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Clinical Report

Detection of interleukin 10 in cerebrospinal fluid of patients with subacute sclerosing panencephalitis

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Subacute sclerosing panencephalitis (SSPE) is a neurodegenerative disorder due to persistent measles virus infection, with high level of measles-specific antibodies in cerebrospinal fluid (CSF). To analyze whether such response arises from a TH2-biased response, the authors determined TH1 (interferon [IFN]-γ) and TH2 (interleukin [IL]-4 and IL-10) cytokines in CSF, taken at diagnosis, of eight SSPE patients (median age, 57.5 month, range 42 to 76 months). All patients presented IL-10 (median 29.3 pg/ml, range 4.3 to 162 pg/ml), but not IL-4 ($<$10 pg/ml); only one case showed IFN-γ (162 pg/ml). These results are consistent with a TH2 bias or with a local, anti-inflammatory or neuroprotective mechanism involving IL-10. *Journal of NeuroVirology* (2005) **11,** 66–69.

Keywords: cerebrospinal fluid; cytokines; measles; subacute sclerosing panencephalitis

Subacute sclerosing panencephalitis (SSPE) is a severe disease induced by persistent infection of the brain by measles virus, a member of *Paramyxovirus* family, that has a gradual progressive and fatal course within 1 to 8 years. Although its pathophysiology is still unclear, demyelination and infiltration of inflammatory cells have been shown in brain biopsies or postmortem histopathological examination, together with evidence of astrogliosis, neuronal loss, degeneration of dendrites, and neurofibrillary tangles (Dyken, 2001).

Despite the long interval between the acute infection and symptoms of SSPE, there is evidence that measles infection of the brain occurs soon after the acute infection, with subsequent spread throughout the brain. One of the hypotheses is that under particular conditions measles virus, usually considered to induce nonproductive neural infection, begins to spread, as evidenced by the increased detection of viral RNA and protein in many brain regions, including the hippocampus, cortex, striatum, and thalamus. Transsynaptic interneuronal transmission of measles virus has been postulated, which may account for the absence of extracellular virus (Lawrence *et al*, 2000). The role of cytokines in the pathophysiology of SSPE is still unclear; however, misbalanced production could lead to ineffective control of measles virus replication in the central nervous system (CNS). During acute natural measles, interferon γ (IFN-γ) is involved in the early phase of infection, followed by prolonged production of TH2 cytokines, including interleukin 4 (IL-4) and IL-10 (Moss *et al*, 2002). High levels of this latter molecule have been shown to persist for several weeks, probably contributing to the defective immune function associated to measles infection.

It is unclear whether similar requirements apply or not to measles infection of the CNS; but if this were the case, a TH2-biased response in the CNS should facilitate infection and neuron damage. The production of oligoclonal bands of anti-measles immunoglobulins in cerebrospinal fluid (CSF) is well established among SSPE patients. Consistent with a TH2-biased response, this production could be indicative of clonal expansion of selected B-cells, probably within the CNS. Indeed, anti–measles virus

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Table 1 Clinical and laboratory findings for SSPE patients

Patient code/sex	*Age of measles disease*	*Age of SSPE onset*	*Initial diagnostic*	*CSF anti-measles antibodies*	*IL-4 (pg/ml)*	*IL-10 (pg/ml)*	*IFN-γ (pg/ml)*	*Outcome*
SSPE-1/m	11 months	5 years 9 months	Progressive myoclonic epilepsy	5.83	<10	4.3	<8	Nonfatal
SSPE-2/m	7 months	3 years 10 months	Acute disseminate encephalomyelitis	3.07	<10	29.9	<8	Fatal
SSPE-3/f	10 months	3 years 7 months	Progressive myoclonic epilepsy	5.01	<10	16.9	<8	Not available
SSPE-4/m	6 months	4 years 4 months	Neuronal ceroid lipofucsinosis	4.22	<10	25.3	<8	Nonfatal
SSPE-5/m	10 months	4 years 9 months	Lennox-Gastaut syndrome	4.01	<10	28.7	<8	Nonfatal
SSPE-6/f	6 months	4 years 9 months	Progressive myoclonic atonic epilepsy	5.97	<10	36.4	162	Nonfatal
SSPE-7/m	10 months	6 years 4 months	Bilateral choriorretinitis, blindness	5.55	<10	97.5	<8	Nonfatal
SSPE-8/m	8 months	5 years 3 months	Behavioral changes, ataxia, atonic episodes	2.75	<10	162	<8	Nonfatal
Controls ($N = 5$)	—	—	Noninflammatory CNS disease	Negative	ND	<3.9	<8	—

