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# Oligoclonal IgG Antibody Response in the Central Nervous System to Different Measles Virus Antigens in Subacute Sclerosing Panencephalitis

**(human cerebrospinal fluid/measles virus antigens/agarose electrophoresis)**

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**ABSTRACT** **Agarose electrophoresis of cerebrospinal fluid from two patients with subacute sclerosing panencephalitis revealed in both cases several homogeneous bands in the intermediate and basic gammaglobulin region. The bands were identified as IgG with various degrees of kappa or lambda light-chain restriction in crossed immunoelectrophoresis.**

**Homogeneous IgG bands were separated by preparative agarose electrophoresis and were found to represent measles virus-specific antibodies. Antibody activities against different virus products (hemolysin, hemagglutinin, and nucleocapsids) were carried by different populations of IgG. In both cerebrospinal fluid materials, antibodies to the virus hemolysin and to virus nucleocapsids were found to be relatively more basic than the antibodies to virus hemagglutinin, which appeared in the basic intermediate gammaglobulin region.**

**We conclude that the oligoclonal IgG bands in cerebrospinal fluid of patients with subacute sclerosing panencephalitis represent homogeneous measles virus antibodies. In some instances, the IgG bands appeared to fulfill criteria of monoclonality on the basis of their electrophoretic homogeneity, selective light-chain type, and specific antibody activity.**

Subacute sclerosing panencephalitis (SSPE) is a comparatively rare disorder of the central nervous system that is associated with a slowly progressing infection with measles virus (1, 2). This disease is, *inter alia*, characterized by increased, and often rising, titers of measles antibodies in serum and cerebrospinal fluid (CSF) (3, 4). Another significant feature is a selective increase of immunoglobulin G (IgG) in CSF and in the brain, appearing as one or several bands on electrophoresis (5–7). The major part of IgG in CSF derives from a nonvascular source, being presumably synthesized locally in the central nervous system (8). The specific production of measles virus antibodies in the nervous system is suspected from the markedly reduced serum/CSF ratio of titers of these antibodies, as compared to the corresponding ratio of antibodies against viruses without connection to SSPE (9, 10).

The present paper reports on the presence in different electrophoretically homogeneous bands of IgG of antibody activities against different measles virus antigens. Recently developed techniques for identification of antibodies against different structural and nonstructural components of measles virus (11) were used.

Abbreviations: SSPE, subacute sclerosing panencephalitis; CSF, cerebrospinal fluid; HLI, hemolysis inhibition; HI, hemagglutination inhibition; CF, complement fixation.

## MATERIALS AND METHODS

*Serum and Lumbar CSF* samples were obtained from two patients (Sa and Lo) with clinically and serologically manifest SSPE. Before further characterization, CSF samples were concentrated 50–100 times by means of vacuum dialysis against phosphate-buffered saline (pH 7.4) at 4° in collodion membrane bags (Sartorius Membranfilter Gmbh, Germany).

*Fractional Methods.* Analytical ("short run") electrophoresis was performed essentially as described by Johansson (12). Glass plates, 10 × 20 cm, were covered with a 0.15-cm layer of 1.2% agarose (2 parts A-37 and 1 part A-45 agarose, Industrie Biologique Francaise) in barbital buffer at pH 8.6, I = 0.05. 5-μl Samples were applied and electrophoresis was run at 15 V/cm for 45 min.

*Crossed Immunoelectrophoresis* was performed by a modification of the antigen–antibody crossed electrophoresis method described by Laurell (13). Electrophoresis in the first dimension was performed under conditions similar to those described above for agarose electrophoresis, but with the time of the run extended to 2 hr. Immediately after this run, 0.4-cm gel strips containing the gamma region (protein cathodal to transferrin) were transferred to the middle of 8 × 8 cm glass plates and embedded in agarose containing antisera of different concentrations. A 20 to 24-hr run in the second dimension was made without cooling at 2–3 V/cm. After washing and drying, the plates were stained with Amidoblack. Rabbit antisera to human IgG, IgA, and IgM (raised in this laboratory) and to human kappa and lambda light-chain determinants (Brostex, Denmark), were used.

