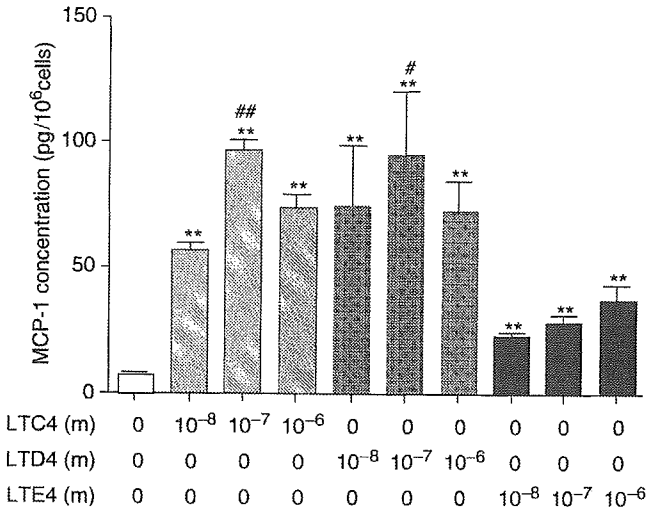

Fig. 1. Monocyte chemoattractant protein-1 (MCP-1) production, as measured by ELISA, in THP-1 cells stimulated with LTC4, -D4, or -E4 for 4 h. Data ($n = 8$) are presented as means ± SD. $^{**}P < 0.01$, compared with the culture fluid of cells not treated with LTC4, -D4, or -E4. $\#\#P < 0.01$, and $\#P < 0.05$, compared with the culture fluid of cells treated with 10^{-6} or 10^{-8} M LTC4, or -D4.

Table 1. Other cytokine productions stimulated by LTD4 in THP-1 cells and peripheral blood $CD14^+$ monocytes/macrophages (pg/mL)

	THP-1 cells		Peripheral blood $CD14^+$ monocytes/macrophages	
	Medium only	$+10^{6}$ M LTD4	Medium only	$+10^{6}$ M LTD4
TNF-α	<2.8	<2.8	<2.8	<2.8
IL-1β	<7.2	<7.2	<7.2	<7.2
IL-2	<2.6	<2.6	<2.6	<2.6
IL-4	<2.6	<2.6	3.0 ± 0.6	2.9 ± 0.5
IL-6	2.9 ± 0.4	3.1 ± 0.6	3.2 ± 0.8	3.3 ± 0.9
IL-8	<3.6	<3.6	4.2 ± 0.5	4.4 ± 0.7
IL-10	<2.8	<2.8	<2.8	<2.8
M-CSF	<9.0	<9.0	<9.0	<9.0
Eotaxin	<5.0	<5.0	<5.0	<5.0

M-CSF, macrophage-colony stimulating factor.

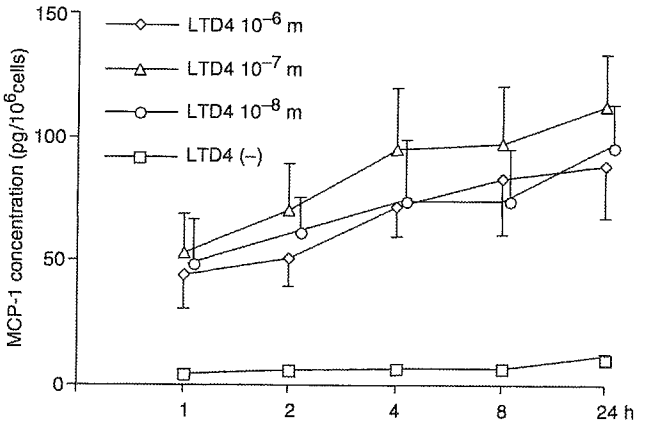

Fig. 2. Kinetics of monocyte chemoattractant protein 1 (MCP-1) production, as measured by ELISA, in THP-1 cells stimulated with LTD4. Data ($n = 8$) are presented as means ± SD.

pretreatments with 10^{-7} and 10^{-8} M pranlukast on MCP-1 production induced by 10^{-6}–10^{-8} M LTD4 were examined in THP-1 cells over 24 h. Pranlukast similarly blocked MCP-1 production induced by 10^{-6}–10^{-8} M LTD4 during the experiment (data not shown).

The purity of the $CD14^+$ cells obtained after negative selection with the MACS system was 87.1 ± 5.4%. The MCP-1 production induced by CysLTs and the inhibitory effect of pranlukast on MCP-1 production were examined by incubating peripheral blood $CD14^+$ monocytes/macrophages with CysLTs for 4 h, with some samples being pretreated with 10^{-6}–10^{-8} M pranlukast (Fig. 4). CysLTs (LTC4, -D4, and -E4) induced MCP-1 in peripheral blood $CD14^+$ monocytes/macrophages (192.2 ± 25.2 pg/10^5 cells, 211.4 ± 20.6 pg/10^5 cells, and 179.5 ± 28.0 pg/10^5 cells, respectively). Pretreatment with pranlukast significantly inhibited MCP-1 production induced by CysLTs in peripheral blood $CD14^+$ monocytes/macrophages in a dose-related manner. CysLTs (10^{-6}–10^{-8} M) did not induce TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, M-CSF, or eotaxin in peripheral blood $CD14^+$ monocytes/macrophages (Table 1).

RT-PCR demonstrated that MCP-1 mRNA expression was significantly increased 4 h after the addition of 10^{-6} M CysLTs in THP-1 cells ($P < 0.05$) (Fig. 5). Moreover, pretreatment with 10^{-6} M pranlukast significantly decreased MCP-1 mRNA expression caused by the stimulation of CysLTs in THP-1 cells ($P < 0.05$).

The effect of MCP-1 on the expression of CCR2B, an MCP-1 receptor [17], was examined using Western blot analysis (Fig. 6). The expression of CCR2B increased significantly 24 h after the introduction of 10^{-6} M CysLTs into THP-1 cells ($P < 0.05$). Moreover, pretreatment by 10^{-6} M pranlukast significantly decreased CCR2B expression stimulated by CysLTs in THP-1 cells ($P < 0.05$).

Discussion

A previous study demonstrated that LTD4 activates mitogen-activated protein kinase in THP-1 cells [13]. Macrophage inflammatory protein-1α (MIP-1α), TNF-α, and nitric oxide were produced in NR8383 cells, a rat AM cell line, exposed to lipopolysaccharide pretreated with LTD4 [6]. Our present study showed that LTC4, -D4, and -E4 induced MCP-1 in THP-1 cells and $CD14^+$ monocytes/macrophages, and increased CCR2B expression in THP-1 cells. LTC4, -D4, and -E4 enhance autocrine MCP-1 production by increasing CCR2B expression in monocytes/macrophages. Our present data suggest that MCP-1 is produced via the bonding of LTC4, -D4, and -E4 to the CysLT receptor because pranlukast inhibits MCP-1 production induced by LTC4, -D4, and -E4. MCP-1 production induced by 10^{-7} M LTC4 and -D4 was significantly higher than that induced by 10^{-6} M LTC4 and -D4. Ménard and Bissonnette [6] reported that MIP-1α production induced by 10^{-11} M LTD4 in AM was more than that induced by 10^{-6}, 10^{-8}, 10^{-10}, 10^{-12}, or 10^{-14} M LTD4. Therefore, it is unlikely that cytokine production induced by CysLTs is dose related. Our present data revealed that LTC4 and LTD4 were equally potent in stimulating MCP-1 release in THP-1 cells, and LTC4, LTD4,

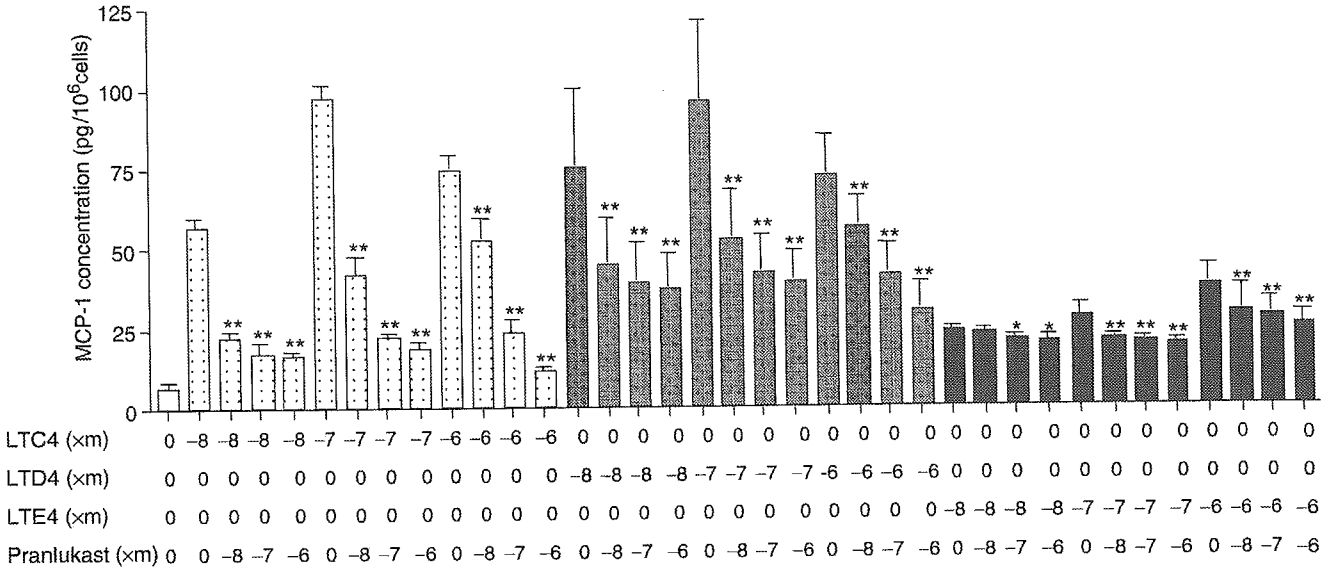

Fig. 3. Inhibitory effect of pranlukast on monocyte chemoattractant protein 1 (MCP-1) release, as measured by ELISA, in THP-1 cells stimulated with LTC4, -D4, or -E4 for 4 h (cells pretreated with pranlukast for 30 min before LTC4, -D4, or -E4 treatments). Data ($n = 8$) are presented as means ± SD. $^{**}P < 0.01$, and $^{*}P < 0.05$, compared with the culture fluid of cells treated with LTC4, -D4, or -E4 only (no pretreatment).

