

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/21628>

Please be advised that this information was generated on 2020-09-19 and may be subject to change.

SHORT PAPER

A Dysbalanced Immune System in Cryptogenic Lennox-Gastaut Syndrome

B. G. M. VAN ENGELLEN, C. M. R. WEEMAES*, W. O. RENIER, J. A. J. M. BAKKEREN*, G. F. BORM† & P. F. W. STRENGERS‡

*Institute of Neurology, *Department of Paediatrics, and †Department of Medical Statistics, University Hospital, Nijmegen, and ‡Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands*

van Engelen BGM, Weemaes CMR, Renier WO, Bakkeren JAJM, Borm GF, Strengers PFW. A Dysbalanced Immune System in Cryptogenic Lennox-Gestaut Syndrome. *Scand J Immunol* 1995;41:209–13

In children with cryptogenic Lennox-Gastaut syndrome we found a functionally impaired humoral immune response to a primary antigen (haemocyanin), despite signs of a triggered immune system consisting of elevated IgG concentrations. This combination of immunological findings, considered to be the expression of a dysbalanced–triggered as well as functionally impaired–immune system, has also been described in an auto-immune disease like systemic lupus erythaematodes in humans, and in genetically epilepsy-prone rats. The interactions between the immune system and the nervous system in Lennox-Gastaut syndrome will be discussed.

Baziel G.M. van Engelen, MD, Department of Neurology, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

INTRODUCTION

The Lennox-Gastaut syndrome (LGS) is an unexplained, age-related epileptic encephalopathy with a tendency to become chronic [1]. Various pathophysiologic mechanisms have been suggested to explain this condition including the hypothesis that it results from an aberrant immune function. Indications supportive for this hypothesis include the reports on antibodies to brain tissue [2, 3], the suggested effect of intravenous immunoglobulin [4], and the reported associations with certain HLA class II alleles in patients with LGS [5]. The publications on humoral and cellular immunity are conflicting, impaired immunity (predominantly IgG₂ and IgA deficiencies) [6–8], normal immunity [9], and increased humoral or cellular immunity [7, 10] have been described. Indirect suggestive evidence for reciprocal interactions between the immune and nervous system in epilepsies are the immunological alterations induced by proper anticonvulsant drugs like phenytoin and carbamazepine, and the anticonvulsant effect of immunosuppressive drugs like adrenocorticotrophic hormone and corticosteroids in otherwise intractable epilepsies (for review, [4]).

For the present study we attempted to select a relatively homogeneous group of cryptogenic LGS patients, and an epilepsy control group receiving the same anticonvulsants. In

addition to studies on humoral and cellular immunity, we measured the *in vivo* primary humoral immune response after subcutaneous injection of the neoantigen, α -Helix pomatia haemocyanin (HPH). The *in vivo* immune response is a sensitive test of total immune responsiveness [11, 12] measuring both the afferent as well as the efferent arc of the immune system, and it is a functional test which might be able to detect a possible modulating influence of the epileptic brain on immune responsiveness. The results in both epilepsy groups are compared with those of normal controls and a possible misregulation of the immune system in LGS is discussed in the light of the conflicting data in the literature.

MATERIALS AND METHODS

Patients. The study population consisted of 12 children (four girls, eight boys, mean age 4.4 years) with cryptogenic LGS receiving valproate, either as monotherapy or in combination with other anticonvulsants, especially nitrazepam. An age and sex matched epilepsy control group consisted of seven children with other types of epilepsy [13] (situation-related seizures ($n=1$), symptomatic generalized seizures ($n=4$), temporal lobe epilepsy ($n=2$)) receiving the same regimen of anticonvulsant drugs as the LGS group. The normal control group was derived from a previously published reference group consisting of 114 healthy children aged 1 month to 16 years [14]. The study protocol was reviewed and approved by the

institutional ethical review board of our hospital. Informed consent was obtained from the children's parents.