*Preparative Electrophoresis* was performed as described above for the first-dimension run in crossed immunoelectrophoresis. IgG content in the samples was adjusted to 10–15 mg/ml, and 100 μl of sample was applied in each experiment. Immediately after the electrophoresis, 0.5 to 1.0-cm wide gel strips were cut along the longitudinal axis of the glass plate, dividing the gammaglobulin region cathodal to transferrin into 12 fractions. The gel strips were frozen and thawed, and protein was eluted through a narrow-gauge cannula. Immunoglobulins in the fractions were quantitated by means of radial immunodiffusion (14). In each experiment, reference gel strips at each end of the plate were transferred to glass plates, fixed, and stained in order to identify the positions of homogeneous gammaglobulin bands.

*Serological Techniques.* The procedures used for determination of antibody against different measles virus products were described in detail (11). The overall occurrence of antibodies against envelope structures of the virus was determined in neutralization (in most cases performed in the presence of anti-human gammaglobulin) and hemolysis inhibition (HLI) tests. Antibodies against the envelope, excluding those reacting with the hemolysin, were measured in hemagglutination inhibition (HI) tests with antigen treated with Tween 80 and ether. Nucleocapsid-specific antibodies were identified in complement-fixation (CF) tests with purified nucleocapsids. "Whole virus" CF tests were done with cell harvests of virus grown in Vero cells frozen and thawed three times, in order to detect additional activities directed against virus structural or nonstructural antigens.

## RESULTS

Electrophoretic separation of the two CSF samples showed the presence of 5–8 homogeneous bands in the intermediate to basic gammaglobulin region (Fig. 1). Some of the bands were markedly basic and migrated more slowly than the most cathodal gammaglobulins seen in normal human serum. Trace gamma zones were seen in one of the sera (Lo), while no bands were detected in serum from the other patient.

Crossed immunoelectrophoresis showed in both cases that the main homogeneous bands were populations of IgG, with apparently restricted kappa or lambda light-chain determinants. The most clear-cut results were obtained in analysis of CSF from patient Sa (Fig. 2), in which the lower number and better separation of bands allowed better characterization. The homogeneous bands gave peak formations with antiserum to IgG. The individual IgG bands varied, however, in their reactions with antisera to light-chain determinants. Some bands reacted with kappa, while others reacted with lambda antisera. Fig. 2 shows the pattern of kappa and lambda IgG populations thus emerging from crossed immunoelectrophoresis. A similar picture of intermingling kappa and lambda IgG populations was also found in case Lo (Fig. 3). This case also illustrates that the prolonged electrophoresis used for crossed immunoelectrophoresis and preparative electrophoresis in some instances resulted in splitting of bands that appeared homogeneous by analytical electrophoresis (Figs. 1 and 3). Thus, some of the basic and intermediate bands appear to be biclonal, with one kappa and one lambda population of IgG placed close together.

Results from crossed immunoelectrophoresis of the patients' sera are not shown. The serum patterns were dominated by the diffuse electrophoretic distribution of kappa and lambda IgG. In both cases, however, slight deviations in the kappa and lambda patterns, corresponding to those in the matching CSF, were seen. Thus, traces of homogeneous immunoglobulin populations upon a high background level of polyclonal IgG were also detected in the serum from patient Sa, in which no bands were seen by analytical electrophoresis.