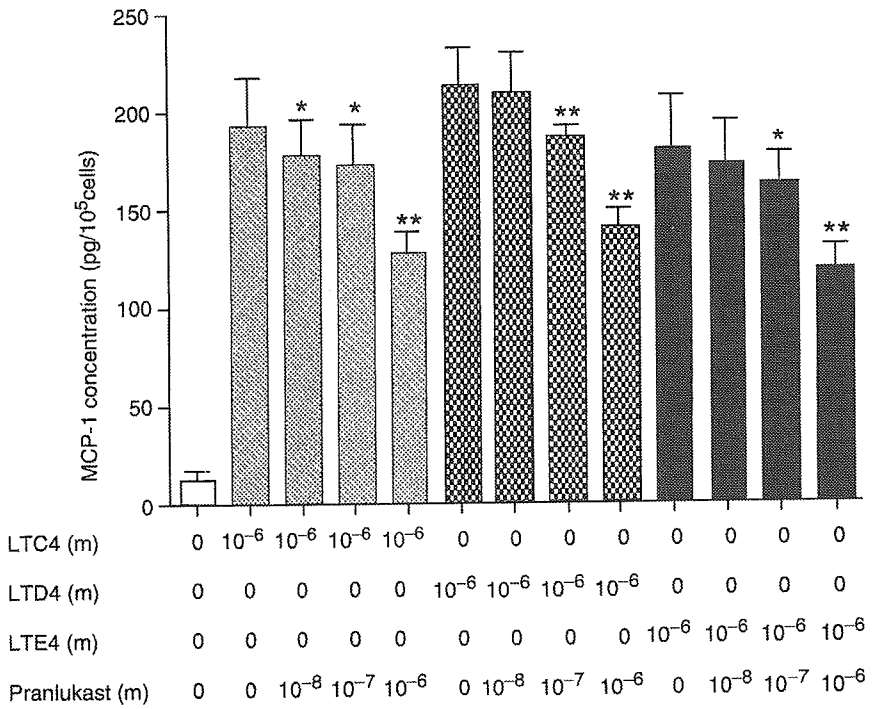

Fig. 4. Monocyte chemoattractant protein 1 (MCP-1) production and the inhibitory effect of pranlukast on MCP-1 release, as measured by ELISA, in peripheral blood $CD14^+$ monocytes/macrophages stimulated with LTC4, -D4, or -E4 for 4 h (cells pretreated with pranlukast for 30 min before LTC4, -D4, or -E4 treatments). Data ($n = 10$) are presented as means ± SD. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with culture fluid of cells treated with LTC4, -D4, or -E4 only.

and LTE4, in peripheral blood $CD14^+$ monocytes/macrophages. LTD4 would be expected to be about tenfold more potent for calcium flux at the CysLT1 receptors than LTC4 (and 100-fold more than LTE4) [18, 19]. The mechanism of MCP-1 production via CysLT1 receptors of monocytes/macrophages stimulated by CysLTs is different from that of the contraction via CysLT1 receptors of airway smooth muscle stimulated by CysLTs. It is likely that the potencies of LTC4, LTD4, and LTE4 for MCP-1 production from monocytes/macrophages are different from those for the contraction of airway smooth muscle via calcium flux.

MCP-1 is part of the C–C chemokine β subfamily and exhibits its most potent chemotactic activity towards monocytes and T lymphocytes [20]. MCP-1 has multiple functions, such as causing an increase in cytosolic calcium levels, stimulating superoxide anion production and lysosomal

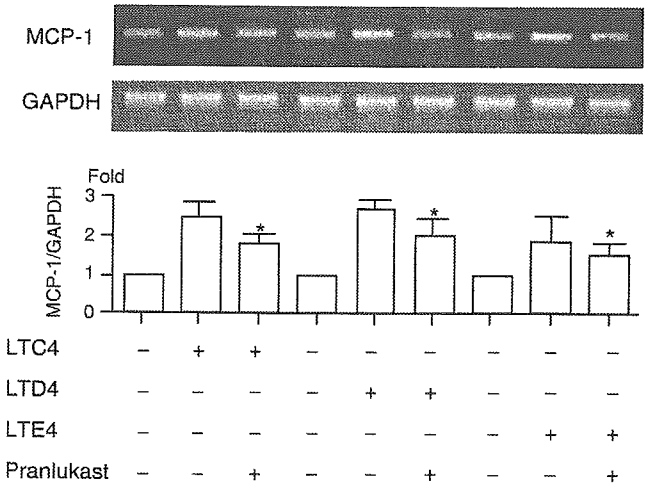

Fig. 5. Monocyte chemoattractant protein 1 (MCP-1) mRNA expression and the inhibitory effect of pranlukast on MCP-1 mRNA expression, as measured by RT-PCR, in THP-1 cells stimulated with 10^{-6} M LTC4, -D4, or -E4 for 4 h (cells pretreated with 10^{-6} M pranlukast for 30 min before LTC4, -D4, or -E4 treatments). Representative data are shown. After densitometric analysis, data ($n = 4$) were expressed as fold-increase above untreated cells and presented as means ± SD. *$P < 0.05$ vs. cells treated with LTC4, -D4, or -E4 only.

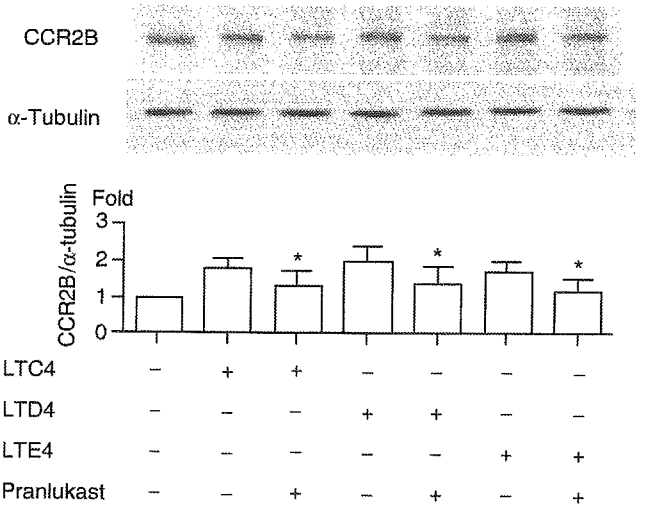

Fig. 6. CCR2B expression and the inhibitory effect of pranlukast on CCR2B expression, as measured by Western blot analysis, in THP-1 cells stimulated with 10^{-6} M LTC4, -D4, or -E4 for 24 h (cells pretreated with 10^{-6} M pranlukast for 30 min before LTC4, -D4, or -E4 treatments). Representative data are shown. After densitometric analysis, data ($n = 4$) were expressed as fold-increase above untreated cells and presented as means ± SD. *$P < 0.05$ vs. cells treated with LTC4, -D4, or -E4 only.

enzyme release in monocytes [21]. MCP-1 provokes mast cell aggregation [22] and histamine release in mast cells [23]. Moreover, MCP-1 induces chemotaxis and calcium mobilization in eosinophils [24]. MCP-1 induces LTC4 in the bronchoalveolar lavage of mice, pulmonary mast cells of mice, and human basophil leucocytes [25, 26]. A positive feedback loop might exist in the expression of MCP-1 and the CysLTs. MCP-1 causes not only the migration of monocytes but also of eosinophils and basophils, and induces basophil histamine release [20]. It is likely that CysLT-induced MCP-1 in monocytes/macrophages, such as AM, is related to the pathogenesis of bronchial asthma. In asthma, AM are activated and participate to create the cytokine milieu [7]. MCP-1 induced by AM and/or AM activated by MCP-1 may modify pulmonary inflammation in asthma. Our present study demonstrated that other cytokines, including TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, M-CSF, and eotaxin, stimulated with CysLTs were not produced in THP-1 cells or peripheral blood $CD14^+$ monocytes/macrophages. A previous paper reported that IL-4 was produced in PBMC from mite antigen-positive asthmatic patients stimulated with the mite antigen [27]. It is likely that cytokines other than MCP-1 were not produced in THP-1 cells or peripheral blood $CD14^+$ monocytes/macrophages because the stimulator was not a specific allergen.

