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Measles virus-specific T helper 1/T helper 2-cytokine production in subacute sclerosing panencephalitis

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Live measles virus-specific T helper 1/T helper 2-cytokine productions by peripheral blood mononuclear cells in response to live measles, mumps or varicella virus were measured in 15 patients with subacute sclerosing panencephalitis and 15 controls by enzyme-linked immunosorbent assays. Most patients with subacute sclerosing panencephalitis had a defect in measles virus-specific production of interferon-γ, one of the T helper 1 type cytokines, despite persistent presence of measles virus, with preserved interleukin-10 (T helper 2 type cytokine) synthesis. Patients with subacute sclerosing panencephalitis were divided into two groups: responders (group A) with significant interferon-γ production (>20 pg/mL) in response to live measles virus and non-responders (group B) with a little or no interferon-γ production. Comparison of the clinical courses between groups A and B revealed that all the patients of group A retained receptive function for a long time, while most patients of group B lost the function rapidly ($P<0.01$). An inverse correlation between interferon-γ production by peripheral blood mononuclear cells and disease progression suggested that interferon-γ plays an antiviral role in subacute sclerosing panencephalitis. *Journal of NeuroVirology* (2000) **6,** 121–126.

Keywords: interferon-γ; interleukin-2; interleukin-10; prognosis

Introduction

Subacute sclerosing panencephalitis (SSPE) is a rare, slow virus infection of the central nervous system caused by measles virus (MV) with mutations. Although the exact pathogenesis of SSPE remains to be determined, immaturity of host immune and central nervous systems has been suggested to play roles in an increased risk of SSPE development among infants who acquired measles before 2 years of age (Britt, 1998; Gascon, 1996).

With respect to cellular immunity, no generalized deficiency of T cell immunity has been reproducibly found in SSPE patients. Lymphoproliferative responses to MV antigens were within the normal ranges (Dhib-Jalbut *et al*, 1988a), while impairment of MV-specific cytotoxic T cells was detected in a significant proportion (two out of three and three of four) patients with SSPE in two independent studies (Ewan *et al*, 1977; Dhib-Jalbut *et al*, 1988b).

Similar to the patients with congenital infections due to rubella virus or cytomegalovirus who showed enhanced antibody production with suppressed cell-mediated immunity (Overall, 1998), SSPE patients exhibit high titer anti-MV antibodies in serum and cerebrospinal fluid (Britt, 1998; Gascon, 1996) with impaired MV-specific cytotoxic T cell response (Ewan *et al*, 1977; Dhib-Jalbut *et al*, 1988b). In such split tolerance characterized by accentuated antibody production with decreased cell-mediated immunity, T helper (Th) 1 function involved in cell-mediated immunity is generally down-regulated, while Th2 function is preserved or enhanced, as reported in animal systems (Peterson *et al*, 1993) as well as in children with congenital infections or prenatal exposure to microfilarial antigens (Overall, 1998; Steel *et al*, 1994). In brain lesions of SSPE, however, both Th1 type (interferon (IFN)-γ, interleukin (IL)-2, tumor necrosis factor-α)

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and Th2 type (IL-6) cytokines were detected (Hofman *et al*, 1991; Nagano *et al*, 1994). In peripheral blood mononuclear cells (PBMC), only one report described IL-1 and IL-2 syntheses by nonspecific mitogen stimulation in patients with SSPE (Brajczewska-Fischer *et al*, 1989). So far, no studies have been carried out on MV-specific Th1/Th2 cytokine production in SSPE.

In the present study, we selected live MV vaccine as a stimulator because live MV and soluble MV antigens might be different in stimulating PBMC to induce cytokine production (Griffin *et al*, 1994), and investigated live MV-specific production of Th1/Th2 cytokines as well as its relation to disease progression in 15 patients with SSPE.