LGS was defined by the characteristic triad: (1) multiple epileptic seizure types (axial tonic, atypical absences, atonic seizures) possibly associated with generalized tonic-clonic seizures and partial seizures; (2) inter-ictal slow spike-waves (fewer than three per second) in awake electroencephalography (EEG); (3) mental retardation or deterioration with personality disorders, with onset between 1 and 5 years of age [1]. Cryptogenic aetiology was defined as epilepsy of unknown cause in infants or children with unremarkable neuro-mental development prior to onset of seizures; normal physical examination; no abnormalities on cerebral CT or MR imaging, except for a slight dilatation of ventricles; no known mental retardation syndromes, chromosomopathy, or inborn errors of metabolism.

Immunological studies. Serum total IgG, IgA and IgM concentrations were determined as described earlier [14]. Serum IgG subclass concentrations were determined by radial immunodiffusion, and calibrated against standard serum containing 6.2 g/l IgG₁; 2.4 g/l IgG₂; 0.64 g/l IgG₃; and 0.46 g/l IgG₄ (HOO-020, CLB, Amsterdam, The Netherlands). Pre-immunization anti-HPH titres were determined with an indirect ELISA technique and expressed as percentage of positive reference samples [12]. Afterwards, 1 ml (1 mg) of HPH was given subcutaneously and serum was obtained 2 weeks (in LGS patients also 4) and 6 weeks after immunization. HPH is a well characterized, easily available primary immunogen, whose safety has been documented [11, 12]. One subcutaneous injection of 1 mL elicits a strong primary humoral (IgG, IgA, IgM) and cellular immune response in normal volunteers. Percentages of B cells were determined by immunofluorescence microscopy using anti-human immunoglobulin antisera (Kallestad Lab. Inc., Austin, TX, USA). The percentages of CD3, CD4, and CD8 positive T cells were determined using monoclonal antibodies (OKT3, OKT4, OKT8, respectively; Coulter Co., Hialeah, FL, USA). Lymphocyte proliferation *in vitro* after stimulation by phytohaemagglutinin and pokeweed mitogen was studied by standard methods.

All immunological studies were performed at a time when neither the patients nor controls showed any clinical signs of intercurrent infection.

Statistical analyses. The results of the immunological investigations in the LGS group and the epilepsy control group were compared with each other and also with the normal control group. Concentrations of immunoglobulins and subclasses were corrected for age by subtracting the expected value as calculated from the data of the reference group [14] from the actual value found. Anti-HPH titres measured in the LGS group and the epilepsy control group, were compared with those of previously described normal control children [15]. All group differences were evaluated using the Kruskal-Wallis test. In addition, Spearman's rank correlation coefficients were determined between IgG, IgA and IgM concentrations, and their respective anti-HPH titres. Significance was set at $P = 0.05$ (two-sided). In the case of multiple testing, the Bonferroni correction was used.

RESULTS

The only significant difference between the LGS group and the normal control group is an increased concentration of total IgG in the LGS group ($P < 0.05$). The epilepsy control

group did not show significantly increased IgG concentrations compared with normal controls, whereas the difference between the epilepsy control group and the LGS group was not significant. IgG₁, IgG₂ and IgG₃ concentrations did not differ significantly between the LGS group and any of the control groups.

The LGS group did not differ from any of the control groups with regard to percentage of B cells (mean 11.3%, standard deviation (SD) 5.5; individual values: 15, 14, 10, 17, 5, 21, 8, 14, 7, 6, 3, and 15%), CD3 positive cells (mean 64.5%, SD 6.4; individual values: 66, 73, 65, 72, 70, 60, 60, 60, 70, 65, 61, and 52%) or CD4/CD8 ratio (mean 2.8, SD 0.9; individual values: 1.8, 5.2, 2.9, 2.8, 2.4, 2.1, 2.3, 2.1, 2.3, 3.1, 3.1, and 3.1). Proliferation of peripheral blood lymphocytes to phytohaemagglutinin and pokeweed mitogen was normal in both the LGS and the epilepsy control groups.