A CysLT1 receptor antagonist inhibits contraction of the tracheal muscle [1]. Pranlukast has some actions in asthma other than as an antagonist of the CysLT1 receptor, including immunomodulation [27]. We have previously demonstrated that pranlukast inhibits TNF-α-induced nuclear factor-κB activation in monocytes/macrophages [28]. Pranlukast could exert its anti-inflammatory action via the inhibition of MCP-1 production induced by CysLT in monocytes/macrophages. Our present data demonstrated that 10^{-6} M pranlukast could block CysLT-induced MCP-1 release completely in THP-1 cells, but could only block about one-quarter of the MCP-1 release in peripheral blood $CD14^+$ monocytes/macrophages. The partial effect of pranlukast in peripheral blood $CD14^+$ monocytes/macrophages suggests that a part of the MCP-1 response may be mediated by CysLT2 receptors, not CysLT1 receptors. The purity of the $CD14^+$ cells obtained after negative selection with the MACS system was 87.1 ± 5.4%. MCP-1 might be produced by the interaction of mononuclear cells other than monocytes. There might be toxic effects of 10^{-6} M pranlukast on cells that have a reduced MCP-1 expression. In adults administered a single oral dose of 225 mg, C_{max} was 642.3 ± 151.0 ng/mL (1.31 ± 0.31 μM) [29], but this value included pranlukast bound to plasma protein. The concentration of free pranlukast was probably at least tenfold lower than this [30]. Therefore, we think that 10^{-7} and 10^{-8} M pranlukast are clinical doses, and 10^{-6} M is a pharmacological dose. However, the contribution of the anti-inflammatory activity of pranlukast at oral therapeutic doses in asthmatic patients remains unclear. We speculate that an additional study, involving the modulation of the regulatory system for monocytes/macrophages, on bronchial asthma is needed.

In conclusion, CysLTs induce MCP-1 and increase CCR2B expression in human monocytes/macrophages *in vitro*, and pranlukast inhibits CysLT-induced MCP-1 production and CCR2B expression in the cells.

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Analysis of cytokine levels in cerebrospinal fluid in mumps meningitis: Comparison with echovirus type 30 meningitis

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Abstract

Background: It is unclear whether or not the CSF cytokine profiles in viral meningitis differ with the kind of causative virus.
Methods: We measured the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, and IL-10 in CSF during the acute stage in 15 children with mumps meningitis (MM), and 34 with echovirus type 30 meningitis (EM).
Results: The CSF IFN-γ, IL-2, IL-6, and IL-10 levels were elevated in MM, and the CSF IFN-γ, IL-2, and IL-6 levels were elevated in EM. The CSF IFN-γ, IL-2, and IL-10 levels in MM were significantly higher than those in EM ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively). The CSF IL-6 levels in EM were significantly higher than those in MM ($p = 0.0255$). The CSF TNF-α and IL-4 levels were not elevated in MM or EM. In MM, the IL-6 level was correlated with the IL-2 and IL-10 levels in CSF ($p = 0.0347$ and $p = 0.0120$, respectively). In EM, the IFN-γ level was correlated with the IL-10 level in CSF ($p = 0.0002$).
Conclusion: CSF cytokine profiles in MM were different from those in EM. Therefore, it is likely that the pathogenesis of MM is different from that of EM.

Keywords: Cerebrospinal fluid; Cytokine; Echovirus type 30 meningitis; Mumps meningitis

1. Introduction

Aseptic meningitis is not rare in childhood. Enterovirus and mumps virus are frequently the causative agents of aseptic meningitis [1]. There have been many previous papers on cerebrospinal fluid (CSF) cytokine profiles in aseptic meningitis [2–6]. However, the causative viruses were not identified in most of the previous studies. There have been few papers on CSF cytokines in meningitis caused by a single viral agent, and all of these papers were related to enteroviruses [7–9]. Our hypothesis is that the CSF cytokine profiles in viral meningitis differ with the kind of causative virus because the immune response of a host to viral meningitis will differ with the kind of causative virus.

To determine whether or not the CSF cytokine profiles in mumps meningitis (MM) are different from those in echovirus type 30 meningitis (EM), we determined the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, and IL-10 as cytokines related to inflammation in CSF during the acute stage in children with MM and EM.

2. Patients and methods

Informed consent was obtained from the parents of the patients and controls enrolled in this study. The protocol was approved by the institutional review board

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of Yamaguchi University Hospital and the ethics committee of Sinnanyo Citizen Hospital.

2.1. Mumps meningitis (MM)

CSF samples were obtained from 15 children with MM (14 males and one female, aged from 2 to 10 years; median, 5.9 years) on admission to our hospital and Sinnanyo Citizen Hospital in Japan, from January 1994 to August 2002 (Table 1). The diagnosis of mumps was based on common clinical symptoms such as painful enlargement of the salivary glands and an increase in the antibody titer determined by enzyme-linked immunosorbent assaying (EIA). The criteria for the diagnosis of aseptic meningitis were (1) clinical symptoms and signs compatible with viral meningitis, (2) a CSF pleocytosis level of >7 leukocytes/μl, and (3) negative CSF bacterial cultures. The day of onset of symptoms associated with meningitis was considered as the first day of illness. CSF samples were taken from children with MM on days 1–2 (median, 2.0) of the illness.

2.2. Echovirus type 30 meningitis (EM)

An outbreak of EM occurred from May to August 1998 in Yamaguchi prefecture, Japan. CSF samples were obtained from 34 children with EM (25 males and nine females, aged from 1 month to 8 years; median, 5.2 years) on admission to our hospital from May to July 1998 (Table 1). The subjects in our present study were completely different from those in our previous study [7]. The criteria for the diagnosis of aseptic meningitis associated with echovirus type 30 were (1) clinical symptoms and signs compatible with viral meningitis, (2) isolation of echovirus type 30 from CSF, the throat, and/or a stool, or a four-fold increase in the antibody titer determined with the neutralization test (NT), (3) a CSF pleocytosis level of >7 leukocytes/μl, and (4) negative CSF bacterial cultures. CSF samples were taken from children with EM on days 1–5 (median, 2.0) of the illness.

2.3. Control subjects

The control subjects were 21 afebrile and noninfected children with neurological disorders (12 males and nine females, aged from 3 months to 15 years: median, 3.7 years). CSF samples were obtained from them on neurological examination and they all had normal CSF cell counts (Table 1).

2.4. Determination of cytokine concentrations

The concentrations of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA) according to the manufacturer's manual, as previously described [10–12], with modification of the data analysis using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). Briefly, a CBA comprises a series of beads exhibiting discrete fluorescence intensities at 670 nm. Each series of beads is coated with a monoclonal antibody against a single cytokine, and a mixture of the six series of beads can detect six cytokines in one sample. A secondary phycoerythrin (PE)-conjugated monoclonal antibody stains the beads proportionally to the amount of bound cytokine. After fluorescence intensity calibration and electronic color compensation procedures, standard and test samples were analyzed with a FACScan flow cytometer equipped with CellQuest software (BD PharMingen). Data were transferred to GraphPad Prism. Starting with standard dilutions, the software performed log transformation of the data, and then fitted a curve to 10 discrete points using a four-parameter logistic model. The calibration curve created for each cytokine was used to determine the cytokine concentrations in the samples. The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1 pg/ml, 2.8 pg/ml, 2.6 pg/ml, 2.6 pg/ml, 2.5 pg/ml, and 2.8 pg/ml, respectively.

2.5. Statistical analysis

All values are medians ± 1 SD. The differences in the results between the groups were analyzed by means of

Table 1
Data for patients with mumps meningitis and echovirus type 30 meningitis, and controls

	Mumps meningitis	Echovirus type 30 meningitis	Controls
Number	15	34	21
Age	5.9 yr, 2 yr–10 yr	5.2 yr, 1 mo–8 yr	3.7 yr, 3 mo–15 yr
Sex (male:female)	14:1	25:9	12:9
Onset of symptoms to sampling of CSF (days)	2.0, 1–2	2.0, 1–5	–
Duration of fever (days)	4.0, 3–5**	2.0, 0–5	–
Hospitalization period (days)	5.0, 3–8*	3.0, 1–8	–
CSF protein (mg/dl)	30.0, 10–53	25.5, 10–84	19.0, 10–34
CSF cell count (/μl)	145, 28–648	79, 10–902	1.0, 0–6

**Significant at $p < 0.0001$ vs. echovirus type 30 meningitis. *Significant at $p = 0.0013$ vs. echovirus type 30 meningitis. (median, ranges).

the Mann–Whitney U test, a p value of less than 0.05 being taken as significant. Correlations were analyzed with use of Spearman's rank correlation coefficient test.

3. Results

The duration of fever and the hospitalization period in MM were significantly longer than those in EM ($p < 0.0001$ and $p = 0.0013$, respectively) (Table 1). There was no significant difference in age, onset of symptoms to sampling of CSF, CSF protein or cell counts between the two groups.

The concentrations of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF of the control subjects were 13.9 ± 14.8 pg/ml, 3.0 ± 1.9 pg/ml, 2.9 ± 1.0 pg/ml, 5.2 ± 2.9 pg/ml, 3.8 ± 2.9 pg/ml, and 3.8 ± 1.5 pg/ml, respectively. The CSF IFN-γ, IL-2, IL-6, and IL-10 concentrations of MM and EM are shown in Fig. 1. The CSF IFN-γ, IL-2, IL-6, and IL-10 levels were elevated in MM, and the CSF IFN-γ, IL-2, and IL-6 levels were elevated in EM. The CSF IFN-γ levels in MM were significantly higher than those in EM (471 ± 1118 pg/ml vs. 61.2 ± 67.3 pg/ml, $p < 0.0001$). The CSF IL-2 levels in MM were significantly higher than those in EM (5.9 ± 4.7 pg/ml vs. 4.0 ± 1.2 pg/ml, $p < 0.0001$). The CSF IL-6 levels in EM were significantly higher than those in MM (1023 ± 1724 pg/ml vs. 556 ± 923 pg/ml, $p = 0.0255$). The CSF IL-10 levels were elevated in MM (84.4 ± 133 pg/ml), but not in EM (4.1 ± 4.7 pg/ml). The CSF TNF-α and IL-4 levels were not elevated in MM or EM.

