
**CELL-MEDIATED AND HUMORAL IMMUNITY IN
WEST SYNDROME**

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The underlying mechanisms of cerebral insult producing infantile spasms and hypsarrhythmia in young children is unknown. The basic etiology of this disorder was reported as "biochemical abnormality of genetic or acquired origin, or a failure of maturation of certain enzymes, and the character of the seizure pattern, a reflection of the immaturity of the nervous system at the onset of the disorder" by Millichap and Bickford (1962). Others, as Low (1958) referred to the hypothesis of an allergic mechanism.

In children with the West syndrome, Gastaut et al (1978) based on computerized tomography, reported diffuse cerebral atrophy predominant in the fronto-temporal region. They concluded that "an unknown factor, therefore, must be assumed to be responsible for the electro-clinical symptoms, and perhaps for the atrophy as well".

It seems that factors of cerebral insults so diverse may act in a highly unspecific manner.

In relation to immunological studies in hypsarrhythmia, it is important to remember Reinskov's report (1963) on the presence of antibody to brain tissue in the sera of four children with this disorder. Unfortunately, systematic studies were not performed.

In this preliminary report, we have studied the immunological status in patients with West syndrome, in order to investigate the possible role of humoral and cell mediated immunity in this neurological disorder.

MATERIAL AND METHODS

The test group consisted of five children, aged between 4 months and 3 1/2 years, with EEG and clinical West syndrome features. They were all submitted to clinical, neurological, electroencephalographic and immunological evaluation.

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Neurological evaluation — EEGs were recorded on a Elema Shoenander instrument, using the 10-20 international system of electrode placement and both bipolar and unipolar derivations. Records were obtained while the patients were awake whenever possible and during spontaneous or barbituric induced sleep.

Urine qualitative tests for inborn metabolic errors were performed in every children (clinistrix, fenistix, clinitest, 2 — 4 dinitrophenylhydrazine and azure A.).

Immunological evaluation — Quantitative analysis of serum IgG, IgA and IgM were performed by radial immunodifusion (Mancini et al, 1956), using "Partigen Plates" (Hoechst, Behringwerke, Marburg). The Ig values were classified as normal, depressed or elevated, by comparison with our laboratory data for healthy person, in accordance with standard values for Brazilian population in different age ranges, as described by Irulegui et al. (1975).

Intracutaneous test with 2 μg of PHA (purified phytohaemagglutinin for clinical use, Wellcome reagents, England), were carried out according to Bonforte et al. (1972). The arithmetic mean between the two largest diameters of the area of induration was calculated in milimeters at 24 hs and nodules equal or larger than 5 mm, were considered as an indication of a positive reaction.

Sensitization with dinitrochlorobenzene (DNCB) was accomplished according to Mendes et al. 1974. Sensitizing doses of 2.000 μg of DNCB dissolved in 0,1 ml of acetone were spread and maintained over a 2 sq. cm. area of skin on the patient's back for 48 hours. Eighteen or more days later, excluding the ACTH therapy days, a patch test was made with 100 μg of DNCB in acetone on a skin site different from the one used for sensitization. Skin reactions consisting of erythema and induration at 48 hours, were accepted as evidence of sensitization.

T and B peripheral blood lymphocytes were studied by rosette formation, as described by Mendes et al. (1974b). By this method, the lower values of our laboratory, obtained from 20 healthy children over 1 year of age, are 13% for B cells and 35% for T cells. Values below this limit, were considered depressed.

Inhibition of peripheral leucocyte migration in the presence of PHA (10 $\mu\text{g}/\text{ml}$) was performed according to Morison (1974). In our experience (more than 50 cases), leucocyte migration index (mean area of migration without PHA) were always lower than 0.55 for normal persons.

Lymphocyte cultures were performed according to Mussati et al. (1976). The stimulation agent employed was PHA-P (Difco, 1:100 dilution) and the results were expressed as blastogenic index, which is the ratio: cpm of stimulated tubes/cpm of control tubes. Prior experience with 50 cases of PHA lymphocyte cultures in normal subjects indicated that subjects with blastogenic indices lower than 10.0 are depressed and those with indices between 10.0 and 20.0 are slightly depressed.