Results

Th1- and Th1-inducing cytokine production in response to live measles, mumps or varicella virus

IFN-γ IFN-γ production was studied *in vitro* after 1–3 days' incubation in the presence of live MV. The maximal levels of IFN-γ (mean ± s.d.) in 15 patients with SSPE and controls with a history of natural measles infection (group C) were 40.3 ± 80.7 pg/mL and 68.8 ± 73.5 pg/mL, respectively (no significant difference). No IFN-γ production was observed in three seronegative controls. Since controls were heterogenous in IFN-γ production and consisted of responders (seven individuals) with significant IFN-γ production (>20 pg/mL) in response to live MV and non-responders (eight individuals) with a little or no IFN-γ production, SSPE patients were also divided into responders (group A, four patients) and non-responders (group B, 11 patients), as shown in Figure 1. It is noteworthy that despite persistent presence of MV and shorter interval from natural measles to examination, the frequency of non-responders among SSPE patients (11/15: 73.3%) was higher than that of controls (8/15: 53.3%), although the difference did not reach statistical significance. No statistically significant differences were present in the ages at examination (A: median 12.5 years, range 9–26 years *versus* B: 12 years, 3–

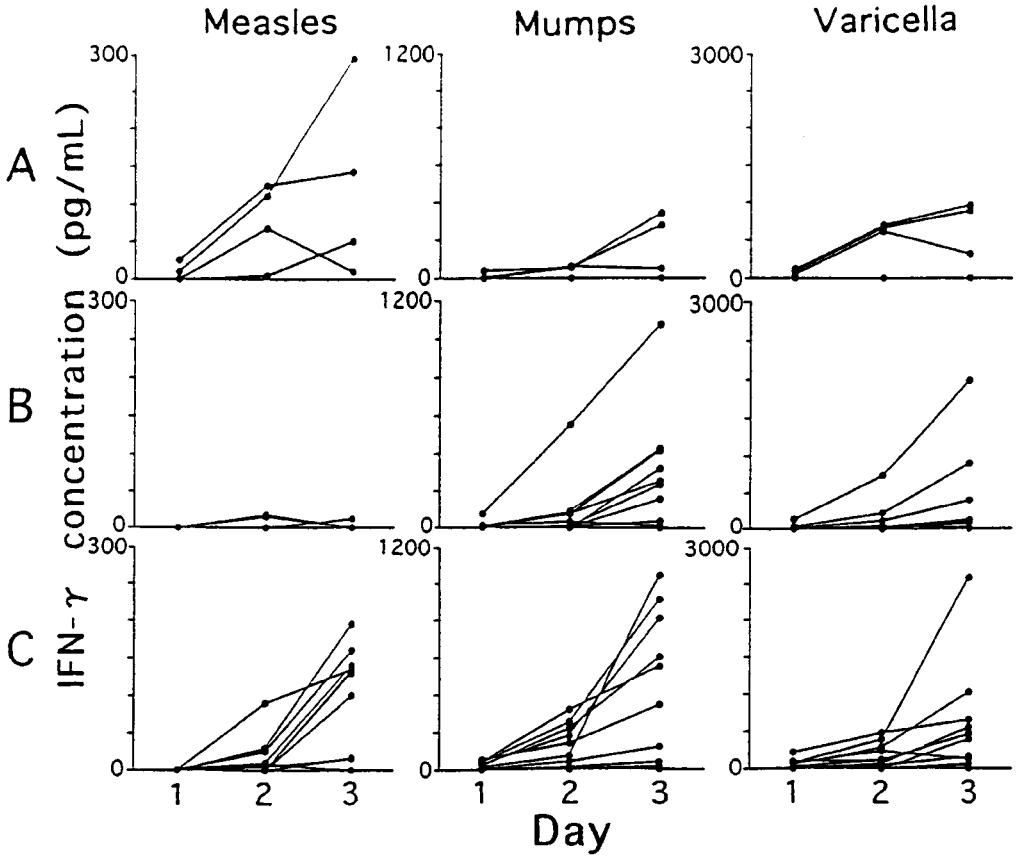

Figure 1 IFN-γ production in response to live measles, mumps or varicella virus. Culture supernatants after 1–3 days' incubation in the presence of measles, mumps or varicella virus were collected and stored in aliquots at −80°C. IFN-γ levels were measured with ELISA, as described in Methods. (**A**) SSPE group A (n=4), (**B**) SSPE group B (n=11), (**C**) controls (n=15). Each dot corresponds to a single subject. Eight of the 11 patients with group B and six of the 15 controls with group C showed complete absence of IFN-γ production (on the bottom line) to live measles.