In MM, the IL-6 level was correlated with the IL-2 and IL-10 levels in CSF ($p = 0.0347$ and $p = 0.0120$) (Fig. 2A and B). In EM, the IFN-γ level was correlated with the IL-10 level in CSF ($p = 0.0002$) (Fig. 3). Moreover, the protein level was correlated with the IL-6 levels in CSF of MM and EM ($p = 0.0498$ and $p = 0.0392$), and the cell count was correlated with IL-10 level in CSF of MM ($p = 0.0320$).

4. Discussion

Our previous study revealed that the numbers of macrophages in CSF were increased, and the concentrations of CSF monocyte chemoattractant protein 1 (MCP-1), which is responsible for the accumulation of macrophages at the inflammatory site [13], IFN-γ, and IL-12 were elevated in EM [7], and that the ratio of CD4/CD8 in CSF of patients with EM was high (3.0 ± 1.2). Sato et al. reported that CSF IL-6, IL-8, and IFN-γ were elevated during the acute phase, and CSF IL-10 and transforming growth factor β1 (TGF-β1) were elevated in the recovery phase in EM [8].

We revealed that the CSF cytokine profiles in MM were different from those in EM. From the clinical point of view, the duration of fever and the hospitalization period in MM were significantly longer than those in EM. MM was clinically more severe compared with EM. We suggest that the immune response of a host to viral meningitis differs with the kind of causative virus.

The previous studies demonstrated that CD8+ cytotoxic T lymphocytes (CTL) play an important role in CSF of MM [14,15]. Taking these reports in consideration, a higher level of CSF IFN-γ in MM may indicate that IFN-γ is mainly produced by CD8+ CTL in CSF in MM. In our present study, IL-2, as Th1 cytokine, levels were elevated in CSF in MM. This finding indicates the activation of CD4+ Th1 cells. Therefore, it cannot be regarded that only CD8+ CTL produce IFN-γ in CSF in MM because activated CD4+ Th1 cells produce IFN-γ [16]. With respect to cytokine levels in CSF, we suggest that CD8+ CTL and/or

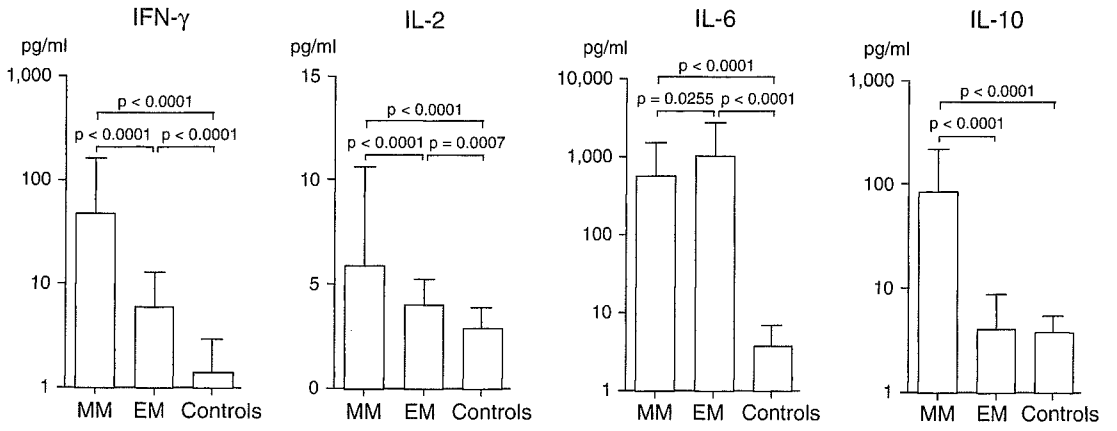

Fig. 1. CSF concentrations of IFN-γ, IL-2, IL-6, and IL-10 in patients with mumps meningitis (MM), echovirus type 30 meningitis (EM), and controls. Data are presented as medians + 1 SD. The CSF IFN-γ, IL-2, and IL-10 levels in patients with MM were significantly higher than those with EM ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively). The CSF IL-6 level in patients with EM was significantly higher than those with MM ($p = 0.0255$).

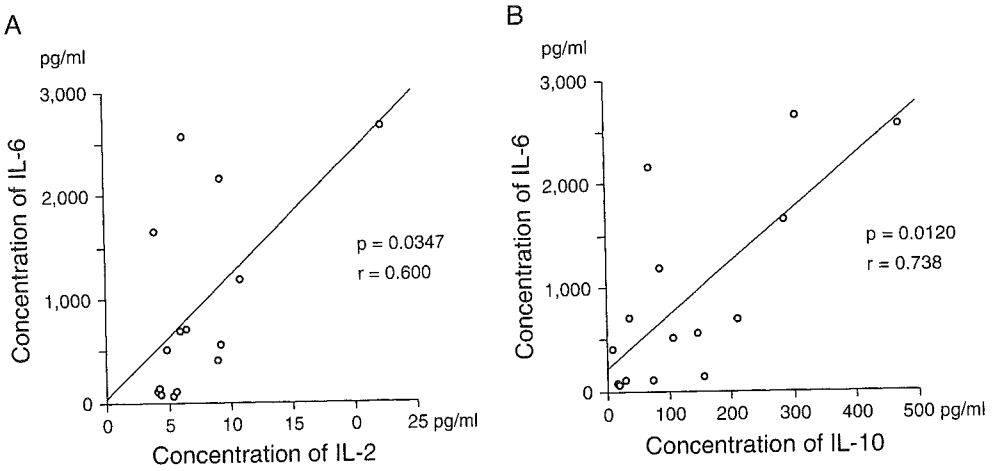

Fig. 2. Relationship between the IL-6 and IL-2 concentrations in CSF of patients with MM (A). Relationship between the IL-6 and IL-10 concentrations in CSF of patients with MM (B).

CD4+ Th1 cells play an important role in CSF in MM during the acute phase. IL-10, which is mainly produced by CD4+ Th2 cells, inhibits cytokine production by CD4+ Th1 cells [17]. Therefore, we suggest that IL-10 is induced in response to higher production of IFN-γ to modulate the balance of Th1 and Th2 in MM. IL-6 may be mainly produced by CD4+ Th2 cells because the IL-6 level is correlated with the IL-2 and IL-10 levels in CSF in MM.

We suggest that the role of CD4+ Th1 cells is dominant compared with that of CD8+ cells as concern lymphocytes in the pathogenesis of EM during the acute phase [7]. Therefore, the level of CSF IFN-γ mainly induced by CD8+ CTL in EM was significantly lower than that in MM. IFN-γ may be mainly produced by CD4+ Th1 cells because the IFN-γ level is correlated with the IL-10 level in CSF in EM. Moreover, monocytes/macrophages will play an important role in the pathogenesis in CSF in EM [7]. The higher level of CSF IL-6 in EM than that in MM may indicate that IL-6 is mainly produced by monocytes/macrophages. Dalal et al. demonstrated that CSF IL-6 and IFN-γ were elevated in echovirus type 4 meningitis, and that the IL-6 level was correlated with the leukocyte count in CSF [9]. We demonstrated that the IL-6 level was correlated with the protein level in CSF in EM, but not with the leukocyte count. The immunological pathogenesis of echovirus type 4 meningitis may be different from that of echovirus type 30 meningitis.

In summary, the CSF cytokine profile in MM was different from that in EM. We suggest that CD8+ CTL and/or CD4+ Th1 cells in MM, and monocytes/macrophages and CD4+ Th1 cells in EM play important roles during the acute phase in the pathogenesis of meningitis. Therefore, the pathogenesis of viral meningitis will differ with the kind of causative virus.

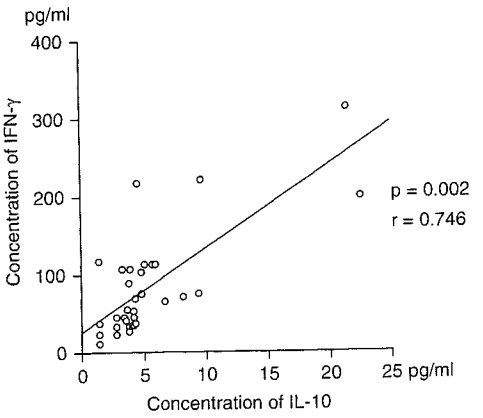

Fig. 3. Relationship between the IFN-γ and IL-10 concentrations in CSF of patients with EM.

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Analysis of serum soluble CD40 ligand in patients with influenza virus-associated encephalopathy

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Abstract

CD40 ligand (CD40L) is mainly expressed on activated platelets and CD4+T cells, and it can be cleaved from the cell surface, releasing a soluble CD40L (sCD40L). Most sCD40L is derived from activated platelets. A previous paper revealed that the platelet number of patients with influenza virus-associated encephalopathy (IE) was correlated with the outcome. We determined the utility of sCD40L as a predictor for the prognosis of IE. We measured the serum concentration of sCD40L and the platelet number on the day of hospitalization in 34 patients with IE, 16 with influenza virus-associated febrile seizures (IFS), 19 with influenza virus infection without complications (Flu), and 7 with Epstein–Barr virus (EBV) infection. The serum sCD40L concentrations in IE and IFS were significantly lower than those in controls, Flu, and EBV infections. Serum sCD40L concentrations in the IE group were 0.70±0.43 ng/ml for deceased patients, 1.73±1.36 ng/ml for those with sequelae, and 3.85±2.91 ng/ml for those without sequelae. There was no significant difference in platelet number between IE patients with and without sequelae, while the platelet number of deceased patients with IE was significantly lower than in controls, Flu, and IFS. Serum sCD40L concentration on the day of hospitalization was more correlated with the outcome of IE than platelet number. Our findings suggest that the serum sCD40L concentration during acute IE is important for predicting the prognosis at an early stage.

