

IMMUNOLOGICAL DISTURBANCE IN WEST AND LENNOX-GASTAUT SYNDROMES

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Infections are frequent in patients with West syndrome. Friedman and Pampiglione⁶ found a high early mortality (1:4) due to bronchopneumonia or other infections, in children with hypsarrytmia. Riikonen²¹ reported that 8 out of 11 children who had infantile spasms and cytomegalovirus infection had severe recurrent respiratory infections. Millichap and Ortiz¹⁴, Hagberg⁸ and Volzke et al²⁵ also have referred the frequency of infections in these children. Beaussart¹ related recurrent infections due to ACTH therapy and Chevie⁴ refers 14 cases of fatal infections, during hormonal therapy. The causes of such susceptibility to infections in these children are unknown. Primary hypogammaglobulinemia A and M was reported by Bugiani et al³ in two siblings with West syndrome. In a previous paper¹⁸, we have described depressed cellular immunity in five children with secondary West syndrome.

The purpose of our investigation is to assess the extent of this immunological dysfunction in children with primary (cryptogenic) and secondary West and Lennox-Gastaut syndromes.

PATIENTS AND METHODS

The test group consisted of 30 patients aged between 4 months and 18 years with West or Lennox-Gastaut syndromes. They were all submitted to clinical, neurological, electroencephalographic and immunological evaluations. The age, sex and etiology of the illness in West and Lennox-Gastaut patients are shown in table 1. One child had a third degree protein-calorie malnutrition¹⁸; nutritional condition of others was satisfactory. The control group for cell-mediated immunity consisted of 19 normal persons aged between 6 months and 18 years. Before immunological evaluation informed consent was obtained from the mothers of all infants enrolled in the study. Immunological tests were planned to avoid the interference of ACTH therapy. Sensitization with dinitrochlorobenzene (DNCB), intracutaneous test with phytohaemagglutinin (PHA) and "in vitro" tests were made two days prior to ACTH therapy, in children with West syndrome. The challenge with DNCB was made 18 to 60 days after the end

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Syndrome	Number of patients	Median age (total range)	Sex		Etiology	
			Male	Female	Primary	Secondary
West	18	11 months (4 months - 3 years)	10	8	3	15
Lennox-Gastaut	12	5 1/2 years (3 years - 18 years)	5	7	3	9

Table 1 — Distribution of West and Lennox-Gastaut patients according to age, sex and etiology of the illness.

of ACTH therapy. Lennox-Gastaut patients were submitted to immunological evaluation when not receiving ACTH. It was impossible to avoid the interference of anti-epileptic drugs. The medication consisted in combinations of phenobarbital with nitrazepan or clonazepan or valproic acid.

Immunoglobulin levels — Quantitative determinations of IgG, IgA and IgM were carried out by single radial immunodiffusion, according to Mancini et al¹¹, using "Partigen Plates" (Behringwerke, Marburg) and the results expressed in mg/ml. The Ig values were compared with standard values for Brazilian population in different age ranges, as described by Irulegui et al⁹. *Cell-mediated immunity evaluation* — *"In vivo" tests*: intracutaneous tests with 2 μg of PHA (Wellcome, England) were carried out according to Bonforte et al². The reactions were read at 24 h, and the two largest diameters of induration were measured in millimeters. A positive reaction was defined as a mean diameter ≥ 5 mm. Sensitization with DNCB was accomplished according to Mendes et al¹². Sensitizing doses of 2000 μg of DNCB dissolved in 0.1 ml of acetone were spread and maintained over a 2 sq cm. area of skin, on the back of patients for 48 h. 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 h were accepted as evidence of sensitization. This test was not performed in control patients due to ethical reasons. *"In vitro" tests*: T and B peripheral blood lymphocytes were studied by rosette formation, as described by Mendes et al¹³. Inhibition of peripheral leucocyte migration in presence of PHA (10 $\mu\text{g}/\text{ml}$) was performed according to Morison¹⁶. The results were expressed as leucocyte migration index, which is the ratio: (mean area of migration with PHA/mean area of migration without PHA) \times 100. Lymphocyte cultures were performed according to Musatti et al¹⁷. The stimulating 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/cmp of control tubes.