Antibody responses to haemocyanin immunization

All pre-immunization anti-HPH titres were negative except for some very low IgG and IgM titres in all three groups (Fig. 1). The peak IgM response after 2 weeks did show significant differences between the groups ($P < 0.01$): it was decreased in the LGS group compared with the epilepsy control group ($P < 0.05$) and to the normal controls ($P < 0.005$), whereas the epilepsy control group and the normal controls did not differ. The IgA response after 2 weeks was significantly different in the three groups ($P < 0.05$) and was reduced in both the LGS ($P < 0.05$) and the epilepsy control group ($P < 0.05$) compared to normal controls, whereas the two epilepsy groups did not differ from each other. The IgG response did not differ significantly between the three groups ($P > 0.05$), although there was a trend towards lower IgG responses in the LGS group compared with normal controls ($P = 0.04$). Concerning the kinetics of the response, the increase in IgG, IgA and IgM anti-HPH titres, respectively between 0 and 2 weeks, showed the same differences as the peak anti-HPH titres after 2 weeks. The decline in all three classes of anti-HPH titres between 2 and 6 weeks was the same for all three groups.

In the LGS group there was a significantly positive correlation between IgM and anti-HPH IgM, anti-HPH IgA and anti-HPH IgG concentrations, respectively (all $P < 0.05$). The Spearman's rank correlation coefficients were 0.65, 0.75 and 0.66, respectively. No such significant correlation existed between the serum IgG and IgA concentrations, and their anti-HPH titres (all $P > 0.05$).

DISCUSSION

The main finding of this study is the impaired humoral immune response in patients with cryptogenic LGS. Until now, this has not been reported in LGS nor in other types of epilepsy. A decreased cellular immune response after dinitrochlorobenzene sensitization has only been described in one