Keywords: Epstein–Barr virus; Influenza virus-associated encephalopathy; Platelet; Soluble CD40 ligand

1. Introduction

CD40 ligand (CD40L) is a transmembrane protein expressed on activated platelets and CD4+T cells [1,2]. CD40L can be cleaved from the cell surface, releasing a soluble CD40L (sCD40L) which is biologically active [2,3]. Several previous papers demonstrated that most serum sCD40L is derived from platelets [4–6].

Many patients with influenza virus-associated encephalopathy (IE) have been reported in Japan [7–9], and recently, some cases have been reported in Europe and the United States [10,11]. Pathological findings revealed that viral antigens and inflammatory cells were undetectable in brain tissues and suggest that direct viral invasion does not induce IE [12,13]. Serum and CSF concentrations of several proinflammatory cytokines and cytokine receptors, such as interleukin-6 (IL-6), IL-1β, and soluble tumor necrosis factor (TNF) receptor 1, are elevated and related to the clinical severity of IE [14–16]. Moreover, the platelet number tends to be correlated with the outcome of IE [8,12,17]. To evaluate the utility of sCD40L in the severity and prognosis of IE, we measured the serum sCD40L concentrations on the day of hospitalization in patients with IE, influenza virus-associated febrile seizures (IFS), influenza virus infection without complications (Flu), and Epstein–Barr virus (EBV) infection.

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2. Materials and methods

2.1. Influenza virus-associated encephalopathy (IE)

Informed consent was obtained from the parents of the patients enrolled in this study. Serum samples were obtained from 34 patients with IE, 16 with IFS, and 19 with Flu on admission to our hospital and eleven research cooperation hospitals in Japan, from December 1999 to March 2005 (Table 1). We divided the patients with IE into three groups, i.e., those who had no sequelae (Group A, $n=18$), those who had neurological sequelae (Group B, $n=10$), and those who were deceased (Group C, $n=6$). The criteria for the diagnosis of IE were: (1) clinical symptoms and signs compatible with acute encephalitis/encephalopathy (defined as a febrile disorder with alteration of consciousness and slow activity on electroencephalography lasting for more than 24 h after an acute onset), and no bacteria or fungi on CSF culture, with all other neurological, vascular, endocrine, toxic and drug-induced disorders having been excluded, and (2) isolation of the influenza virus from the throat, or a four-fold increase in the antibody titer determined by means of the hemagglutination inhibition test and/or virus antigen detection in the throat with the latex agglutination test. The day of onset of neurological symptoms was considered as the first day of illness. Serum samples were taken from the patients with IE on days 1.2±0.5 (range, 1–3) of the illness.

2.2. Influenza virus-associated febrile seizures (IFS)

Influenza virus-associated febrile seizures were defined as seizures with fever and impaired consciousness lasting less than 24 h without neurological sequelae, and influenza virus infection was proven by the above-mentioned method. Sixteen patients enrolled with IFS (10 males and 6 females, aged from 11 months to 10 years: mean, 5.2 years) (Table 1). The day of seizure onset was considered as the first day of illness. Serum samples were taken from the patients with IFS on days 1.1±0.3 (range 1–2) of the illness.

2.3. Influenza virus infection without complications (Flu)

Nineteen patients enrolled with influenza virus infections had fever and upper respiratory symptoms (9 males and 10 females, aged from 14 months to 9 years: mean, 5.4 years) (Table 1). Influenza virus infection was proven by the latex agglutination test. The day of fever onset was considered as the first day of illness. Serum samples were taken from these patients on days 2.1±1.1 (range, 1–4) of the illness.

2.4. Epstein–Barr virus (EBV) infection

The disease control subjects of viral infections were 7 patients who had EBV infection (three males and four females, aged from 2 to 12 years: mean, 5.9 years) (Table 1). The diagnosis was based on clinical presentation of a sore throat, fever, and bilateral cervical lymphadenopathy accompanied by atypical lymphocytes in the peripheral blood. All patients were positive for IgM and IgG antibodies to the EBV capsid antigen and negative for antibodies to the EBV nuclear antigen during the acute stage. The day of fever onset was considered as the first day of illness. Serum samples were taken from the patients with EBV infection on days 6.8±3.8 (range, 2–13) of the illness.

2.5. Control subjects

The control subjects were 25 healthy children (14 males and 11 females, aged from 3 months to 11 years: median, 5.4 years).

2.6. Determinations of the sCD40L concentration and platelet number

The serum concentrations of sCD40L were measured with a sandwich-type ELISA kit (R&D Systems, Minneapolis, MN). The detection limit was 4.2 pg/ml. Platelet number was assessed in the blood samples by standard automated techniques.

Table 1
Data for subjects

Group	Number	Age (years) (range)	Sex male:female	Type
IE-Group A	18	5.8±4.5 (6 months–13 years)	12:6	H1N1=1, H3N2=6, A=5, B=6
IE-Group B	10	5.9±6.6 (9 months–19 years)	4:6	H1N1=2, H3N2=4, A=3, B=1
IE-Group C	6	5.4±3.8 (2 years–10 years)	5:1	H3N1=5, A=1
IFS	16	5.2±3.0 (11 months–10 years)	10:6	A=10, B=6
Flu	19	5.4±3.1 (14 months–9 years)	9:10	A=13, B=6
EBV infection	7	5.9±3.2 (2 years–12 years)	3:4	
Controls	25	5.4±3.2 (3 months–11 years)	14:11	

IE=influenza virus-associated encephalopathy; IFS=Influenza virus-associated febrile seizures; Flu=influenza virus infection without complications; EBV=Epstein-Barr virus.
Group A=patients without sequelae; Group B=patients with sequelae; Group C=deceased patients.

2.7. Statistical analysis

All values are the means±S.D. Differences in the results between groups were analyzed by the Mann–Whitney *U*-test, with a *p*-value of less than 0.05 being taken as significant. Correlations were analyzed with Spearman's rank correlation coefficient test.

3. Results

The serum sCD40L concentrations of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization are shown in Fig. 1. The serum sCD40L concentrations of IE-Group A, IE-Group B, IE-Group C, and IFS were significantly lower than those of Flu ($p=0.0015$, $p=0.0002$, $p=0.0034$, and $p=0.0123$, respectively), EBV infection ($p=0.0105$, $p=0.0047$, $p=0.00269$, and $p=0.0235$, respectively) and controls ($p=0.0003$, $p<0.0001$, $p=0.0004$, and $p=0.001$, respectively). In the IE group, the serum sCD40L concentration of Group C was significantly lower than those of Groups A ($p=0.0077$) and B ($p=0.0409$), and that of Group B was significantly lower than that of Group A ($p=0.044$). The serum sCD40L concentration of IE-Group C was significantly lower than that of IFS ($p=0.0066$). There were no significant differences in serum sCD40L concentrations among Flu, EBV infection, and controls.

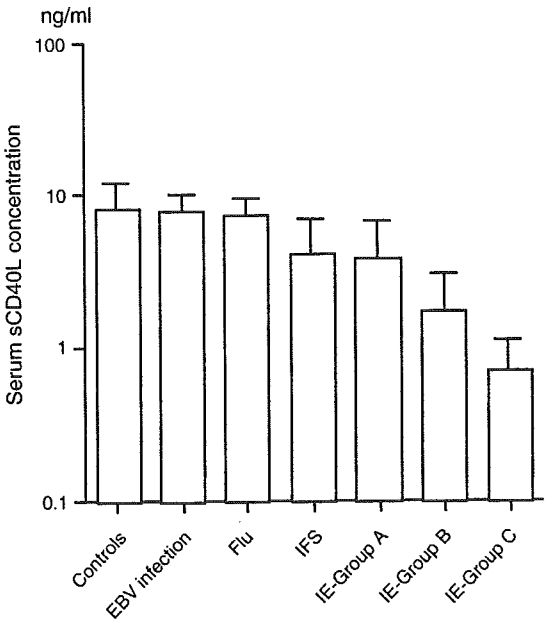

Fig. 1. The serum sCD40L concentrations of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization, and controls. Data are presented as means+1 S.D. IE-Group A, IE without sequelae; IE-Group B, IE with a poor prognosis; IE-Group C, the deceased patients with IE. The serum sCD40L concentration of controls was 8.23±3.61 ng/ml, EBV infection, 8.00±1.93 ng/ml, Flu, 7.49±1.96 ng/ml, IFS, 4.15±2.68 ng/ml, IE-Group A, 3.85±2.91 ng/ml, IE-Group B, 1.73±1.36 ng/ml, and IE-Group C, 0.697±0.428 ng/ml, respectively.

