RESEARCH NOTE

Absence of Antibody-dependent Enhancement (ADE) of Viral Infectivity in the Epidemic Neuropathy in Cuba

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Key words: antibody-dependent enhancement - enterovirus - epidemic neuropathy

During 1992-1993, a new disease called Epidemic Neuropathy (EN) was reported in Cuba. The patients complained of weight loss, blurred vision, sensitivity to light and gradual loss of visual activity. Other patients presented complaints of pain in the upper and lower limbs, dysesthesia and paresthesia, burning sensation on the feet soles and other symptoms. More than 50,000 cases were reported from the entire country. The disease was observed mainly in adults. The etiology of EN appears to be related to several factors, including nutritional deficiencies and a probable neurotoxic factor. In addition, an enterovirus identified as coxsackie A9 and an agent that produced a slow progressive CPE, were isolated from some of the patients' CFS samples (G Llanos et al. 1993 *Epidemiol Bul PAHO 14*: 1-4). Since the etiopathogenesis of EN is not known, and some patients had evidence of immune complex formation (Grupo Operativo Nacional, Ministerio de Salud Pública de Cuba, Informe sobre Neuropatía Epidémica, julio de 1993), we study whether or not an ADE phenomenon was related to this disease.

The aim of this study is twofold. First to study the possible immunoamplification of the strain 47/93/IPK (identified as Coxsackie A9) in peripheral blood lymphocytes (PBL) from healthy donors in the presence of subneutralizing concentrations of antibodies against this virus; and second, to study the possible presence in sera of EN patients of immunenhancing antibodies to the same strain grown in U-937 cells.

For the first experiment, PBLs from ten healthy donors were obtained by the Ficoll-Hypaque method (A Boyum 1968 *Scand J Clin Lab Investig 21*: suppl 97). Cells were resuspended in RPMI at 10^6 cells/ml. Volumes of strain 47/93/IPK (moi=0.01) were mixed with different dilutions (10^3-10^6) of a serum of EN patient with 1/160 neutralizing titer to this virus. After 1 hr incubation at 37°C, mixtures were added to cell suspensions previously dispensed in 24 wells Costar tissue culture plates. These cells were incubated at 37°C in a humidifed incubator with 5% CO_2 for three days after which were frozen and thawed three times. Virus was titered in Vero cells and the titers calculated by Reed and Muench method.

For the second study volumes of strain 47/93/IPK (moi=0.01) were mixed with serum dilutions of EN patients (1/50-1/500000). After 1 hr incubation at 37°C, mixtures were added to U937 cell suspensions in RPMI ($5x10^6$ cells/ml). Cell incubation and virus titration were done as described above. In both studies each combination was tested in duplicated and a virus control with PBS was included. The immunenhancement response was considered positive when viral titer (in enhancing conditions) was two or more log ID50 greater than the control viral titer.

The immunenhancement activity of the strain 47/93/IPK is shown in Table I. Only in three (30%) of donors the immunenhancement reaction at 1/1000-1/10000 serum dilutions was positive. No immunenhancement antibodies were detected in

TABLE I

Enhancing activity to 47/93/IPK (cox A9) strain in health donors

Case	Sex	Age	Enhancing rate[a]
1	F	41	2
2	F	38	0
3	F	27	2
4	F	35	1
5	F	41	1
6	M	31	0
7	M	34	0
8	M	25	1
9	M	35	2
10	M	41	1

[a]: viral titer in enhancing conditions/control viral titer

Received 16 August 1994
Accepted 5 May 1995

TABLE II

Immunenhancement antibody detection study in EN sera patients

caso	Nt titer[a]	enhancing rate[b]
152	160	0
153	>320	1
164	>320	0
167	>320	0
192	>320	1
245	-	1

[a]: titer of neutralizing antibodies to 47/93/IPK strain
[b]: viral titer in enhancing conditions/control viral titer

sera studied in the second experiment (Table II). The ability of strain 47/93/IPK to replicate in U-937 cells was previously confirmed (data not shown).

Antibody dependent enhancement results from the interaction of three components: virus, antibody and Fc receptor. This phenomenon was not previously reported in enteroviruses although there is a large number of viruses in which *in vitro* virus enhancement has been observed (M Bendinelli, H Friedman Plenum Press 1988, I Kurane et al. 1991 *Rev Med Virology 1*: 211-221, SB Halstead 1988 *Science 239*: 476-481). During the EN outbreak, immune complexes were detected in some EN patients suggesting the possibility that an ADE phenomenom could be related (Grupo Operativo Nacional, *loc. cit.*). Our preliminary results suggest that, at least in the PBL of some of healthy donors studied, it was possible to observe an increase of viral replication under immunenhancement conditions. Although this finding indicates the possibility of this phenomenon, it does not mean that it occurs *in vivo*. Furthermore, no enhancing antibodies were detected in sera patients under the conditions of this experiment.

The EN outbreak ceased in September 1993. So far the etiopathogenesis of the disease is not known although toxic, metabolic and nutritional factors have been implicated. The finding of an agent, in addition to Coxsackie A9 virus, in the CSF of a high number of patients does not allow us to eliminate the possible infection etiology of this disease.

The role of this agent is not clear at this moment, however this preliminary results do not suggest an immunenhancement mechanism involving Coxsackie A9 infection. More studies are needed in order to clarify the etiology and the pathogenesis of the disease.