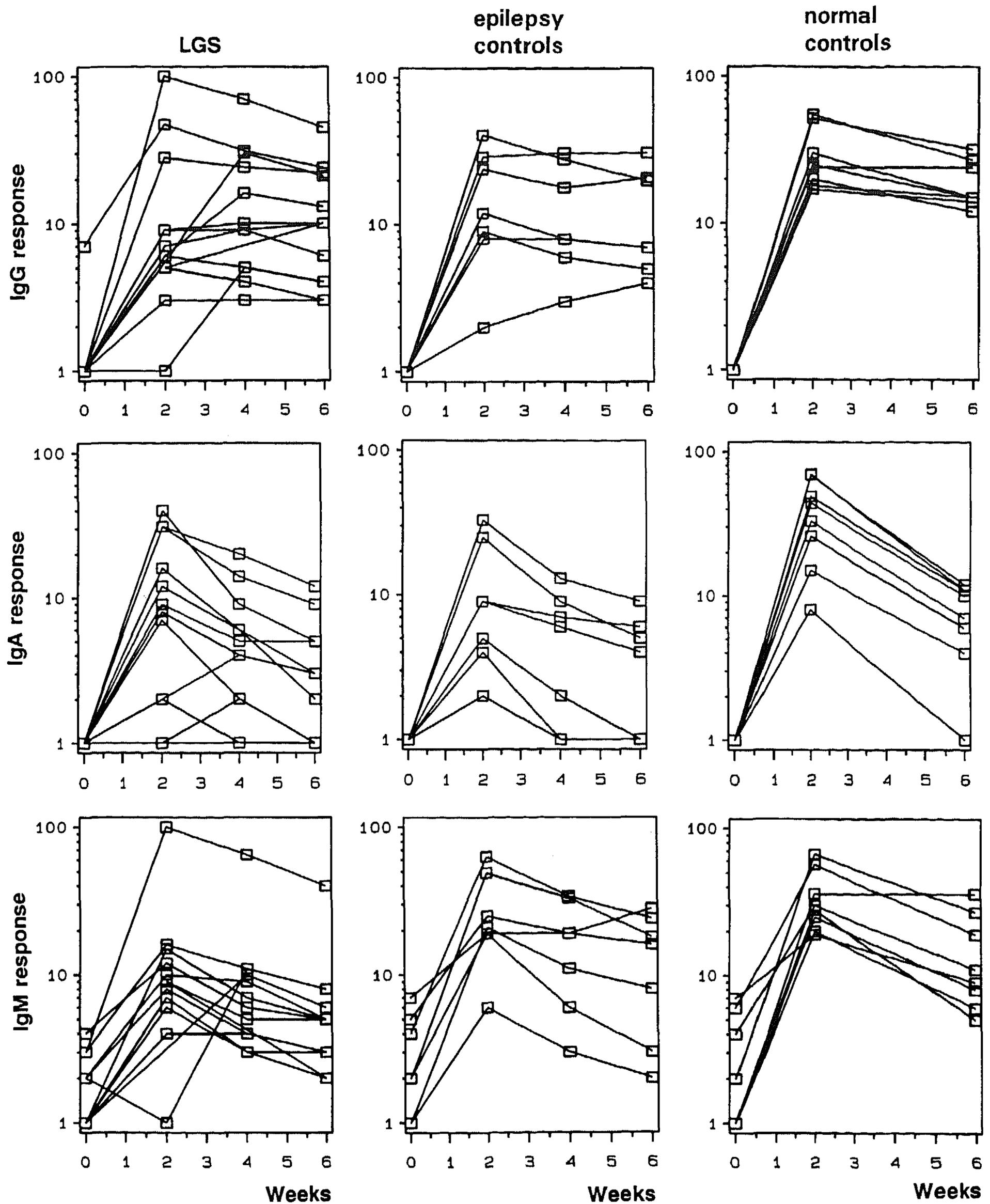


Fig. 1. *In vivo* IgG, IgA and IgM primary immune responses to haemocyanin (as percentage of positive reference samples) in the LGS group ($n=12$), epilepsy control group ($n=7$), and in normal controls ($n=8$).

report on a group of patients with cryptogenic as well as symptomatic types of LGS and West syndrome [8]. Factors underlying the impaired humoral immune response in LGS patients are not yet known, and some can be ruled out from

the present study. The immune response does not have a delayed time course (anti-HPH titres declined between 2 and 4 weeks); there is no decrease in the number of different types of immune cells; no decrease in IgM concentration (IgM

enhances the humoral immune response [16]); and no defect in lymphocyte proliferation *in vitro*. Since HPH is a T cell dependent antigen the impaired humoral immune response does not necessarily point to a primary B cell defect. A disturbance in the afferent arc of the immune system, a defective antigen processing or presentation by macrophages or a deficient interaction between B and T cells, may well account for the decreased immune responsiveness.

In addition (unexpected in the light of the impaired immune responses), the increased total IgG concentration in the LGS group points to a triggered immune system. This increase in total IgG in epilepsy has also been reported by others [7] and by our previous study [14] on a more extended epilepsy group consisting of the present LGS patients and patients with West syndrome. Such a triggered immune system can be caused by infectious or autoimmune processes [17]. Infectious causes have been excluded during the investigation period in our study.

In the current study, a drug effect cannot be entirely ruled out, but seems unlikely because both the LGS group and the epilepsy control group received the same anticonvulsants. Moreover, valproate and nitrazepam were used, drugs that are not known to have an effect on immunoglobulins.