CASE REPORTS

V.C.P. — Is a boy, born of a 38 weeks pregnancy. He weighed 1,700 g. at birth and his Apgar score at first minute was 4. After seven hours, he developed respiratory distress syndrome and bronchopneumonia at five days of life. He had normal

psychomotor development up to seven months of age, when he got bacterial meningitis and a few days after recovery, chickenpox. Then, the child began to show neuromotor development arrest, so that 15 days after chickenpox recovery, he had lost all his neuromotor acquisition. At one year of age, the child was first seen in neuropediatric clinic. He was in an almost permanent infectious disease state (bronchopneumoniae, otitis, diarrheas and tonsillitis). On clinical examination he was a well nourished microcephalic boy (CP = 42 cm) with normal facies and grossly normal hair. No cutaneous abnormalities were noticed. There was an abundant bronchial secretion and a toe nail mycosis. On neurological examination the child had normal fundi, impaired active movements, hypertonia in all extremities, exaggerated deep tendon reflexes with knee and ankle clonus and a positive Babinski sign in both feet. No spasms or seizures were reported. Results of laboratory tests for inborn metabolic errors screening were all normal. In the first immunological study performed, he had negative response to DNCB sensitization and impaired lymphocyte transformation induced by PHA in autologous plasma. Skull X Rays and cerebrospinal fluid examination were normal.

At two years of age his parents first noticed spasms, characterized by upward, leftward or rightward gaze, followed by flexion of the arms and head, several times a day. EEG, performed in barbituric induced sleep, showed a pattern of hypersarrhythmia, with synchronous burst suppression activity. The child received a course of ACTH 25 units per day, nitrazepan 0,5 mg/kg/day and phenobarbital 50 mg per day. ACTH was withdrawn within five days due to a new infection. At this time, immunological evaluation showed a fall in T cell number, in the peripheral blood. A second EEG at this time was unchanged. Therapy with nitrazepan was continued, due to improvement of seizures. At 2 years and 7 months of age athetoid movements in both arms were first noticed, with a neurological examination otherwise unchanged. Several EEGs performed later during barbituric induced sleep and resting, showed generalized, bilateral, and synchronous sharp and slow wave complexes, bursts of high voltage fast spikes discharges, mixed with a hypersarrhythmic pattern. Computerized axial tomography showed minimal diffuse enlargement of the ventricles.

After the age of 3 years and 6 months, the resting and sleep EEGs showed slow delta waves background activity with posterior predominance, bursts of desynchronization of background activity, bursts of recruiting high voltage fast rhythm and generalized and synchronous sharp and slow wave complexes.

In his last immunological examination, depressed PHA lymphocyte stimulation in autologous plasma, positive sensitization to DNCB and increased IgA serum levels, were still present.

The child is now 4 years old. There has been no significant improvement in his psychomotor performance, he has brief periods of cessation of movements, with upward gaze and smiles, several times a day. He has had no serious infections since the age of 2 years and 10 months.

S.A.B. — Is a girl, born of normal pregnancy and delivery. She weighed 3,000 g at birth and her Apgar score in the first minute was 8. Wassermann test performed in the umbilical cord blood showed a positive result and the child was properly treated with penicillin.

At 1 1/2 month of age she developed bacterial meningitis. Despite this fact she had a normal psychomotor development until 4 months of age, when there was abrupt onset of multiple brief spasms, characterized by flexion of arms, legs and head. She stopped to follow objects, to smile, to sit with support and to support her head. Examination at admission (4 months and 10 days of age) disclosed a well nourished girl, with normal facies and normally sized head; hair was grossly normal; no cutaneous abnormalities were noticed.

There was an intense bronchial secretion. On neurological examination the child showed normal fundi, hypertonia in lower extremities, exaggerated deep tendon reflexes in legs and feet. Deep tendon reflexes in arms and face were normal and symmetrical.

Skull X-Rays and cerebrospinal fluid examination were normal. Blood Wassermann test was negative, inborn metabolic errors screening was negative. EEG, performed in barbituric induced sleep showed a pattern of hypsarrhythmia, with synchronous burst suppression activity. Immunological study showed a negative response to sensitization with DNCB and a reduced percentage of T lymphocytes. She was given clorazepam 12 mg per day, phenobarbital 50 mg per day and ACTH, starting with 25 units per day, until 75 units per day. With this therapy, there was disappearance of the hypsarrhythmia.

The last EEG showed normal background activity and a central focus in the left hemisphere. The child is now 9 months old. There has been improvement of her psychomotor development.

