furthermore, 2 pair of samples obtained from same patients (patient 13: 1 sample in untreated state and 1 sample in relapsed state; patient 19: 2 samples in relapsed state at 69 years old and at 71 years old; table e-1) were involved in this study. Those would lead to biased results. Further prospective validating studies with large scale will be warranted in the future.

The findings of this study suggest that IL-12 could be a causative cytokine for the pathogenesis of demyelinating polyneuropathy and other systemic

Table 3 **Effect of treatments on serum IL-12 and VEGF levels and their associations to clinical manifestations**

Patient	Pretreatment	IL-12		VEGF		Hughes grade[a]			Treatment period, d
		Before	After	Before	After	Before	At estimation	6 mo	
HCT with PBSCT									
2	None	69.1	6.8	1,920	58	3	2	2	91
22	Steroids	117.0	45.6	4,097	1,002	4	3	3	58
23	Steroids	24.2	22.1	402	459	3	3	3	141
24	Steroids	42.1	7.2	977	94	4	3	3	96
Thalidomide									
9	None	43.4	48.5	1,411	1,428	2	2	2	112
10	None	32.3	22.4	665	575	2	2	2	70
11	None	67.5	23.2	2,910	563	3	3	2	82
13	None	42.0	23.4	1,183	511	3	3	3	120
14	None	72.2	23.9	2,125	490	2	2	2	72
25	Steroids	99.2	77.6	4,273	2,673	4	4	4	87

Abbreviations: HCT = high-dose chemotherapy; IL = interleukin; PBSCT = peripheral blood stem cell transplantation; VEGF = vascular endothelial growth factor.
[a] 2, able to walk; 3, able to walk with aids; 4, chairbound.

symptoms in POEMS syndrome, and that IL-12 can be effectively used as a biomarker of the disease activity associated with POEMS syndrome. This also opens up a promising new molecular targeting therapy for the disorder.

AUTHOR CONTRIBUTIONS

K.K., S.S., M.M., and S.K. designed the study. K.K., S.S., K.S., and M.M. performed experiments. K.K. and S.K. drafted the manuscript. S.M., K.S., S.I., Y.F., Y.N., Y.S., A.N., and C.N. contributed the clinical data and specimens. S.T., H.Y., M.M., and F.N. made contribution of vital tools and supervised the experiments.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. **Go to Neurology.org for full disclosures.**

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