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Oligoclonal IgG in the Cerebrospinal Fluid of Guinea Pigs with Experimental Allergic Encephalomyelitis (41510)

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Abstract. Oligoclonal IgG bands were demonstrated in the cerebrospinal fluid of guinea pigs with experimental allergic encephalitis before clinical signs developed. No bands were found in the serum of these animals nor were bands found in the CSF or serum of control animals. Immunofixation techniques demonstrated that the bands were immunoglobulins of the γ G class.

Experimental allergic encephalitis (EAE) is an acute neurological disease which can be produced in guinea pigs, rats, and other animals by injection of brain tissue or extracts from brain tissue such as myelin basic protein in complete Freund's adjuvant. The disease has been proposed as a possible experimental model for multiple sclerosis (MS) (1). The presence of oligoclonal IgG bands in the cerebrospinal fluid (CSF) of patients with MS is one of the most common immunological findings in this disease (2). Most patients with optic neuritis, acute herpes encephalitis, Epstein–Barr encephalitis, subacute sclerosing panencephalitis, and neurosyphilis also have bands present (3–5). Oligoclonal IgG is present in some patients with Guillain–Barre, myasthenia gravis, and Parkinson's disease but is not present in normal individuals (3, 6, 7). We report here the occurrence of oligoclonal IgG in the spinal fluid of guinea pigs with EAE.

Materials and Methods. Guinea pig myelin basic protein (BP) was prepared as previously described (8). Adult strain 13 guinea pigs were sensitized by injection of 0.7 mg BP in complete Freund's adjuvant (3.5 mg of heat-killed mycobacteria ($H_{37}R_v$) total) or 0.1 mg BP in complete Freund's adjuvant (0.1 mg of heat killed mycobacteria) in five sites in the nuchal region and in each hind foot pad. Injection of this material causes EAE in 100% of strain 13 guinea pigs within 21 days. At the time CSF was obtained (Day 11) none of the guinea pigs had developed clinical signs of disease and no circulating antibodies to BP were detectable (9). Control animals were injected with either the same emulsion containing no BP or with an emulsion containing BP but no mycobacteria. None of the control animals displayed any clinical signs of EAE.

The guinea pigs were anesthetized using ether inhalation for cisternal puncture. The cisternal subarachnoid space was entered with a 23-gauge needle and the sample was allowed to fill the needle. CSF samples from control and EAE animals were centrifuged in a Beckman microfuge at 10,000 rpm for 2 min and carefully transferred to conical plastic vials and stored at −20°; only samples with no evidence of hemolysis or red cells were included in this study. Blood was obtained by cardiac puncture and was allowed to clot at room temperature. The sample was centrifuged and serum was stored at −20°.

A modification of the SDS–polyacrylamide gel electrophoresis was used for the determination of oligoclonal IgG (10). With this new method it is possible to test unconcentrated CSF. Briefly, the electro-

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phoresis was carried out in vertical gel slabs. Acrylamide content of the stacking gel was 5% and the separating gel was 9%. Samples (50 μl of unconcentrated CSF or serum diluted 1:200) were prepared for electrophoresis by mixing 10 parts CSF or diluted serum with one part buffer (70% sucrose, 10% SDS, and bromophenol blue as dye). These specimens were placed directly on the stacking gel with no further treatment. We tested CSF samples from EAE and control animals with comparable IgG concentrations, i.e., 3 μg of IgG contained in 50 μl CSF. Oligoclonal bands were detected in EAE animals while no bands were found in control animals. These results indicate that the appearance of oligoclonal bands is independent of the IgG concentration. Purified guinea pig IgG were used as controls. After electrophoresis with a constant current of 10 to 30 mA per gel for 3–4 hr, the slabs were removed and stained for protein with Coomassie brilliant blue. Two or more bands in the IgG region were considered positive.

Immunofixation of IgG was done on the gel surface. Strips of cellulose acetate membrane were impregnated by soaking in the appropriate undiluted antiserum (anti-guinea pig IgG heavy and light chains, H & L) prepared in goat (Cappel Laboratories). The gels were overlaid with the strips for 2 hr in a moisture chamber at 4°. Antibody excess was carefully washed out overnight in phosphate-buffered saline (PBS) (pH 7.2) at room temperature. To detect the reaction between the oligoclonal IgG and specific immune sera, the washed gel was incubated with peroxidase conjugated rabbit anti-goat IgG (Miles-Yeda LTD, Israel) for 2 hr in a moisture chamber at 4°. The gel was washed overnight with frequent changes of PBS (pH 7.4) and reacted with diaminobenzidine (11). The enzymatic reaction was stopped by washing with cold tap water when color developed in the gel. The dark brown bands demonstrated by this technique confirmed the presence of IgG bands. Side-by-side comparisons were done on identical samples in which one lane was stained with Coomassie brilliant blue and another lane with peroxidase-conjugated antisera, to prove that IgG was present.