RESULTS AND DISCUSSION

The polyclonal stimulation of lymphocytes by PHA was depressed (blastogenic index < 15) in 10 out of 18 patients (55%) with West syndrome (Fig. 1). The majority of Lennox-Gastaut patients (78%) presented a good response to PHA stimulation. In this study lymphocyte transformation was little influenced by the patient's plasma. We detected blastogenic inhibition activity in one out of 8 cultures of West patients performed in both autologous and homologous plasma. This inhibitory activity was detected when the blastogenic index was reduced in patient's plasma compared to normal plasma. Our findings suggest that depressed blastogenic response to PHA may be the consequence of an intrinsic T-cell dysfunction. The percentage of T lymphocytes was significantly lower in West and Lennox-Gastaut patients than in controls (Fig. 2). No alteration in the proportion of B lymphocytes was observed in these patients. In our study, leucocyte migration inhibition test in the presence of PHA was

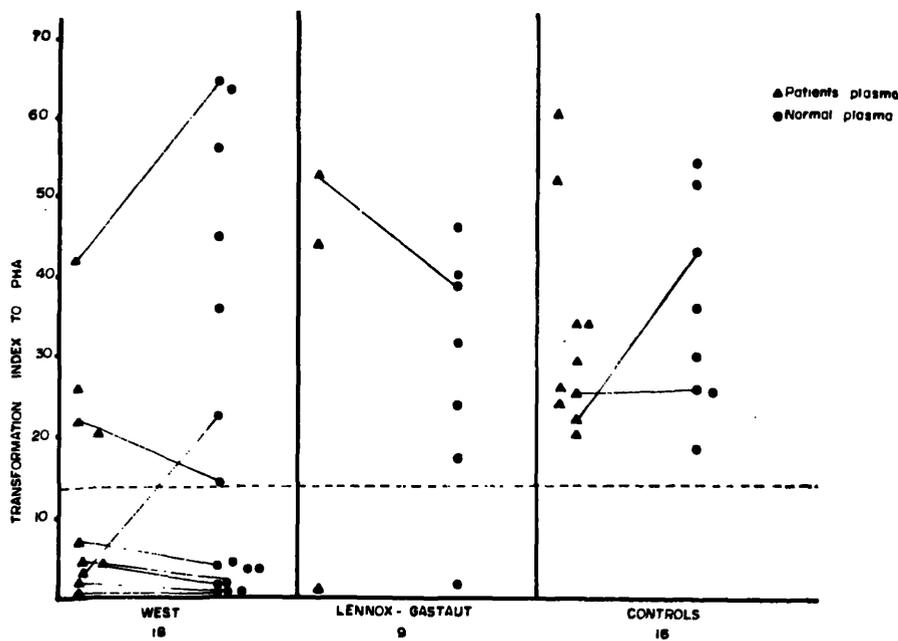
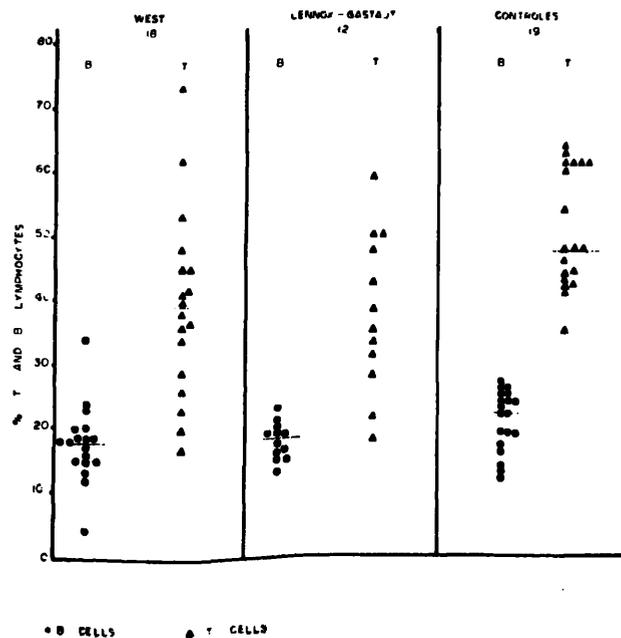


Fig. 1 — Lymphocyte blastic transformation index to PHA, in healthy controls, West and Lennox-Gastaut patients. Solid line shows simultaneous cultures of the same patient, containing either patient or normal plasma. Statistical analysis by Mann-Whitney U Test. (Control x West) $p < 0.025$. Values above dotted lines were considered as positive.