Serological activities in electrophoretically separated fractions of CSF IgG are shown in Figs. 2 and 3. All fractions containing homogeneous IgG bands carried measles virus antibody activities. The antibodies to different measles virus

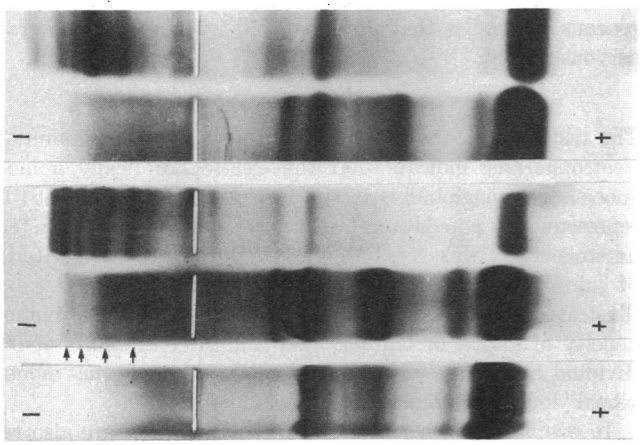


FIG. 1. Agarose gel electrophoretic patterns in CSF and serum from patients Sa (*top*) and Lo. A normal human serum is included for comparison (*bottom*). Note the basic distribution of homogeneous gamma zones in the two CSF samples. In one of the sera (Lo), trace homogeneous zones in the gamma region (*arrows*) are seen.

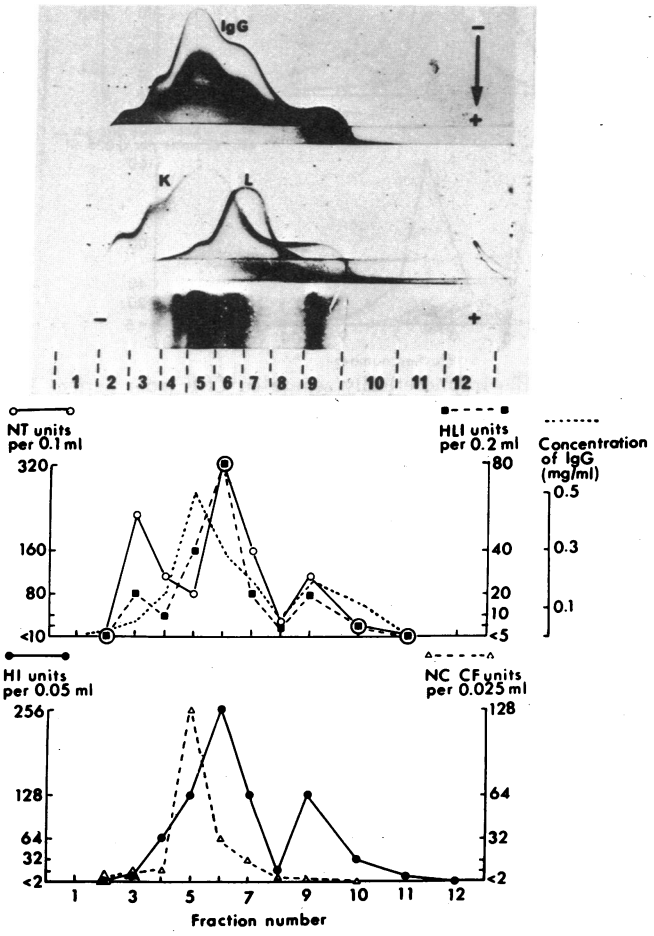


FIG. 2. Electrophoretically separated CSF from patient Sa. *Top:* the pattern from crossed immunoelectrophoresis with antiserum against human IgG, and the superimposed patterns from crossed immunoelectrophoresis with antisera against human kappa (*K*) and lambda (*L*) light-chain determinants. *Bottom:* measles virus-specific antibody activities found in fractions separated by preparative electrophoresis. The cutting pattern for obtaining fractions is indicated on the stained gel strip. IgG content in individual fractions is shown by *stippled lines*. Antibody activities are shown by the following symbols: Neutralization (NT) (O—O), HLI (■– – –■), HI (●—●), and nucleocapsid (Δ– – –Δ) CF.