Results. A modified SDS–polyacrylamide electrophoresis method was used to detect oligoclonal bands in the unconcentrated CSF of guinea pigs in which EAE was induced. The animals were clinically well at the time they were studied. With the sensitizations used clinical signs of EAE are observed by 21 days after inoculation. Oligoclonal IgG bands were observed in the CSF of all 14 guinea pigs obtained 11 days after inoculation with 0.7 mg BP (Table I). Three bands were detected in 5 and two bands in 9 guinea pigs using this concentration (Fig. 1). Two oligoclonal bands were also found in three of four guinea pigs which were inoculated with 0.1 mg BP. These results suggest that the variation in BP concentrations did not affect the appearance of oligoclonal bands in these animals. None of the control guinea pigs had detectable components in the IgG region of the CSF.

The electrophoretic pattern of the sera of the inoculated guinea pigs showed no difference when compared to the control groups; all of the sera were considered to have no oligoclonal IgG (Table I).

The globulin class of the oligoclonal bands was determined by immunofixation with purified anti-guinea pig IgG heavy and light chain-specific sera. The localization of the color in the approximate IgG area was observed in all samples showing specific oligoclonal IgG patterns (Fig. 1); those without oligoclonal IgG were negative. This confirmed the class of the oligoclonal IgG.

Discussion. Oligoclonal IgG bands were demonstrated in the CSF but not in the sera from 17 of 18 guinea pigs inoculated with myelin basic protein in complete Freund's adjuvant. The observation that oligoclonal IgG did not occur in the CSF of any of the control animals shows an association with EAE. Staining with anti-guinea pig IgG heavy and light chains confirmed the specificity of the bands as IgG. The appearance of oligoclonal IgG in the CSF of basic protein-sensitized guinea pigs before the onset of EAE suggests that this antibody may contribute to the pathogenesis of the disease. Similar oligoclonal bands were found in three of four guinea pigs and reported in an abstract by Rostami (12).

TABLE I. DEVELOPMENT OF OLIGOCLONAL BANDS IN CSF OF GUINEA PIGS SENSITIZED WITH BASIC PROTEIN IN COMPLETE FREUND'S ADJUVANT

	GP sensitization BP (mg)/MTbc (mg)		No. of animals	GP with oligoclonal bands	
				CSF	Sera
EAE	0.7/3.5	CFA	14	14	0
	0.1/0.1	CFA	4	3	ND
Controls	0/0	—	1	0	0
	0/3.5	CFA	6	0	0
	0.7/0	IFA	7	0	0
	0.1/0	IFA	2	0	0

Note. Abbreviations used: CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; BP, basic protein; GP, guinea pig; MTbc, mycobacteria; ND, not done.

Oligoclonal bands were also detected in brain extracts and CSF of guinea pigs with chronic relapsing experimental allergic encephalitis (R-EAE) (13). It will be important to determine the antigenic specificity of the oligoclonal IgG in the sensitized guinea pigs since this finding may clarify the possible role of the IgG in these animals and may contribute to an understanding of the importance of oligoclonal IgG in MS.

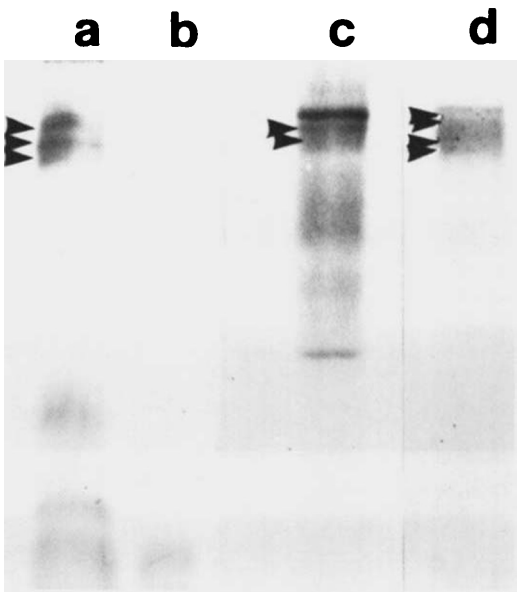

FIG. 1. Oligoclonal IgG in CSF of guinea pigs inoculated with basic protein in complete Freund's adjuvant (BP-CFA). SDS–PAGE electrophoresis of CSF, (a) CSF of guinea pig inoculated with 0.7 mg BP-CFA; (b) CSF of control guinea pig; (c) CSF of guinea pig inoculated with 0.1 mg BP-CPA; (d) immunofixation with an indirect peroxidase assay in CSF from guinea pig inoculated with BP-CFA. Note: The discrete oligoclonal bands (arrows) are in the IgG area. The IgG concentrations of CSF samples a, b, c, were similar, i.e., 3 μg IgG contained in 50 μl CSF.