Fig. 2 — Percentage of T and B peripheral blood lymphocytes, in healthy controls, West and Lennox-Gastaut patients. Statistical analysis by Mann-Whitney U Test. T cells: (West x Control) $p < 0.05$; (Control x Lennox) $p < 0.05$. Horizontal bars represent the median value.



found to be slightly depressed only in West syndrome (Fig. 3). These data suggest a defect in lymphokine production in this group of patients. Results of delayed cutaneous reactions to PHA and DNCB are shown in Table 2. The percentage of positive response to PHA was significantly lower in patients with West syndrome than in normal controls. The high frequency of positive reactions observed in our control group is similar to that reported by Benforte et al² and Lawler et al¹⁰. Reactivity to DNCB was reduced in both groups of patients, specially in that with West syndrome. DNCB sensitization has been reported to be positive in 87% of normal children of 1-5 years of age²³ and in 92% of children aged between 1-17 years²². It is remarkable that cutaneous reaction to DNCB was the more sensitive parameter to detect immunodeficiency in our patients. A negative DNCB sensitization test may be attributable to either impairment of afferent or efferent limb of the immune response²⁰.

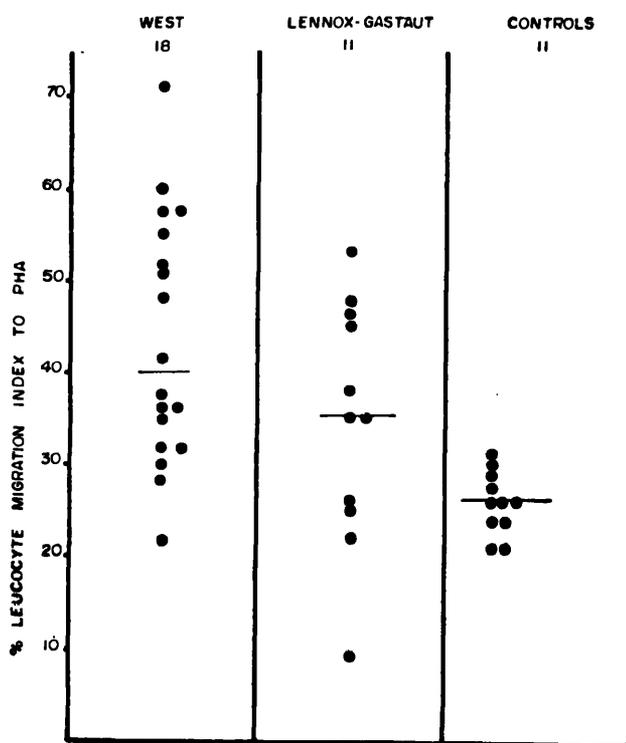


Fig. 3 — Leucocyte migration index to PHA, in healthy controls, West and Lennox-Gastaut patients. Statistical analysis by Mann-Whitney U Test. (Control x West) $p < 0.05$; horizontal bars represent the median value.

Group	% Positive reactions	
	DNCB	PHA
West	12 (2/16)	66 (10/15)*
Lennox-Gastaut	50 (5/10)	88 (8/ 9)
Control	N. D.	100 (12/12)

Table 2 — Percentage of positive cutaneous reaction to DNCB and PHA in West and Lennox-Gastaut patients. Statistically different from control ($p = 0.037$) as determined by Fischer exact probability test; N.D. = not determined.

Table 3 shows the number of depressed cell-mediated immunological tests per patient. Few children treated on a outpatient basis had not all the tests performed, due to lack in the previously estipulated return days. So, these

Group	Number of patients	Number of depressed tests			
		0	1	2 to 3	4 to 5
West	18	0	3	9	6
Lennox-Gastaut	12	2	3	6	1

Table 3 — Number of depressed cell-mediated tests per patient in West and Lennox-Gastaut syndromes.

numbers may represent minimal deficits. We considered depressed values those below the total range of controls. It is noteworthy that all cases of West syndrome showed depression in at least one out of 5 tests of cellular immunity. T cell dysfunction in children with Lennox-Gastaut syndrome was less severe, with only one patient exhibiting pronounced immunosuppression (4-5 depressed test). Our results also demonstrated that the impairment of cellular immunity varies from patient to patient. Similar findings has been reported in ataxia-telangiectasia¹⁸, but reason for such variability in the immune deficits remains unknown. Depressed cellular immunity has been described in some neurological disorders, such as primary gliomas, subacute sclerosing panencephalitis and ataxia-telangiectasia¹⁹. The significance of this association is not clearly understood.