A triggered as well as functionally impaired immune system, which may be called a dysbalanced immune system, is in accordance with and may partly explain the conflicting literature on immunological investigations in epilepsy. A study showing an elevated B cell number has been interpreted as conflicting with the report on an impaired cellular immune responsiveness [8, 10]. Because we have measured both, we have been able to reproduce in LGS patients just this combination of a triggered but nevertheless functionally impaired immune system, which has also been described in an autoimmune disease like systemic lupus erythematodes in humans [12], and in genetically epilepsy-prone rats [17]. Therefore, we think that this combination of findings, instead of being the expression of conflicting results, may have a biological significance pointing to a misregulation of the immune system. A possible explanation may be that high IgG concentrations cause a feedback inhibition of the immune response leading to a diminished capacity to respond to new antigenic challenges [16, 17]. Nevertheless, we could not confirm a significant negative correlation between IgG concentrations and immune responses in the LGS group. In addition, also the epilepsy control group showed increased IgG concentrations, however, without impaired humoral immune responses. We did find a significant positive correlation in the LGS group between IgM concentrations and immune responses, which is in accordance with the reported enhancing effect of IgM antibodies on the humoral immune response [16].

In conclusion, the present study found indications not of an immune deficiency, but rather of an immune dysbalance or misregulation in LGS. Interestingly, LGS a severe form of epilepsy, can be defined neurologically also as a dysbalance,

in this case of excitation and inhibition in neural networks leading to functional disturbances [18]. There are several possibilities that may account for the association between an immune and neural dysbalance [19]. One possibility is that the dysbalanced immune system alters neural function [20–22] and causes epilepsy. Certain antibodies intracerebrally injected in experimental animals induce epileptiform activity (for review [23]). Secondly, the neural dysbalance i.e., epilepsy may modify immune function [24]. As a consequence of epilepsy central nervous system (CNS) antigens may become exposed to and trigger the immune system. In experimental animals, lesions of the anterior hypothalamus modify asthma and reduce humoral and cellular responses to foreign antigens [25]. Thirdly, the disturbances both in the immune and nervous system could be due to a common underlying biological process. This has been described in experimental mice that develop severe autoimmune disease and learning disabilities, each of which possibly originate from neuropathological anomalies consisting of ectopic collections of neurons [26]. Whatever the exact interaction might be, one could speculate that the association between the neural dysbalance and the immune dysbalance in LGS may offer the potential of new treatment approaches in this type of intractable epilepsy by addressing the nervous system via the immune system.

ACKNOWLEDGMENTS

This study was supported by a grant (CLEO A69) from the Dutch National Commission for Epilepsy Research. We are indebted to Professor Dr T. H. The (Department of Clinical Immunology, University Hospital, Groningen, The Netherlands) for performing anti-HPH measurements.

REFERENCES

- 1 Beaumanoir A, Dravet C. The Lennox-Gastaut syndrome. In: Roger J, Bureau M, Dravet C, Dreifuss FE, Perret A, Wolf P, eds. *Epileptic syndromes in Infancy, Childhood and Adolescence*. London: John Libbey, 1992:115–32.
- 2 Mota NGS, Rezkallah-Iwasso MT, Peracoli MTS, Montelli TCB. Demonstration of antibody and cellular immune response to brain extract in West and Lennox-Gastaut syndromes. *Arq Neuropsiquiatr* 1984;42:126–31.
- 3 Pliopys AV, Greaves A, Yoshida W. Anti-CNS antibodies in childhood neurologic diseases. *Neuropediatrics* 1989;20:93–102.
- 4 van Engelen BGM, Renier WO, Weemaes CMR, Gabreels FJM, Meinardi H. Immunoglobulin treatment in epilepsy, a review of the literature. *Epilepsy Res*, (in press).
- 5 van Engelen BGM, de Waal LP, Weemaes CMR, Renier WO. Serologic HLA typing in cryptogenic Lennox-Gastaut syndrome. *Epilepsy Res* 1994;17:43–7.
- 6 Duse M, Tiberti S, Plebani A *et al.* IgG2 deficiency and intractable epilepsy of childhood. *Monogr. Allergy* 1986;20:128–34.
- 7 Fontana A, Grob PJ, Sauter R, Joller H. IgA deficiency, epilepsy and hydantoin medication. *Lancet* 1976;1:228–31.