23 years), the ages at onset of SSPE (A: median 7 years, range 6–15 years *versus* B: 7 years, 3–13 years), the ages of natural measles infection (A: mean ± SD, 1.4 ± 0.5 years *versus* B: 1.6 ± 1.1 years), and the durations of disease (A: median 6 years, range 2–11 years *versus* B: 3 years, 0–16 years) between groups A and B. No significant differences were also observed in the ages at examination and the ages at natural measles infection between control responders and non-responders (data not shown). Group A consisted of three patients with stage II and one with early stage III, while group B included four with stage I/II, four with stage III and three with stage IV. Of the 11 patients in group B, three patients including two with stage III and one with stage IV produced a little IFN-γ production ($20 \geqslant \sim > 5$ pg/mL). Of the three, one with stage IV who had shown slow neurological deterioration (retention of response to call for 9 years) produced a little IFN-γ (15 pg/mL) to MV even after 16 years from onset. Of the eight with no significant IFN-γ production to live MV, two patients were examined at onset and subsequently showed rapid deterioration (loss of receptive function within 6 months). Only one of the four in group A and five of 11 in group B received continuous local IFN-α therapy. Blood samples from five of the 15 with SSPE were available at follow-up examinations, showing similar IFN-γ production to live MV.

On the other hand, both SSPE groups A and B showed significant IFN-γ production in response to live mumps virus (A: 171.3 ± 165.9 pg/mL, B: 304.1 ± 311.4 pg/mL) or varicella virus (A: 809.0 ± 179.0 pg/mL, B: 431.0 ± 661.0 pg/mL) similar to the levels of controls (C: mumps 330.3 ± 362.8 pg/mL, varicella 525.1 ± 656.4 pg/mL).

IL-2 No differences were observed in IL-2 production as a response to measles, mumps or varicella virus between SSPE group A or B and controls, as shown in Figure 2.

IL-12 Minimal IL-12 production to MV was observed in one of 15 SSPE patients and none of controls (data not shown).

Th2-cytokine production to live measles, mumps or varicella virus

IL-10 IL-10 production was evaluated using the same supernatants in which Th1-cytokine production was measured. IL-10 production to measles, mumps or varicella virus showed no differences between SSPE group A or B and controls (Figure 3).

IL-4 IL-4 production to MV was observed in only one of 15 SSPE patients and none of 15 controls (data not shown).

Correlation between IFN-γ production and disease progression

To clarify the difference in disease progression from onset between SSPE patients, groups A and B, the durations of retaining receptive function defined as a positive response to call or weak stimuli were compared. As shown in Figure 4, all the patients of group A with significant IFN-γ production in response to live measles virus retained receptive

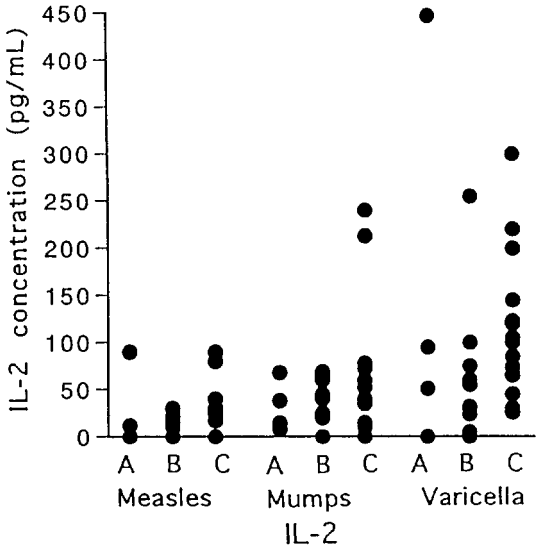

Figure 2 IL-2 production to live measles, mumps or varicella virus. Maximal IL-2 levels during 3 days' incubation were determined by ELISA. SSPE group A, SSPE group B, controls.

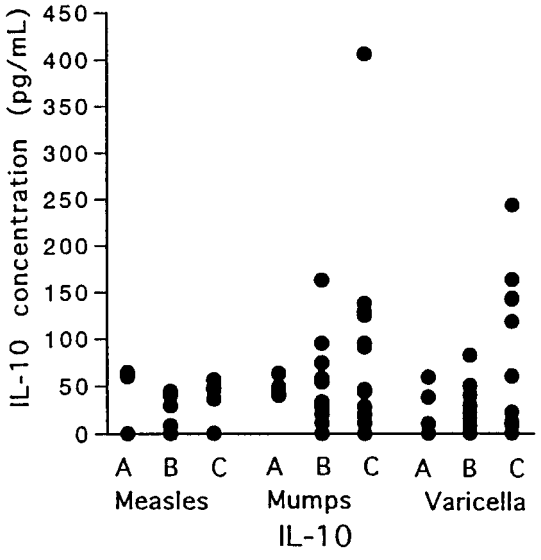

Figure 3 IL-10 production to live measles, mumps or varicella virus. Maximal IL-10 levels during 3 days' incubation were studied, as shown in Figure 2. SSPE group A, SSPE group B, controls.