A.D.P. — Is a boy, born of a normal pregnancy and delivery. He weighed 2,850 g at birth. At 2 months of age he developed brief spasms of reflexion of arms and head with eye blinks several times a day. Also, he had seizures with hypertonia, cyanosis and crying, for 30 minutes until 7 months of age. There was arrest of psychomotor development: he never supported his head only smiled and followed objects. The child was not medically treated. He had also one episode of bronchopneumonia. He was first seen at this hospital at 1 year and 1 month of age.

Examination revealed a well nourished microcephalic boy (CP = 42 cm) with generalized hypertonia and impairment of movements. There was exaggerated deep reflexes, with knee and ankle clonus and a positive Babinski sign in both feet.

The child had normal fundi. Several flexion spasms were observed and also brief periods of cessation of movements; no cutaneous abnormalities were noticed.

Results of laboratory tests for inborn metabolic errors, skull X-Rays and cerebrospinal fluid examination were normal.

EEG, performed in barbituric induced sleep, showed generalized synchronous sharp and slow wave complexes discharges, mixed with hypsarrhythmic pattern and synchronous burst suppression activity. There was also sharp and slow wave complexes occurring only in the right temporal region.

Immunological study showed impairment of cell-mediated immunity, detected by decrease in the following tests: DNCB sensitization, PHA lymphocyte stimulation and leucocyte migration inhibition.

The child received a course of clonazepan 12 mg per day, phenobarbital 5 mg/kg/day and ACTH 25 units per day, during 20 days.

A second resting and spontaneous sleep EEG showed slow background activity and generalized synchronous sharp and slow wave complexes discharges.

The child is now 1 year and 10 months old, he is free of flexion spasms seizures, but there has been no improvement of his psychomotor development.

F.D.S. — Is a boy born of normal pregnancy. Delivery was complicated: the umbilical cord was around his neck. The child did not cry immediately after delivery. He weighed 3,800 g. at birth. He had no signs of psychomotor development at all. At 3 months of age, there was onset of seizures with generalized hypertonia, cyanosis and clonus of extremities, lasting for 5 minutes, several times a day. The child was treated with phenobarbital, with slight improvement.

He was first admitted at 11 months of age. Examination revealed a well nourished microcephalic boy (CP = 45 cm), with no cutaneous or hair abnormalities and normal fundi. There was an abundant bronchial secretion.

The child had generalized hypotonia and normal deep tendon reflexes. Several spasms, characterized by flexion of the arms and eye blinks, were noticed. Inborn metabolic errors screening was negative. Skull X-Rays and cerebrospinal fluid examination were normal.

EEG, performed in barbituric induced sleep showed a pattern of hypsarrhythmia, with synchronous burst suppression activity.

Immunological study showed a negative response to sensitization with DNCB. The child began a course of ACTH, 25 units per day, nitrazepan 0.5 mg/kg and phenobarbital 50 mg per day on an outpatient basis. He had several episodes of fever and respiratory infections.

We lost contact with the patient after two consultations, during which it was observed that his parents were not giving him the medication.

E.A.P. — Is a boy born of a normal pregnancy and delivery. He had a normal development up to 3 months of age, when presented with diarrhea, bronchopneumonia and urinary infection. He was admitted to the pediatric clinic where he remained for 3 months. He had also bacterial meningitis at the hospital.

A few days after discharge, his parents noticed the occurrence of brief spasms characterized by flexion of trunk, legs and arms, with upward gaze, several times a day. There was an arrest of psychomotor development.

Examination at 7 months of age showed an undernourished, microcephalic child (weight 4,520 g; CP = 39 cm) in opisthotonus.

He had normal fundi, generalized hypertonia, exaggerated deep tendon reflexes, normal facies and grossly normal hair. There was an abundant bronchial secretion. Several flexion and extension spasms seizures were reported. There was also periods of cessation of movements with upward gaze and eye blinks.

EEG, performed in barbituric induced sleep showed hypsarrhythmia, with synchronous burst suppression activity. Inborn metabolic errors screening showed normal results. Skull X-Rays and cerebrospinal fluid examination were normal. Bone-marrow

examination for storage cells was negative. Therapy with nitrazepan 0.5 mg/kg. phenobarbital 50 mg per day and ACTH 25 units per day was introduced, but ACTH was withdrawn within 3 days because of new infections (otitis and bronchopneumonia). Nitrazepan and phenobarbital were continued. There was no improvement in the frequency of seizures, but there was evidence of inadequate care of the child, with discontinued intake of medication. He returned only at 2 years and 3 months of age. He presented with a 3rd degree protein-caloric undernutrition (weight: 8,150 g), with unchanged seizures.