- 8 Montelli TCB, Mota NGS, Peracoli MTS, Torres EA, Rezkallah-Iwasso MT. Immunological disturbance in West and Lennox-Gastaut syndromes. *Arq. Neuropsiquiatr* 1984;42:132-9.
- 9 Illum N, Taudorf K, Heilmann C *et al.* Intravenous immunoglobulin: a single-blind trial in children with Lennox-Gastaut syndrome. *Neuropediatrics* 1990;21:87-90.
- 10 Hrachovy RA, Frost JD, Shearer WT, Schlactus JL, Mizrahi EM, Glaze DG. Immunological evaluation of patients with infantile spasms. *Ann Neurol* 1985;18:414.
- 11 Reeves WG, ed. *Recent Developments in Clinical Immunology*, Amsterdam: Elsevier Biomedical Press, 1984:1-27.
- 12 Kallenberg CGM, Limburg PC, van Slochteren C, van der Woude FJ, The TH. B cell activity in systemic lupus erythaematosus: depressed *in vivo* humoral immune response to a primary antigen (haemocyanin) and increased *in vitro* spontaneous immunoglobulin synthesis. *Clin Exp Immunol* 1983;53:371-83.
- 13 Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for classification of epilepsies and epileptic syndromes. *Epilepsia* 1989;30:389-99.
- 14 Haraldsson A, van Engelen BGM, Renier WO, Bakkeren JAJM, Weemaes CMR. Light chain ratios and concentrations of serum immunoglobulins in children with epilepsy. *Epilepsy Res* 1992;13:255-60.
- 15 Weemaes CMR, The TH, van Munster PJJ, Bakkeren JAJM. Antibody responses *in vivo* in chromosome instability syndromes with immunodeficiency. *Clin Exp Immunol* 1984;57:529-34.
- 16 Heyman B. The immune complex: possible ways of regulating the antibody response. *Immunol Today* 1990;11:310-13.
- 17 Rowland RRR, Tokuda S, Weiss GK, Tso-Olivas D, Savage DD. Evidence of immunosuppression in the genetically epilepsy-prone rat. *Life Sci* 1991;48:1821-6.
- 18 Lopes da Silva FH, Kamphuis W, Wadman WJ. Epileptogenesis as a plastic phenomenon of the brain, a short review. *Acta Neurol Scand* 1992;140:34-40.
- 19 Reichlin S. Neuroendocrine-immune interactions. *N Engl J Med* 1993;329:1246-53.
- 20 Besedovsky H, Sorkin E, Felix D, Haas H. Hypothalamic changes during the immune response. *Eur J Immunol* 1977;7:323-5.
- 21 Korneva EA. Electrophysiological analysis of brain reactions to antigen. *Ann N Y Acad Sci* 1987;496:318-37.
- 22 Saphier D, Ovadia H, Abramsky O. Neural responses to antigenic challenges and immunomodulatory factors. *Yale J Biol Med* 1990;63:109-19.
- 23 Aali JA. Epilepsy and the immune system. In: Aarli JA, Behan WHM, Behan PO, eds. *Clinical Neuroimmunology*, Oxford: Blackwell, 1987:385-403.
- 24 Jankovic BD, Isakovic K. Neuro-endocrine correlates of immune response, I. Effects of brain lesions on antibody production, Arthus reactivity and delayed hypersensitivity in the rat. *Int Arch Allergy* 1973;45:360-72.
- 25 Felten DL, Cohen N, Ader R, Felten SY, Carlson SL, Roszman TL. Central neural circuits involved in neural-immune interactions. In: Ader R, Felten DL, Cohen N, eds. *Psychoneuro-immunology*. San Diego: Academic Press, 1991:3-25.
- 26 Sherman GF, Galaburda AM, Behan PO, Rosen GD. Neuroanatomical anomalies in autoimmune mice. *Acta Neuropathol* 1987;74:239-42.

Received 4 July 1994

Accepted in revised form 19 October 1994