ABSENCE OF ANTI-PURKINJE CELL ANTIBODIES IN PATIENTS WITH CEREBELLAR ATAXIA FOLLOWING FALCIPARUM MALARIA

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Abstract. Immunological mechanisms have been implicated in the pathogenesis of delayed cerebellar ataxia following falciparum malaria (DCA). We tested serum and CSF samples obtained from 39 Sri Lankan patients with DCA for the presence of antibodies (Ab) directed against cerebellar Purkinje cells by an immunofluorescence (IF) technique and Western blot analysis. For the IF test 7μ thick frozen sections of histologically normal cerebellum obtained at post mortem were used. Proteins obtained from crude preparations of Purkinje cells isolated from the cerebellum were used for Western blot analysis. Sera obtained from patients known to have anti-neuronal antibodies associated with cerebellar degenerations and paraneoplastic disorders (anti-Hu and anti-Yo Ab) and sera from normal blood donors served as positive and negative controls, respectively. All serum and CSF samples obtained from patients with DCA were negative for Ab directed against cerebellar Purkinje cells. Humoral mechanisms are, therefore, unlikely to be important in the pathogenesis of this delayed complication of falciparum malaria.

INTRODUCTION

Delayed onset cerebellar ataxia (DCA) is a recognized complication of falciparum malaria (Senanayake 1987; Edirisinghe *et al,* 1987; de Silva *et al,* 1988; Wijesundere, 1989; Senanayake and de Silva, 1994). The syndrome is characterized by a self-limiting, acute onset, isolated cerebellar ataxia without any evidence of cerebral involvement. Severe gait and truncal ataxia are striking features, suggesting that the lesion affects predominantly midline cerebellar structures. Ataxia occurs 7 to 28 days following an otherwise uncomplicated attack of falciparum malaria, and can last for up to 8 weeks. The majority of patients have an afebrile period before onset of cerebellar symptoms. Several factors suggest involvement of immunological mechanisms in the pathogenesis of the condition; delay between the onset of fever and onset of ataxia, selec-tive cerebellar involvement, favorable response to corticosteroids seen in some patients (Senanayake, 1987) and elevated cytokine levels in the sera and cerebrospinal fluid of patients durings ataxia and which fall, subsequently, on clinical recovery (de Silva *et al,* 1992a).

DCA complicating falciparum malaria, which first appeared in Sri Lanka in 1984 (Senanayake *et al,* 1984), reached epidemic proportions during the next few years (de Silva *et al,* 1992b). Epidemic' DCA coincided with an epidemic of falciparum malaria which occurred between 1984 and 1988 in Sri Lanka (Anti-Malaria Campaign 1982-1991). Since 1990, hardly any cases have been reported. This suggested that a new strain of *P. falciparum* (which caused the malaria epidemic) was probably responsible for DCA. Not all individuals who contracted falciparum malaria during this period developed DCA. This indicates that host susecptibility factors also play an important role in the pathogenesis of this condition. These facts have led to the hypothesis that molecular mimicry existed between antigens of a new strain of *P. falciparum* and epitopes within the cerebellum, and that, in susceptible individuals, antibodies (Ab) formed during the attack of malaria cross reacted with these cerebellar epitopes giving rise to cerebellar dysfunction.

The objective of this study was to investigate the presence of Ab directed against cerebellar Pur-kinje cells in patients with DCA.

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MATERIALS AND METHODS

Thirty-nine patients with DCA following an attack of falciparum malaria who were admitted to the Teaching Hospital, Peradeniya, Sri Lanka, were investigated. Thirty-four of the patients were males and their median age was 29 years (range 18-56). During routine investigation (which included lumbar puncture), 2ml of venous blood (from all 39) and 2ml of cerebrospinal fluid (CSF, from 15 patients) were obtained after informed consent. All samples, which were stored at -70°C and transported in dry ice, were tested for antibodies directed against cerebellar Purkinje cells using an immunofluorescence (IF) technique and Western blot analysis. Serum from patients with anti-neuronal Ab associated cerebellar degenerations and paraneoplastic disorders (anti-Hu, anti-Yo and anti-Ri Ab) and from normal blood do-nors served as positive and negative controls, respectively, in all experiments.

Immunofluorescence

Seven micron thick frozen sections of histologically normal cerebellum obtained at post-mortem were used. Sections were first fixed in acetone. They were then incubated with 10% normal goat serum for 20 minutes to block non-specific binding. Following this the sections were incubated in turn with serum (patients and controls, diluted 1:500) or CSF (patients only, diluted 1:25) for 2 hours, and fluorescein-labeled goat anti-human IgG (diluted 1:200) for 1 hours, with a phosphate buffered saline wash in between. All incubations were performed at room temperature. The slides were then mounted in aqueous media and examined under a fluorescence microscope.

Western blot analysis

Protein (500 μg) obtained from crude preparations of Purkinje cells isolated from normal cerebellum were seperated in reducing conditions using 10% polyacrylamide gel electrophoresis. Proteins were then transferred to a nitrocellulose filter (Towbin *et al,* 1979) and blocked with 5% dry Carnation milk (Carnation Company, CA, USA). The filters were then cut into strips, and each strip was incubated with serum (patients and controls, diluted 1:500) of CSF (patients only, diluted 1:25) for 2 hours at room temperature. The strips were then washed and incubated with iodine-125 labeled protein A (0.1 μCi /ml) for 1 hour also at room temperature. After a further wash the strips were dried, apposed to Kodak XAR 5 film (Sigma), and exposed at -70°C.

RESULTS

On Western blot analysis, no consistent bands were seen in any of the serum of CSF samples obtained from patients with DCA. There was no immunohistochemical evidence for the presence of Ab directed against cerebellar Purkinje cells in the patients' serum of CSF either.

DISCUSSION

Cerebellar ataxia is well known to occur following viral, bacterial, fungal and other protozoal infections (Ukadgaokar *et al,* 1981; Wadia *et al,* 1985; Weiss and Guberman, 1978). In these instances too it has been suggested that immunological mechanisms, either humoral or cell mediated, are responsible for cerebellar dysfunction, although no conclusive evidence for this has been presented.

Our results suggest the absence of anti-Purkinje cell Ab in patients with DCA following falciparum malaria. An earlier study using an immunofluorescence technique (de Silva *et al,* 1992c) found no evidence of adsorbed malarial antigens or immune complexes on glial cell preparations made from CSF of patients with DCA. The same study also found no evidence of Ab directed against the cerebellum in serum or CSF of patients with this syndrome. However, in that investigation only immunohistochemical methods were used for detection of Ab, and the authors themselves admit to the lack of sensitivity when employing only this technique. When all these results, including the findings of the present study, are taken together, it becomes very unlikely that humoral mechanisms play an important role in the pathogenesis of DCA following falciparum malaria. The finding of raised concentrations of tumor necrosis factor α, interleukin 2 and interleukin 6 in sera and CSF of patients with DCA (de Silva *et al,* 1992a) probably indicate that cell mediated immune responses are the important mechanisms which give rise to

cerebellar dysfunction in DCA following falciparum malaria.

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