He had athetoid movements of extremities, tongue and neck, hypotonia, depressed deep tendon reflexes, ankle jerk and Babinski sign.

EEG performed in spontaneous sleep, showed generalized, synchronous sharp and slow wave complexes, mixed with hypsarrhythmic pattern and synchronous burst suppression activity.

Immunological tests performed at 2 years and 9 months of age showed a negative response to sensitization with DNCB, impaired lymphocyte transformation induced by PHA and elevated serum levels of IgG and IgA.

Therapy was changed to clonazepam, 9 mg per day. The child is now 4 years old. He has had until now moniliasis, several urinary infections and seven episodes of bronchopneumonia. There has been no improvement of his psychomotor development nor of his seizures.

RESULTS

Results of immunological study are shown in table 1.

DISCUSSION

The first case described (V.C.P.) had his immunological deficiency diagnosed prior to the onset of the flexion spasms.

All the children had probably pre-existing cerebral lesions; three had bacterial meningitis, one was born with the umbilical cord around his neck and one had microcephalia of unknown cause.

EEG examinations showed that three children are changing from West to Lennox-Gastaut syndrome.

All children had had frequent episodes of infectious diseases particularly serious in two of them (VCP and EAP). The child in better conditions had one bronchopneumonia.

Great incidence of infections in patients with West syndrome has been reported. Beaussart (1960) related recurrent infections interfering with ACTH therapy. Lison (1968) comments that infections of so great frequency can not be attributed only to the physical conditions of these children and considered therapy with benzodiazepines as related with respiratory infectious episodes. Millichap and Ortiz (1966), Hagberg (1967) and Volzke et al (1967) also have referred the frequency of infections in these children. Friedman and Pampiglione (1971) in a report about the clinical outcome of children with hypsarrhythmia,

Patients	Age	Skin tests		Lymphocytes		PHA blastogenic index		Leucocyte migration inhibition	Immunoglobulins (mg%)				
		PIIA	DNCB	T% T/mm ³	B% B/mm ³	Autologous Plasma	Homologous Plasma		IgG	IgA	IgM		
ADP	1 1/2 year	7	negative	73	2964	16	650	2*	1*	0.71*	1020	34	106
EAP	2 year 9 months	10	negative	34	1887	19	1054	4*	2*	0.86	2500**	200**	118
FDS	11 months	11	negative	37	1998	18	846	20	20	0.35	1200	91	105
SAB	4 months	8	negative	23*	966	23	966	42	62	0.52	890	40	150
VCP 1st	1 year	7	negative	40	2780	24	1668	3*	22	0.32	900	36	108
VCP 2nd	2 year 3 months	5	negative	18*	1185	19	1250	5*	19	0.48	1067	67	140
VCP 3rd	3 1/2 years	NP	positive	30	1166	13	505	1*	16	NP	1650	306**	166

Table 1 — Summary of immunological data: NP == not performed; * == depressed values; ** == values exceeding the upper normal range; PHA == phytohaemagglutinin; DNCB == dinitrochlorobenzene.

found a high early mortality (1:4) and referred that most of them succumbed to bronchopneumonia or other infections. Recently, Riikonen (1978) reported that 8 of the 11 children who had infantile spasms and cytomegalovirus infection, had severe recurrent respiratory infections.

The infectious diseases may be related to defects in cell mediated immunity, since all our children examined showed alterations in immune response. All showed a negative response to DNCB sensitization, that is a method used to investigate cell-mediated immunity "in vivo", since 95% of normal individuals give a positive response. (Convit et al, 1971; Mendes and Raphael, 1971; Lawlor et al, 1973). This may be interpreted as a inability of these children in being sensitized with a new antigen. Our children had a positive response to intracutaneous PHA, showing that the mechanisms involved in this response were preserved. Since PHA stimulates lymphocytes nonspecifically, prior exposure is not required for elicit a cell-mediated immune response "in vivo" (Burgio et al, 1971; Bonforte et al, 1972).

There was variability in the responses of lymphocyte blastic stimulation with PHA. Two children (F.D.S. and S.A.B.) had normal responses and three showed depressed values (A.D.P., E.A.P. and V.C.P.). The responses of A.D.P. and E.A.P. suggest a functional deficiency of thymus dependent lymphocytes in undergoing blastic transformation induced by this mytogen. The three evaluations performed in V.C.P. have shown that cell-mediated immunity depression is due to the presence of an autologous plasmatic blocking factor of blastic transformation.