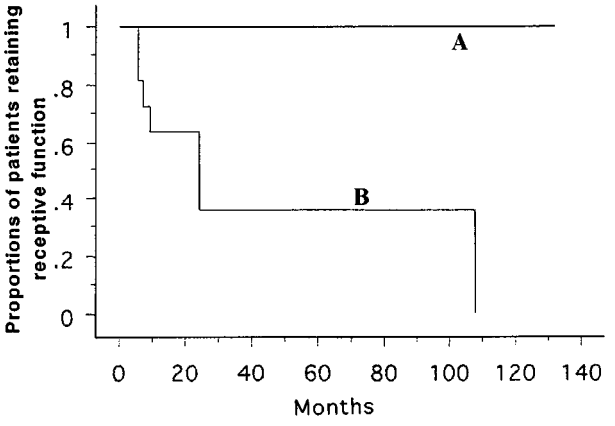

Figure 4 Comparison of the proportions of patients retaining receptive function between SSPE groups A and B. SSPE group A (**A**) patients showed significantly longer duration of retaining receptive function from onset than group B (**B**) patients by Kaplan-Meier method and logrank test ($P<0.01$). Durations are expressed by months after disease onset.

function, while most patients of group B with a little or no IFN-γ production lost the function rapidly ($P<0.01$). On the other hand, no significant differences were observed in the rates of disease progression between SSPE patients with and without IL-2 or IL-10 production to live MV (data not shown). In addition, because only one patient was being given IFN-α treatment in group A, and two of five with IFN-α administration and two of six without IFN-α treatment in group B retained long-term receptive function, no correlation was observed between IFN-α treatment and disease progression.

Discussion

Most SSPE patients showed a defect in MV-specific IFN-γ (Th1 type cytokine) production despite persistent presence of MV, with preserved IL-10 (Th2 type cytokine) synthesis. Contrary to our expectation, some SSPE patients did produce significant amounts of IFN-γ to live MV. Interestingly, such patients retained significantly long-term receptive function despite longer duration of the disease, while those with a little or no IFN-γ production lost this function rapidly, as shown in Figure 4 ($P<0.01$).

Cell-mediated immune response plays the predominant role in control of persistent viral infections. IFN-γ exerts its effects through enhancement of lysis of virus-infected cells and cytokine-mediated virus clearance, by augmentation of cytotoxic effector cells with upregulation of major histocompatibility antigens on target cells and inhibition of viral gene expression and replication, respectively (Gascon *et al*, 1996). Recently, cytokine-mediated virus clearance is considered to play a more dominant role than lysis of virus-infected cells in persistent viral infections (Borrow, 1997). In fact, IFN-γ was suggested to be a major antiviral cytokine in experimental MV-induced encephalitis (Finke *et al*, 1995) and in viral clearance from oligodendroglia (Parra *et al*, 1999). Since MV may spread transneuronally in the central nervous system in SSPE (Allen *et al*, 1996), it is possible that IFN-γ exerts its effects mainly through inhibition of viral spread. It may explain for the inverse correlation between IFN-γ production and disease progression observed in patients with SSPE.

As one of the host factors implicated in the pathogenesis of SSPE, immature immune system at the time of the initial measles infection probably plays a role since MV infection in early infancy leads to development of SSPE in a higher frequency than when measles occurs later in life, just as hepatitis B virus persists in a high frequency when hepatitis B virus infection occurs at an early age (Overall, 1998). In animals with immature immune system, selection of either the tolerance or protective immunity pathway is determined in part by the dose and form of antigen presented (Ridge *et al*, 1996; Sarzotti *et al*, 1996; Forsthuber *et al*, 1996). Such tolerance is not the result simply of immunological immaturity, but is correlated with the induction of a nonprotective Th2 cytokine response. Therefore, a relative dominance of Th2 response over Th1 response toward MV might represent an important host factor attributable in part to an immature immune system that is coping with the initial measles infection. Th1/Th2 imbalance observed in most SSPE patients may reflect a persistence of a relative dominance of Th2 response at the initial measles infection.