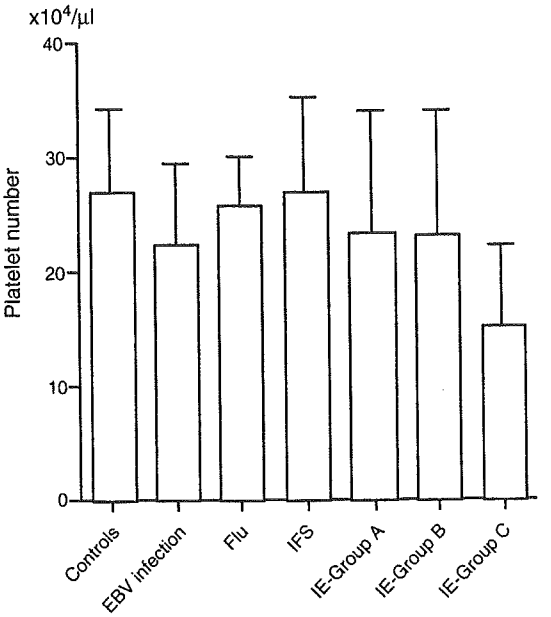

Fig. 2. The platelet numbers of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization, and controls. Data are presented as means+1 S.D. IE-Group A, IE without sequelae; IE-Group B, IE with a poor prognosis; IE-Group C, the deceased patients with IE. The platelet number of controls was $27.0\pm7.5\times10^4$/μl, EBV infection, $22.9\pm6.6\times10^4$/μl, Flu, $25.9\pm4.4\times10^4$/μl, IFS, $27.0\pm8.4\times10^4$/μl, IE-Group A, $23.4\pm10.9\times10^4$/μl, IE-Group B, $23.3\pm11.0\times10^4$/μl, and IE-Group C, $15.2\pm7.2\times10^4$/μl, respectively.

The platelet numbers of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization are shown in Fig. 2. The platelet number of IE-Group C was significantly lower than those of IFS ($p=0.0078$), Flu ($p=0.0233$), and controls ($p=0.0045$) (Fig. 2). There were no significant differences in platelets numbers among IE-Group A, IE-Group B, IFS, Flu, EBV infection, and controls.

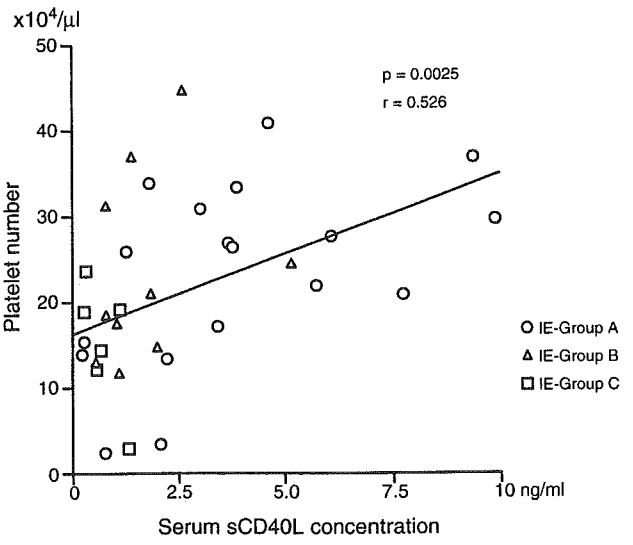

Fig. 3. Relationship between the serum concentration of sCD40L and platelets number in IE groups.

In the influenza virus infection group, including Flu, IFS, and IE, the serum concentrations of sCD40L were correlated with the platelet numbers ($p=0.0006$, $r=0.465$). In the entire IE group, the serum concentrations of sCD40L were correlated with the platelet numbers ($p=0.0025$, $r=0.526$) (Fig. 3). However, In IE groups with a poor prognosis, including Groups B and C, the serum concentrations of sCD40L were not correlated with the platelet numbers ($p=0.2273$, $r=0.312$).

4. Discussion

The CD40–CD40L interaction is pivotal in the cellular immune response. CD40L was first described as an antigen, which is expressed on activated CD4 T cells, and CD40L interacts with CD40 expressed on B cells and induces the class switch [6]. A broader role for CD40 signaling was revealed through the finding that CD40 is expressed on numerous cell types, including monocytes/macrophages, dendric cells, fibroblasts keratinocytes, endothelial cells, and vascular smooth muscle cells [18–20]. Stimulation of these cell types through CD40 induces cell functions which contribute to inflammatory responses, including the expression of adhesion molecules, and also the release of proinflammatory cytokines, such as IL-6, IL-1β, IL-8, IL-12, and TNF-α [19–24]. Serum and plasma sCD40L levels are elevated in systemic lupus erythematosus [25,26], rheumatoid arthritis and associated vasculitis [27], mixed connective tissue disease [28], systemic sclerosis [29], cystic fibrosis [30], advanced squamous cancer of the lung [31], autoimmune thrombocytopenic purpura [32], and chronic idiopathic urticaria [33]. In these disorders, the importance and immunological mechanism of the CD40–CD40L interaction have been discussed. However, >95% of plasma sCD40L is derived from platelets [34]. Therefore, it is likely that the sCD40L level depends on platelet number and activation. In fact, serum sCD40L concentrations were well correlated with platelet numbers and thrombopoiesis in patients undergoing allogenic stem cell transplantation [6].

Our present study demonstrated that serum sCD40L concentrations on the day of hospitalization were well correlated with the severity and prognosis of IE. A previous paper reported that a decrease in platelet number was correlated with a poor prognosis of IE [8]. However, the platelet number reported in the paper made no mention of the sampling time [8]. Platelet number tends to decrease gradually in IE, and therefore, the platelet numbers of patients with IE who had a poor prognosis on the day of hospitalization were often normal [12,17]. Our present study demonstrated that the platelet number of IE-Group B on the day of hospitalization was not decreased, while the serum sCD40L concentration was decreased. It is likely that the serum sCD40L concentration is decreased ahead of platelet number in IE with a poor prognosis. The condition of patients with severe IE often deteriorates rapidly within 2 days after the development of neurological signs [8]. Therefore, it is important to predict a poor prognosis in IE as soon as possible.

Why are serum sCD40L concentrations decreased ahead of platelet numbers? sCD40L is mainly released by activated platelets [28,34]. Apoptosis under hypercytokinemia has been suggested as a possible mechanism of IE [35,36]. In IE, the function of platelets may become poor and inactive, and then, the platelet number may decrease through megakaryocyte apoptosis. The definite mechanism leading to the decrease of platelet number in IE remains unclear.

In summary, serum sCD40L levels on the day of hospitalization were well correlated with the outcome of IE compared to the platelet numbers. Our findings suggest that the decreased serum sCD40L levels in IE are important for predicting a poor prognosis in the early phase.

Acknowledgments

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Concise Report

Intravenous immunoglobulin does not increase FcγRIIB expression on monocytes/macrophages during acute Kawasaki disease

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Objectives. **Intravenous immunoglobulin (IVIG) therapy has been reported to be effective for reducing the incidence of coronary artery lesions in Kawasaki disease (KD), an acute febrile vasculitis of unknown aetiology. Regarding the mechanism of IVIG in immune thrombocytopenic purpura (ITP), it has been reported that IVIG increases the expression of the inhibitory Fc receptor, FcγRIIB (CD32B), on splenic macrophages in a murine ITP model. Regarding the mechanism of IVIG during acute KD, we investigated whether or not IVIG increases the expression of FcγRIIB in peripheral blood $CD14^+$ monocytes/macrophages.**

Methods. **The expression of FcγRIIB in peripheral blood $CD14^+$ monocytes/macrophages was determined before and after IVIG therapy in 13 patients with acute KD by flow cytometry.**

Results. **The percentage of $CD14^+CD32B^+$ monocytes/macrophages among peripheral blood mononuclear cells, the absolute number of $CD14^+CD32B^+$ monocytes/macrophages and the percentage of $CD14^+CD32B^+$ monocytes/macrophages among $CD14^+$ monocytes/macrophages in patients with acute KD before IVIG therapy were significantly increased compared with those after IVIG therapy and in controls. $CD14^+CD32B^+$ monocytes/macrophages decreased to within the normal range soon after IVIG therapy.**

Conclusions. **IVIG therapy in patients with KD did not increase the expression of FcγRIIB in peripheral blood $CD14^+$ monocytes/macrophages during the acute stage.**

KEY WORDS: Monocytes/macrophages, Kawasaki disease, FcγRIIB, Intravenous immunoglobulin.

Kawasaki disease (KD) is an acute febrile vasculitis of unknown aetiology that may lead to cardiovascular disorders [1]. A coronary artery lesion (CAL) is the most important complication of KD and may cause significant coronary stenosis, resulting in ischaemic heart disease [2]. Intravenous immunoglobulin (IVIG) therapy has been reported to be effective for reducing the incidence of CAL in KD patients [3–5]. However, there have been few reports on the mechanism of IVIG in acute KD [4, 6, 7]. IVIG is effective for other disorders, including immune thrombocytopenic purpura (ITP) and Guillain–Barré syndrome. The mechanism of IVIG in the murine ITP model has been documented. In the murine model of ITP, IVIG increases the expression of the inhibitory Fc receptor, FcγRIIB, on splenic macrophages [8]. The cross-linking of FcγRIIB and the activation of the Fc receptor, FcγRIII, by platelet–antibody immune complexes inhibits the activating signal through the recruitment of SH2-containing 5′-phosphoinositol-phosphatase and the breakdown of phosphatidylinositol 3,4,5-trisphosphate, resulting in abrogation of phagocytosis [9]. FcγRII, which exists in three isoforms, i.e. A, B and C, is a low-affinity receptor for the Fc fragment of IgG [10]. All forms are present on monocytes, placental trophoblasts and endothelial cells [11]. In addition, the FcγRIIB form is present on B lymphocytes and mast cells [11]. The FcγRIIA and FcγRIIC forms are found on neutrophils [11] and do not have the inhibitory motif that is present in FcγRIIB [12].