Figure 4 shows IgG, IgA and IgM levels in the groups studied. Shaded areas indicate normal mean \pm total range in accordance with standard values for Brazilian population in different age ranges. Patients above and below 3 years of age are plotted separately, with appropriate normal areas (10 controls in each group). We found few cases of depressed immunoglobulin levels in our patients. Low levels of IgG, IgA and IgM were detected in 10% of West patients whereas only one Lennox patient presented hypoglobulinemia G. These findings contrast with those referred by Tartara et al²⁴ who studied serum immunoglobulin levels in 86 epileptic patients. They found depressed

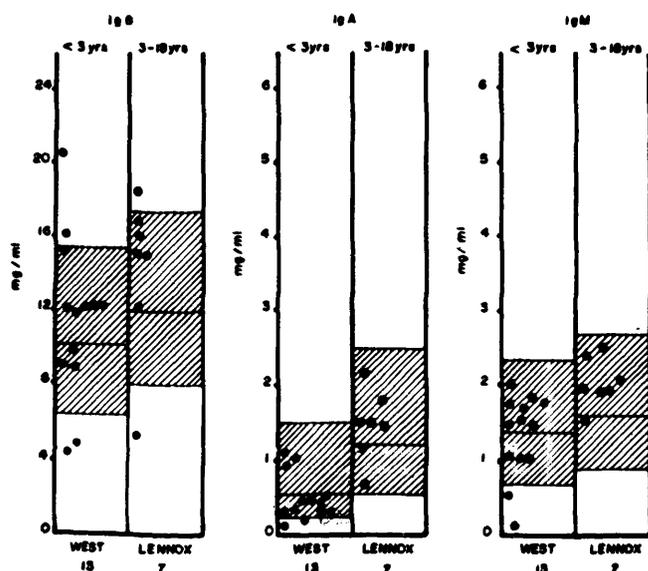


Fig. 4 — Serum immunoglobulin levels in West and Lennox-Gastaut syndromes. Shaded areas indicate normal mean \pm total range.

values in 42.4% of cases, not related to clinical type of epilepsy. On the other hand, Bugiani et al³ reported primary deficiency of IgA and IgM in two siblings with West syndrome.

The degree of depression of cellular and humoral immunity in West and Lennox-Gastaut syndromes correlates with their predisposition to infections. In 9 children, infections were so frequent and serious that they overshadowed neurological symptoms (broncopneumonias, diarrheas, meningitis, urinary infections, mycosis). It was surprising, therefore, to find that at the opposite extreme there were 6 children that had not an increase in susceptibility to infections when compared with normal individuals. Intermediate susceptibility to infections, such as one episode of bronchopneumoniae or urinary infections, was also found in 15 children.

There was no association with malignancy except for one child, in which retinoblastoma was diagnosed at the same time that West syndrome. In one case of West syndrome, previously described in detail¹⁵, the cell-mediated deficiency was diagnosed prior to the onset of the flexion spasms. The immunological evaluation of three children was performed without any medication. Changes of immunological behavior induced by phenobarbital do not seem to occur^{5,7}. Benzodiazepines and valproic acid effects on immunological function, have not been defined, to our knowledge. The immunological dysfunction detected in this study confirms our previous report¹⁵ that the thymus-dependent immune response is frequently depressed in West syndrome and extends this observation to Lennox-Gastaut syndrome.

SUMMARY

Cell-mediated and humoral immunity were investigated in 18 patients with West syndrome, 12 with Lennox-Gastaut syndrome and 19 healthy controls. The study included determination of T and B peripheral blood lymphocytes, serum levels of IgG, IgA and IgM, skin sensitization with DNCB, intracutaneous PHA, leucocyte migration inhibition test and lymphocyte blastogenic transformation in the presence of PHA. Cell-mediated deficiency was detected in 28 children whereas low levels of immunoglobulins were observed only in 6 children. Immunological disturbances were more prominent in children with West syndrome.

RESUMO

Distúrbios imunológicos nas síndromes de West e Lennox-Gastaut.

Investigamos a imunidade humoral e celular em 18 pacientes com síndrome de West, 12 com síndrome de Lennox-Gastaut e 19 controles. Os exames realizados foram os seguintes: percentagem de linfócitos T e B no sangue periférico, níveis séricos de IgG, IgA e IgM, sensibilização cutânea com o DNCB, PHA intra dérmica, teste de inibição de migração de leucócitos e transformação blástica de linfócitos em presença de PHA.

Detectamos deficiência de imunidade celular em 28 crianças, (18 com síndrome de West e 10 com síndrome de Lennox-Gastaut) e baixos níveis de imunoglobulinas em apenas 6.

A depressão imunitária mostrou-se mais intensa nas crianças que apresentam síndrome de West.

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