antibodies and oligoclonal bands in CSF are used as diagnostic criteria for SSPE (Garg, 2002). In agreement with this hypothesis, at least in mice, IFN-γ is critically required to control measles infection of the CNS, because normal mice overcome experimental encephalitis caused by measles virus, but this ability is abrogated by IFN-γ neutralization, resulting in increased susceptibility to encephalitis and impaired clearance of virus from the CNS (Finke *et al*, 1995). Polymorphism in the TH2 cytokine–related genes has been studied. In Japanese patients the frequency of IRF-1 allele 1 in the IL-4 promoter gene was associated with increased IL-4 synthesis, which could confer genetic susceptibility to SSPE to the host (Inoue *et al*, 2002).

The occurrence of measles epidemics in 1997 to 1998 in Argentina resulted in the increased of SSPE cases among affected children. Between 2002 and 2003 we detected 13 cases, some of which have been described in detail elsewhere (Barrero *et al*, 2003). In this report we evaluated whether a biased pattern of cytokines was present in the CNS by measuring the CSF level of the TH2 cytokines IL-4 and IL-10 and the TH1 cytokine IFN-γ.

Diagnosis of SSPE was based on Dyken's criteria, defined as (1) progressive cognitive decline and stereotypal myoclonus; (2) characteristic electroencephalogram (EEG) changes; (3) raised cerebrospinal fluid (CSF) globulin levels without pleocytosis; (4) raised CSF measles antibody titers; and (5) typical histopathologic findings in brain biopsy.

Samples of CSF obtained during the diagnostic procedure were stored at -70°C. Specific measles antibodies titer was assessed by automated qualitative enzyme-linked fluorescent immunoassay (BioMeriéux, Marcy l'Etoile, France) and expressed in arbitrary fluorescence units. IL-4, IL-10, and IFN-γ detection was performed with commercial enzyme immunoassay (EIA) kits (R&D Systems, Minneapolis, MN), used according to the manufacturer's instructions. The sensitivity limits of the assays were 10 pg/ml for IL-4, 3.9 pg/ml for IL-10, and 8 pg/ml for IFN-γ. CSF obtained from five children (four girls, one boy, aged between 5 days and 12 years) with noninflammatory CNS diseases (perinatal asphixia, haloperidol intoxication, one case of cerebral pseudotumor, and two cases of routine assessment in healthy children born to mothers with suspicion of genital herpes) were used as control.

Clinical and laboratory findings of eight SSPE patients are described in Table 1. All had born during measles outbreak and had suffered measles exanthema when they were between 6 and 10 months old. Median age at SSPE onset was 57.5 months (range 42 to 76 months) and the median lag period was 50 months (range 32 to 66 months). CSF cytochemical analyses were reported as normal for age. By the time of this report all patients except patient SSPE-2 are alive. In patients SSPE-4, SSPE-5, and SSPE-6, genetic characterization of brain tissue–associated measles virus was performed, and phylogenetic relationships clustered these viruses with the wild-type D6 genotype isolated during the 1998 outbreak (Barrero *et al*, 2003).

All CSF samples of SSPE patients presented detectable values of IL-10, with a median of 29.3 pg/ml (range: 4.3 to 162 pg/ml). None of the samples had IL-4 and only one patient (SSPE-6) presented detectable IFN-γ (who also had the highest values for both anti-measles immunoglobulin G (IgG) in CSF and total CSF IgG). IFN-γ and IL-10 were not detected in controls. CSF IL-4 was not assayed in controls, due to the limited available volumes of CSF.