Previous studies have demonstrated that activation of monocytes/macrophages plays an important role in the pathogenesis of KD. The immunological features of monocytes/macrophages observed in patients with KD can be summarized as follows: (i) infiltration by these cells is notable in affected tissues in autopsy cases and skin biopsy specimens [13]; (ii) there are elevated levels of a variety of serum cytokines, such as TNF-α, interleukin (IL)-1 and IL-6, during acute KD [14–18]; (iii) peripheral blood mononuclear cells (PBMC) spontaneously secrete high levels of TNF-α and IL-1 [19, 20]; (iv) increases in the number of peripheral blood $CD14^+$ monocytes/macrophages, serum TNF-α level, IL-6 activity in serum, and secretion of IL-1 from mononuclear cells are more evident in KD patients with CAL than in ones without CAL [14, 17, 20, 21]; (v) KD patients with high levels of soluble TNF receptors in their serum seem to be susceptible to CAL even if they receive IVIG therapy [22]; (vi) immunocytochemical and immunoelectron microscopic studies have shown that monocytes partly differentiate into macrophages in the peripheral circulation during the acute stage of KD [23, 24]; (vii) increased numbers of peripheral blood $CD14^+CD16^+$ (FcγRIII) monocytes/macrophages are part of the regulatory system for monocyte/macrophage function

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during acute KD [25]; and (viii) nuclear factor-κB (NF-κB) activation is higher in peripheral blood $CD14^+$ monocytes/macrophages than in $CD3^+$ T cells in KD patients during the acute stage [26]. These findings suggest that the activation of monocytes/macrophages plays an important role during acute KD. Regarding the mechanism of IVIG during acute KD, we investigated whether or not IVIG increases the expression of FcγRIIB on peripheral blood $CD14^+$ monocytes/macrophages.

Patients and methods

Thirteen children (five girls and eight boys; aged from 3 months to 4 yr; mean age 1.6 yr) with acute KD on admission to our hospital between July 2003 and May 2004 were included in this study. The children's parents gave informed consent for their participation in the study. The patients met the specific diagnostic criteria for KD [1]. The day of onset of fever was considered as the first day of illness. All patients received standard Japanese treatment with high-dose IVIG (Polyglobin N; Bayer Yakuhin, Osaka, Japan) at 1000 mg/kg/day for 2 days, and oral aspirin (30 mg/kg/day), and all responded to IVIG therapy. No patients had CAL. Samples were obtained at the acute stage, i.e. on days 3–6 (mean ± S.D., 4.6 ± 0.9 days) before treatment with IVIG. Samples were also obtained after IVIG therapy, i.e. on days 6–9 (mean ± S.D., 7.4 ± 1.4 days). The control subjects were 18 healthy children (10 males and eight females, aged from 4 months to 4 yr; median 1.8 yr). Peripheral blood labelled with phycoerythrin-conjugated anti-$CD14^+$ antibodies was labelled with an anti-FcγRIIB (CD32B) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The antibody is an affinity-purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of CD32B of human organ. The cells were then labelled with a second fluorescein isothiocyanate-conjugated antibody (Pharmingen, San Diego, CA, USA). Immunofluorescence staining was analysed with a FACScan flow cytometer equipped with CellQuest software (Becton-Dickinson Biosciences, San Diego, CA, USA). We analysed 5000 cells for each subject in the flow cytometric studies. The values obtained from flow cytometric analyses are expressed as mean ± S.D. Statistical analysis was performed with the Wilcoxon matched pairs test and the Mann–Whitney *U* test, a *P* value of less than 0.05 being taken as significant.

The research was reviewed and approved by the Institutional Review Board of Yamaguchi University Hospital. The parents of children who were patients with KD and of healthy controls gave informed consent for their participation in the study according to the Declaration of Helsinki.

Results

Figure 1 shows the results of flow cytometric analyses of PBMC from a 21-month-old girl with KD before and after IVIG therapy, and at the convalescent stage. The patients had an increased percentage of $CD14^+CD32B^+$ monocytes/macrophages before IVIG therapy. The results of flow cytometric analyses are presented in Table 1. The percentage of $CD14^+$ monocytes/macrophages among PBMC, the absolute number of $CD14^+$ monocytes/macrophages, the percentage of $CD14^+CD32B^+$ monocytes/macrophages among PBMC, the absolute number of $CD14^+CD32B^+$ monocytes/macrophages, and the percentage of $CD14^+CD32B^+$ monocytes/macrophages among $CD14^+$ monocytes/macrophages in patients with acute KD before IVIG therapy were significantly increased compared with those after

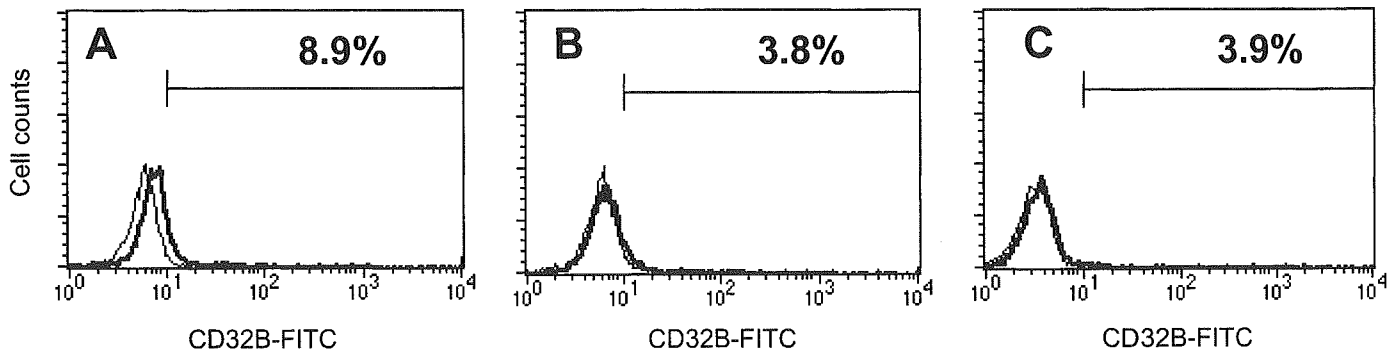

FIG. 1. Flow cytometric analyses of PBMC from a 21-month-old girl with KD before (A, day 5) and after (B, day 7) IVIG therapy, and at the convalescent stage (C, day 39). The percentages of $CD14^+CD32B^+$ monocytes/macrophages among $CD14^+$ monocytes/macrophages are given. Anti-CD32B antibody binding is shown as a black line and the background isotype antibody (a non-specific goat IgG antibody) binding as a grey line for each sample. An area of the black line on the right of a cut-off line is the percentage of positive cells.

TABLE 1. $CD14^+$ and $CD14^+CD32B^+$ monocytes/macrophages in patients with acute KD and control subjects

	KD ($n=13$)		Controls ($n=18$)	*P* value		
	Before IVIG Group A	After IVIG Group B	Group C	A vs B	A vs C	B vs C
Mononuclear cells (/μl)	6074 ± 2258	6044 ± 2661	6075 ± 2097	0.8753	0.8653	0.9325
$CD14^+$ cells among PBMC (%)	20.7 ± 7.6	12.2 ± 5.6	7.2 ± 2.9	0.0019	<0.001	0.0167
$CD14^+$ cells (/μl)	1242 ± 625	674 ± 355	423 ± 190	0.0088	<0.001	0.0223
$CD14^+CD32B^+$ cells among PBMC (%)	1.7 ± 1.6	0.5 ± 0.5	0.3 ± 0.2	0.0047	<0.001	0.4583
$CD14^+CD32B^+$ cells (/μl)	97 ± 88	28 ± 38	18 ± 12	0.0229	<0.001	0.4846
$CD14^+CD32B^+$ cells among $CD14^+$ cells (%)	7.6 ± 4.3	3.5 ± 2.6	4.3 ± 2.7	0.0309	0.0209	0.4981

IVIG therapy ($P=0.0019$, $P=0.0088$, $P=0.0047$, $P=0.0229$ and $P=0.0309$, respectively) and the controls ($P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$ and $P=0.0209$, respectively). $CD14^+CD32B^+$ monocytes/macrophages decreased to within the normal range soon after IVIG therapy.

Discussion

We have already reported that $CD14^+$ and $CD14^+CD16^+$ (FcγRIII) monocytes/macrophages among PBMC of patients with acute KD before IVIG therapy were increased compared with at the convalescence stage, and $CD14^+CD16+$ monocytes/macrophages decreased to within the normal range soon after IVIG therapy [25]. Activation of NF-κB through FcγRIII has been reported [27], which agrees with our previous finding that the number of peripheral blood $CD14^+CD16^+$ monocytes/macrophages and NF-κB activation in $CD14^+$ monocytes/macrophages decreased soon after IVIG therapy in KD [25, 26]. Recently, we revealed that FcγRIII expression decreased transiently on the membranes of U-937 cells, a human monocytic leukaemia cell line, and peripheral blood $CD14^+$ monocytes/macrophages after the addition of IVIG *in vitro* [28].

In the present study, we demonstrated that IVIG therapy in patients with KD did not increase the expression of FcγRIIB in peripheral blood $CD14^+$ monocytes/macrophages during the acute stage. On the contrary, $CD14^+CD32B^+$ monocytes/macrophages slightly increased during acute KD before IVIG therapy, and returned to within the normal range soon after IVIG therapy. We speculate that the slight increase in $CD14^+CD32B^+$ monocytes/macrophages during acute KD before IVIG therapy reflects negative feed-back in response to activated monocytes/macrophages associated with the increase in $CD14^+CD16^+$ monocyte/macrophage numbers. Alternatively, the slight increase in $CD14^+CD32B^+$ monocytes/macrophages during acute KD before IVIG therapy may reflect activated $CD14^+CD32A^+$ monocytes/macrophages, since the anti-CD32B antibody may react against not only CD32B but also CD32A because the extracellular and transmembrane regions of CD32A and CD32B are similar or identical [29]. Our results suggest that $CD14^+CD32B^+$ monocytes/macrophages, including $CD14^+CD32A+$ monocytes/macrophages, do not increase in numbers after IVIG therapy in acute KD.