One child (A.D.P.) showed deficiency in leucocyte migration inhibition test with PHA. There was no correlation in response of PHA lymphocyte blastic stimulation with the leucocyte migration inhibition in the presence of PHA. Both tests appraise T lymphocyte functional activity "in vitro", but disagreement of responses has been referred. Curtis and Hersh (1973) in normal persons immunized with haemocyanin, and Valdimarsson et al (1973) in patients with disseminated mucocutaneous candidiasis found similar results.

There was also disagreement in PHA reactivity "in vivo" and "in vitro". This finding was described before, by Airo et al (1967) in patients with chronic lymphocytic leukemia and by Bonforte et al (1972) in patients with sarcoidosis. They found strong positive responses to intracutaneous PHA and depressed responses to lymphocyte blastic stimulation "in vitro".

Serum levels of IgA, IgG, IgM were within normal values. The high values in one children may be attributed to infectious diseases.

Peripheral T and B blood lymphocytes were within normal values, except for the low T lymphocyte percentual observed in S.A.B. and V.C.P. (2nd evaluation). This latter result was obtained during ACTH therapy, that seems to affect T cell sub-population production (Claman & Moorhead, 1972). The interpretation of S.A.B. T lymphocyte percentual value is difficult since the values for normal young children are unknown. Diaz-JouAnen et al (1975), and Fleischer et al (1979) have found that newborn infants had lower T cell percentual values in relation to adult normal values.

The immunological evaluations of three children (S.A.B., F.D.S. and V.C.P. 1st) were performed without any medication; in two (A.D.P. and E.A.P.) and in the 3rd of V.C.P., evaluations were made during therapy with phenobarbital and benzodiazepines.

Changes of immunological behaviour induced by phenobarbital do not seem to occur (Crob and Herold, 1972; Fontana et al, 1976). Benzodiazepine effects on immunological function have not been studied.

One of these children (E.A.P.) presented a 3rd degree protein calorie under-nutrition when submitted to the immunological tests. The abnormalities in the immune response detected by a negative sensitization with DNCB, impaired lymphocyte blastic transformation, T cell values slightly depressed and high levels of serum IgG, may be associated to his nutritional deficit. Other authors have found depressed cell-mediated immunity together with a raising in the Ig levels in patients with malnutrition (Faulk et al., 1976; Mullen et al., 1978, Chandra, 1979).

The demonstration of a precipitating antibody to extract of brain tissues in four patients with hypsarrhythmia, reported by Reinskov (1963) supports the hypothesis of a autoimmune mechanism in West syndrome. Our study was not planned to investigate the relationship between autoimmunity and West Syndrome, however the depressed cell-mediated immunity observed in our children could be a predisposing factor to the development of an autoimmune disorder. Some authors have pointed out the association between immuno-deficiency state and autoimmunity. Allisson et al (1971), Stiller et al (1975) have suggested that failure in the thymus dependent system may be an important factor in the releasing of autoantibody production.

In this preliminary study, we have found immunological abnormalities associated with secondary West syndrome. Further investigations are needed in order to clarify the extent of this association and to investigate a possible role of an autoimmune process in this neurological disorder.

SUMMARY

The immunological status of five children with West syndrome consequent to previous cerebral lesions was investigated. Three children had West syndrome and two were in transition from West to Lennox-Gastaut syndrome. All of them showed cellular immunological deficiencies in the following tests: sensitization to DNCB, intracutaneous reaction to PHA, inhibition of leucocyte migration, blastic transformation of lymphocytes, T and B lymphocytes in peripheric blood and levels of serum immunoglobulins. These immunological deficiencies, of different degrees of severity, were associated to frequent infections in these children. A possible association between the immunological deficiencies and autoimmunity is discussed.

RESUMO

Imunidade celular e humoral na síndrome de West

Realizamos avaliações de imunidade celular e humoral em 5 crianças com síndrome de West secundária a lesões cerebrais. Todas apresentaram deficiência de imunidade celular, detectada pelas seguintes provas: sensibilização ao DNCB, reação intracutânea à PHA, inibição da migração de leucócitos, transformação blástica de linfócitos, determinação de linfócitos T e B no sangue periférico e dosagem de imunoglobulinas séricas. Estes exames foram realizados durante a síndrome de West em três crianças e durante evolução de síndrome de West para síndrome de Lennox-Gastaut em duas. Estas alterações imunitárias, de graus variáveis, associam-se a infecções frequentes nessas crianças. Discute-se uma possível associação desses defeitos imunitários com auto-imunidade.

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