Inflammation in the CNS is rigorously controlled by several mechanisms. To limit immune cell entry and to restrict T cell–neuron interactions, multiple anatomical and biochemical barriers exist within the brain, including the presence of the blood-brain barrier, limited lymphatic drainage, and the paucity of class I major histocompatibility complex (MHC) molecules on resident brain cells. The immune response appears able to resolve an infection without apparent neuronal damage by a noncytolytic role of the antiviral T-cell response. Although the basis of such virus clearance remains poorly understood, the process appears to be mediated by IFN-γ (Patterson *et al*, 2002).

Here we show that CSF of patients with SSPE present high values of IL-10 at diagnosis, without IL-4 and IFN-γ. The lack of IFN-γ in CSF in all but one case is consistent with the assumption that an effective control of measles virus replication within the CNS is absent. A possible explanation is the lack of maturity at the time of the initial infection, resulting in inappropriate levels of IFN-γ. The production of IFN-γ is dependent upon the age, being clearly defective at birth and still at low age (Smart and Kemp, 2001). All our patients suffered of measles when they were less than 1 year old. In addition, changes in the epidemiological SSPE pattern was observed in Turkey in recent years: although the incidence has decreased, the age at onset has shortened (Anlar *et al*, 2001). Factors affecting the duration of the latency period should be further investigated.

Moreover, the analysis of IFN-γ and IL-10 production by blood leukocytes show that most SSPE patients are unable to produce IFN-γ when challenged with measles antigen, whereas IL-10 production is preserved (Hara *et al*, 2000). Nevertheless, to some extent, CNS production of IFN-γ must exist, because this cytokine has been detected by immunohistochemistry in brain biopsy of SSPE cases (Anlar *et al*, 2001). In contrast, the lack of IL-4 is intriguing and raises several possible explanations. Like IL-10, IL-4 is a common TH2 cytokine, which was expected to be high. A first caveat is the sensitivity of the EIA assay employed. Levels below its sensitivity of detection (as measured by ultrasensitive assay) could be of interest. A possibility is the time course of sampling: IL-4 could have been lost in CSF by the time of sampling, as reported for serum IL-4 during acute measles infection (Moss *et al*, 2002). Although possible, this explanation seems rather improbable, because all values were below the assay's sensitivity and IL-10 was easily detectable in all samples. IL-10 values were heterogeneous, but present in all cases. No positive results were obtained with CSF samples of control children and the reported values for IL-10 in normal human CSF is low or absent even in children (Bell *et al*, 1997; Ishiguro *et al*, 1996). Because simultaneous serum samples had not been stored, the lack of appropriate paired samples hindered us to comparatively analyze the concentration of IL-10 in both compartments and the eventual detection of a concentration gradient between them. Such a gradient has been shown for IL-10 in traumatic brain injury, another inflammatory neural disorder. Although there are many reports about cytokines in CSF of SSPE patients, including IL-1, IL-2, and tumor necrosis factor (TNF)-α, as well as lymphocytes subsets (Mehta *et al*, 1997; Tekgul *et al*, 1999), data on CSF level of TH2 cytokines are scarce.

Among many other possibilities, the presence of IL-10 can be the expression of TH2 cells activity in the CNS. IL-10 is one of the main regulatory products of these cells, contributing to the generation of an antibody response while decreasing the activity of TH1 cells.

On the other hand, IL-10 level in CSF could also represent the extent of a local anti-inflammatory or neuroprotective response within the neural compartment, through regulation or defense against neural damage in SSPE. Indeed, IL-10 is synthesized in the CNS, where it plays a role in limiting clinical symptoms of several inflammatory, infectious and neurodegenerative diseases (see Strle *et al*, 2001, for review). In addition to leukocytes, IL-10 and its membrane receptor can be produced by both microglia and astrocytes. Though controversial, some data support a protective role for IL-10 in several models of CNS cell death, including neurons (Grilli *et al*, 2000) and glia (Molina-Holgado *et al*, 2001). Although no direct evidence supports this interpretation, it is an exciting possibility and certainly deserves further research.

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