It is unclear whether the IVIG treatment caused the reduced numbers of $CD14^+CD32B^+$ monocytes/macrophages since we did not evaluate a control group of patients treated with only aspirin. Such a study is not possible in Japan because the Research Committee on Kawasaki Disease recommends that IVIG treatment be given to patients with KD [30]. A previous study demonstrated that there was no significant difference in the expression of $CD14^+CD32^+$ monocytes/macrophages before and after IVIG therapy [31]. We focused on $CD14^+CD32B^+$ monocytes/macrophages with an inhibitory motif, but not $CD14^+CD32^+$ ones. Regarding FcγR expression on peripheral blood monocytes/macrophages during acute KD, the main effect of IVIG therapy may be based on a decrease in $CD14^+CD16^+$ (FcγRIII) monocytes/macrophages, but not an increase in $CD14^+CD32B^+$ (FcγRIIB) monocytes/macrophages.

The authors have declared no conflicts of interest.

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Clinical and electrophysiologic correlates of IVIg responsiveness in CIDP

Abstract—To identify clinical and electrophysiologic features related to IV immunoglobulin (IVIg) responsiveness in chronic inflammatory demyelinating polyneuropathy (CIDP), the authors conducted a multicenter study on 312 patients with CIDP (199 responders and 113 nonresponders). Muscle atrophy and decreased compound muscle action potential were pronounced in nonresponders of IVIg. Male gender, longer disease duration, and slow progression of symptoms were also associated with IVIg unresponsiveness. Features suggesting axonal dysfunction in peripheral nerves indicated IVIg unresponsiveness in CIDP.

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Chronic inflammatory demyelinating polyneuropathy (CIDP) is characterized by insidious onset, chronicity with progressive or remittent clinical course, and segmental demyelination in peripheral nerves.[1] Among treatments for CIDP such as corticosteroids, plasmapheresis, and IV immunoglobulin (IVIg), IVIg is commonly used as an initial therapy because of relatively few side effects, immediate therapeutic response, and convenient administration without special equipment. However, some patients fail to show therapeutic response to IVIg,[2,3] and the features related to IVIg responsiveness are not well understood.[2-4] Thus, we investigated the clinical and electrophysiologic correlates of CIDP patients showing a good response or little or no response to IVIg.

Methods. We studied CIDP patients from June 2002 to April 2004 as members of the multicenter study group for hereditary neuropathy in Japan, working under the Auspices of the Ministry of Health, Labor, and Welfare of Japan. The Ethics Committee of the Nagoya University Graduate School of Medicine approved the study design in full. All patients fulfilled the diagnostic criteria established by the Ad Hoc Subcommittee of American Academy of Neurology AIDS Task Force.[1] Patients with monoclonal gammopathy of undetermined significance, anti-myelin-associated glycoprotein, and anti-sulfate-3-glucuronyl paragloboside antibodies were excluded, together with patients with severe diabetes mellitus, alcoholism, drug poisoning, hereditary neuropathy, and other diseases causing neuropathy. The subjects of the current investigation were the 312 of 372 patients initially entered, who met diagnostic and inclusion criteria and treated with IVIg (400 mg/kg/day for 5 days). The majority of the patients (90.7%) were treated initially by IVIg without any other prior therapy, whereas some patients (9.3%) who had received other treatments such as plasmapheresis or corticosteroids before IVIg were followed for >4 weeks before IVIg treatment to be sure they were not on some other form of treatment.

Clinical features including motor and sensory impairment and muscle atrophy were assessed. Weakness was estimated according to Medical Research Council criteria in proximal muscles (deltoid, biceps, and triceps muscles in upper limbs, iliopsoas and quadriceps muscles in lower limbs) as well as distal muscles (thenar, interosseous, and finger flexion muscles in upper limbs, ankle dorsiflexor and toe dorsiflexor muscles in lower limbs).[6] Activities of daily living (ADLs) involving upper limbs were evaluated according to the arm disability score of the overall disability sum score (ODSS).[7] ADLs involving lower limbs were evaluated according to the modified Rankin Scale.[8] We assessed ADLs 1 to 14 days before IVIg and reassessed then 4 to 6 weeks after IVIg for evaluation of clinical efficacy. Those patients who improved by ≥1 point in the ODSS or the modified Rankin Scale were termed responders, and those with no change, a minimal improvement of <1 point, or showing a worse score were termed nonresponders.

For electrophysiologic study, the previously described standardized method was adopted.[5,6] Motor nerve conduction was evaluated for the median, ulnar, and tibial nerves, whereas sensory nerve conduction was evaluated for median, ulnar, and sural nerves. Motor nerve conduction velocity (MCV), distal latency, compound muscle action potential (CMAP), and presence of conduction block was also assessed. Sensory nerve conduction velocity and sensory nerve action potential also were assessed. Control values were obtained from normal subjects for median (n = 191; 48.7 ± 16.5 years old), ulnar (n = 166; 48.9 ± 15.8 years old), tibial (n = 121; 49.9 ± 15.0 years old), and sural (n = 133; 50.6 ± 15.6 years old) nerves as previously described.[6] All electrophysiologic data were obtained 1 to 14 days before IVIg was started. For some patients who were assessed 4 to 6 weeks after IVIg, nerve conduction velocity findings were compared before and after IVIg.

The two-tailed Fisher exact test and Mann–Whitney *U* test were used to evaluate relative differences between responders and nonresponders and between data before and after IVIg, using

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Table 1 *Clinical findings*

Clinical features and CSF	Responders	Nonresponders	*p* value*
All patients (responders: n = 199; nonresponders: n = 113)			
Age, y	52.5 ± 18.3	55.5 ± 16.7	NS
Sex, M/F	1.8/1.0	2.9/1.0	<0.05
Duration from onset to IVIg, mo	7.8 ± 4.0	9.8 ± 3.5	<0.0005
Progression after onset,† %	45.9	22.9	<0.0005
MRC score,‡ 0–5			
Upper limb, proximal	4.1 ± 1.0	4.4 ± 0.9	<0.0005
Upper limb, distal	3.5 ± 1.0	3.8 ± 1.2	<0.005
Lower limb, proximal	4.0 ± 1.0	4.3 ± 1.1	<0.01
Lower limb, distal	3.4 ± 1.1	3.3 ± 1.4	NS
Muscle atrophy, % of patients			
Upper limb	28.9	42.4	<0.01
Lower limb	25.9	47.8	<0.0005
ADL score			
Arm disability score (ODSS)	2.3 ± 1.5	1.5 ± 1.5	<0.0001
Modified Rankin Scale	2.6 ± 1.2	2.2 ± 1.3	<0.005
CSF protein, mg/dL	94 ± 71	132 ± 158	NS
Patients with similar duration (<12 mo) (responders: n = 115; nonresponders: n = 29)			
Age, y	52.2 ± 9.0	50.3 ± 18.0	NS
Sex, M/F	1.5/1.0	2.5/1.0	<0.05
Duration from onset to IVIg, mo	4.5 ± 2.1	4.8 ± 2.2	NS
Progression after onset,† %	22.2	12.1	<0.01
MRC score, 0–5			
Upper limb, proximal	4.0 ± 1.0	4.4 ± 0.9	<0.05
Upper limb, distal	3.5 ± 1.0	3.8 ± 1.2	NS
Lower limb, proximal	3.9 ± 1.1	4.2 ± 1.1	<0.05
Lower limb, distal	3.4 ± 1.2	3.3 ± 1.5	NS
Muscle atrophy, % of patients			
Upper limb	20.2	48.9	<0.05
Lower limb	20.4	48.3	<0.01
ADL score			
Arm disability score (ODSS)	2.4 ± 1.5	2.0 ± 1.7	<0.01
Modified Rankin Scale	2.8 ± 1.2	2.6 ± 1.3	NS
CSF protein, mg/dL	129 ± 202	191 ± 206	NS

* *p* value indicates a significant difference between responders and nonresponders.
† Rate of patients with disability <3 mo after the onset.
‡ Mean score of the examined muscles.

ODSS (overall disability sum score) = 0, normal; 1, minor symptoms or signs in one or both arms but not affecting any function (dressing upper part of body, washing and brushing hair, turning a key in a lock, using knife and fork, doing/undoing buttons and zips); 2, moderate symptoms or signs in one or both arms affecting but not preventing any function listed; 3, severe symptoms or signs in one or both arms preventing at least one but not all functions listed; 4, severe symptoms or signs in both arms preventing all functions listed but some purposeful movements still possible; 5, severe symptoms and signs in both arms preventing all purposeful movements. Modified Rankin Scale = 0, normal; 1, nondisabling symptoms not interfering with lifestyle; 2, minor disability from symptoms leading to some restrictions of lifestyle but not interfering with patients' capacity to look after themselves; 3, moderate disability from symptoms that significantly interfered with lifestyle or prevented fully independent existence; 4, moderately severe disability from symptoms that clearly precluded independent existence, although patients did not need constant attention day and night; 5, severe disability involving total dependence, including constant care day and night. IVIg = IV immunoglobulin; MRC = Medical Research Council; ADL = activities of daily living.