On the other hand, there are several virus factors that might be involved in persistence of MV in SSPE. MV itself has a unique feature that infects dendritic cells and induces apoptosis and syncytia formation of these cells, leading to profound inhibition of IL-12 production by dendritic cells and T cell proliferation (Karp *et al*, 1996; Grosjean *et al*, 1997; Fugier-Vivier *et al*, 1997). As a result, Th2 polarization of the cytokine responses during and after MV infection (Brajczewska-Fischer *et al*, 1989; Griffin *et al*, 1993) could result in insufficient elimination and persistence of MV in a certain individual with immature immune system.

Another mechanism of viral persistence may be the generation of viral mutants. However, epidemiological data do not support the view that mutated MV causes SSPE to occur in clusters but that mutations occur after the initial MV infection. In fact, an infant who had received perinatally acquired MV infection from the mother developed SSPE at 11 months of age and died at 16 months, while no symptoms appeared in the mother (Zwiauer *et al*, 1995). In progressive rubella encephalitis, of which symptoms are very similar

to those of SSPE, infants with congenital rubella syndrome develop the encephalitis but their mothers never do, despite the infection with the same rubella virus (Britt, 1998). As such slow virus infections develop not in mothers with mature immune system but in infants with immature immune system despite the infection of the same virus, it is likely that virus-host immune balance plays a critical role in the development of SSPE. MV mutations may occur from viral strategies to generate genetic diversity together with selective pressure of the host immune responses when host immune system is not sufficient enough to cope with MV. Further investigation is required to determine the significance of IFN-γ in the development and disease progression of SSPE.

Materials and methods

Subjects

Fifteen patients, five females and ten males, who met the diagnostic criteria for SSPE (Britt, 1998; Gascon, 1996), were studied. The median age at examination was 12 years (range: 3–26 years). The ages at onset of SSPE ranged between 3 and 15 years (mean $\pm$ SD, 7.9 $\pm$ 3.1 years). Fourteen had natural measles which occurred between the ages of 0.7 and 4 years (mean $\pm$ SD, 1.5 $\pm$ 0.9 years) and the measles history was unknown in one. None received measles vaccination. The median duration from diagnosis to examination was 4 years with a range from 0 to 16 years. Seven patients were in stage I/II, five in stage III and three in stage IV. Six of the 15 were being given IFN-α treatment at the time of examination.

For cytokine assays, 12 healthy individuals and three patients with non-immunologic disorders (seven females and eight males) who had a history of natural measles without measles vaccination were studied as seropositive controls. The median age at the time of natural measles infection among control individuals who remembered the ages was 5 years (range 2–7 years). Three infants who had neither natural measles nor measles vaccination were included as seronegative controls. The median age in control individuals was 22 years with a range of 1–43 years. All subjects were Japanese. Informed consent was obtained from subjects or their parents.

Cytokine production by PBMC in response to live MV was performed according to the method previously described with modifications (Nakayama *et al*, 1987). Briefly, PBMC at 2×10^6/ml were incubated with one-tenth volume of freshly reconstituted live measles, mumps or varicella vaccine for 2 h at 37°C and washed twice with RPMI 1640 medium containing 10% fetal calf serum. Live measles (>10 000 50% tissue culture infective dose per ml) and varicella (>2000 plaque forming unit per ml) vaccines were purchased from the Research Foundation for Microbial Diseases of Osaka University (Biken), Osaka. Live mumps vaccine (>10 000 50% tissue culture infective dose per ml) was from Takeda Pharmaceutical Co., Osaka, Japan. The volumes of live vaccine added were demonstrated in preliminary experiments. Live virus-infected PBMC were suspended at a concentration of 1×10^6/ml and added to an equal volume of untreated PBMC (1×10^6/ml) and cultured for 3 days. Cultured cells from SSPE patients or controls showed over 95% of viability. Supernatants of PBMC cultured in the presence or absence of live virus vaccine were obtained at 1, 2 and 3 days following initiation of culture. IFN-γ and IL-2, 4, and 10 were measured in triplicates with enzyme-linked immunosorbent assay (ELISA) kits from Genzyme Diagnostics, Cambridge, MA, USA. IL-12 was measured with Quantikine human IL-12 Immunoassay (R&D systems, Minneapolis, MN, USA) because the assay kit accurately detected functionally active heterodimeric IL-12 (Tsang and Weatherbee, 1996).

Statistical analysis was performed by Student *t*-test, Welch's *t*-test or Mann-Whitney *U*-test. To compare the difference in disease progression of two groups, Kaplan-Meier method and logrank test were employed.

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