A recent study of CSF and serum from rabbits with EAE showed oligoclonal IgG bands in some of these animals (14). The animals were tested prior to sensitization and at the time of development of clinical disease. A few rabbits had bands in their CSF and/or serum prior to sensitization. Of 11 rabbits given whole nervous tissue, 7 developed new bands in their serum and CSF. The absence of bands in the CSF of some of the animals in these studies and presence of bands in the serum may relate to the different species of animals studied or the collection of specimens at the time of appearance of symptoms. Our animals were sampled prior to development of symptoms and bands were always present in the CSF but not the serum. Serial studies of sensitized animals will have to be conducted to determine the time of appearance of bands. Another study (15) demonstrated anti-BP activity in the oligoclonal bands in serum and CSF from rabbits with EAE.

The use of the modified stacking polyacrylamide gel electrophoresis permitted the tests in this study to be carried out with 50-μl samples of unconcentrated CSF obtained by cisternal puncture. In the preparation of specimens for electrophoresis, the procedure avoided disruption of the IgG molecules to subunits as well as prevented IgG from aggregating during storage. This

was done by mild treatment with SDS which did not break it down to the constituent light and heavy chains. Bands are only present in the IgG region. This was further confirmed by using marker proteins of known molecular weight and by immunofixation with antibodies to guinea pig IgG heavy and light chains. The concentrations and defined porosity of the stacking and separating gels used for these studies did not permit entry of dimeric IgG (~ mol wt = 340,000 daltons) or IgM (~ mol wt = 900,000 daltons). The oligoclonal bands were the result of increased production of IgG by certain clones of plasma cells. The bands appear as distinct lines, sometimes with a background of diffuse polyclonal IgG. The SDS treatment modifies the IgG to permit it to enter the gel and migrate as a single entity. The IgG, however, is not irreversibly denatured because it can be eluted from gels without loss of functional capability. Thus, this procedure should be useful to study small serial samples from individual animals.

1. Paterson PY, Day ED, Whitacre CC. Neuro immunologic diseases: Effector cell responses and immunoregulation mechanisms. Immunol Rev 55:90, 1981.
2. Johnson KP, Arrigo SC, Nelson BJ, et al. Agarose electrophoresis of cerebrospinal fluid in multiple sclerosis: A simplified method for demonstrating cerebrospinal fluid oligoclonal immunoglobulin bands. Neurology 29:273–277, 1977.
3. Link H, Muller R. Immunoglobulins in multiple sclerosis and infections of the nervous system. Arch Neurol 25:326–344, 1971.
4. Link H, Norrby E, Olsson J. Immunoglobulins and measles antibodies in optic neuritis. N Engl J Med 289:1103–1104, 1973.
5. Olsson JE, Link H. Immunoglobulin abnormalities in multiple sclerosis. Relation to clinical parameters: Exacerbations and remissions. Arch Neurol 28:392–399, 1973.
6. Laterre EC, Callewaert A, Heremans JF, et al. Electrophoretic morphology of gamma globulins in cerebrospinal fluid of multiple sclerosis and other diseases of the nervous system. Neurology 20:982–990, 1970.
7. Williams A, Houff S, Lees A, Calne D. Oligoclonal banding in the cerebrospinal fluid of patients with postencephalitic Parkinson's. J Neurol Neurosurg Psychiatry 42:790, 1979.
8. Diebler GE, Martenson RE, Kies MW. Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. Prep Biochem 2:139–165, 1972.
9. Kies MW, Driscoll BF, Lisak RP, Alvord EC. Immunologic activity of myelin basic protein in strain 2 and strain 13 guinea pigs. J Immunol 115:75–79, 1975.
10. Iivanainen MV, Wallen W, Leon ME, et al. Micromethod for detection of oligoclonal IgG in unconcentrated CSF by polyacrylamide gel electrophoresis. Arch Neurol 38:427–430, 1981.
11. Graham RC, Karnovsky MF. The early stage of absorption of injected horseradish peroxidase in the proximal tube of the mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302, 1966.
12. Rostami A, Lisak RP, Blanchard N, Guerrero F, Zweiman B, Pleasure D. Oligoclonal IgG in cerebrospinal fluid (CSF) of animals with experimental allergic encephalomyelitis (EAE). Neurology 31 (2):126–127, 1981.
13. Mehta P, Pansmann H, Wisniewski H. Immunologic studies of chronic relapsing EAE in guinea pigs: Similarities to multiple sclerosis. J Immunol 127:334, 1981.
14. Whitacre CC, Mattson DH, Paterson PY, Roos RP, Peterson DJ, Arnason BGW. Cerebrospinal fluid and serum oligoclonal IgG bands in rabbits with experimental allergic encephalomyelitis. Neurochem Res 6:87–96, 1981b.
15. Petrasek J, Panitch H, Norrby E. Formation of oligoclonal antibody in experimental allergic encephalomyelitis. Neurology 31 (2):126, 1981.

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