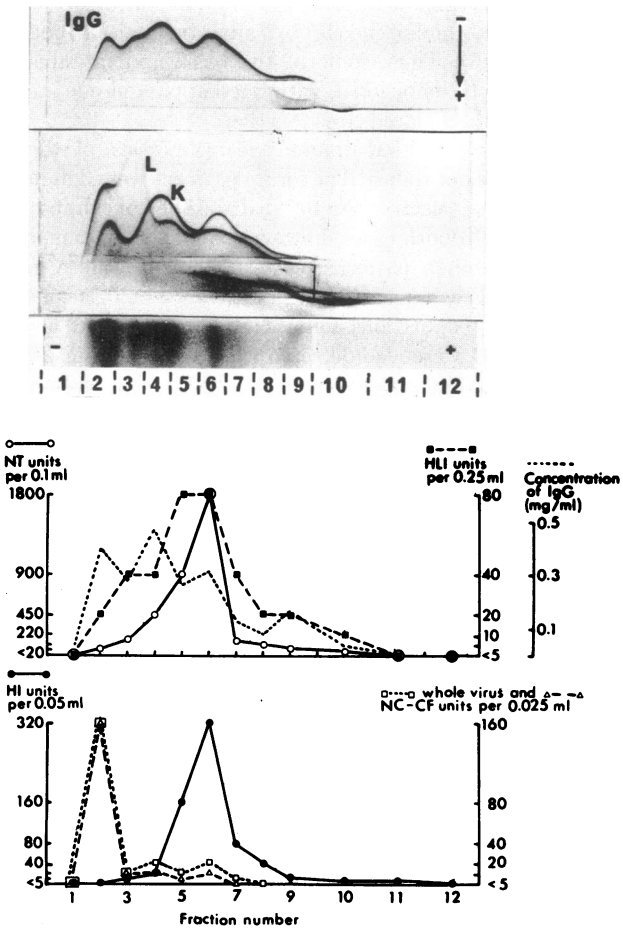


FIG. 3. Electrophoretically separated CSF from patient Lo. CF antibody activities to whole virus material are shown by symbol □- - -□. For explanation of other symbols, see Fig. 2. Some homogeneous bands, e.g., in fractions *2* and *4*, appear biclonal with kappa and lambda IgG populations placed close together (see *text*).

products were, however, unevenly distributed. Fig. 2 shows the sequence of antibodies with various specificities found in the CSF from patient Sa. From the cathodal to the anodal region, these were directed against (*a*) virus hemolysin (high HLI and neutralizing, with only trace of HI and nucleocapsid CF activities); (*b*) nucleocapsids (high nucleocapsid CF and relatively lower HI, HLI, and neutralizing activities); and (*c*) a hemagglutinin component (high HI, HLI, and neutralizing and low nucleocapsid CF activities). In addition (*d*) another population of antibodies corresponding to the position of a more anodic IgG band carrying activities similar to those of (*c*) was found. The individual antibody peaks corresponded to fractions that contained homogeneous IgG bands. Fig. 2 also shows that the maximum antibody activity against nucleocapsids was found in the most-pronounced IgG band. The distribution of antibodies with CF activity to whole virus material (not shown) was parallel to that of nucleocapsid antibodies.

The results from the second case (Lo) are seen in Fig. 3. Also in this case, a distinctly uneven distribution of antibody activities was found. A sharp peak of antibody activity to nucleocapsids was found in the most basic fraction, which contained an apparently biclonal population of IgG (fraction 2, Fig. 3). In this fraction, no HI and only trace of neutralizing activity was seen. The somewhat higher HLI activity in this fraction may be due to contamination from the neighboring fraction (fraction 3, Fig. 3), which exhibited relatively high HLI and some neutralizing, but only a trace of HI activity. As shown by the figure, the plateau of HLI activity in this region corresponded to the location of a homogeneous IgG band of lambda light-chain type (fraction 3), and a more diffuse, apparently biclonal IgG band (fraction 4). More anodal to these fractions a peak of antibody activity to a hemagglutinin component (fraction 6), corresponding to an IgG band with basic-intermediate mobility, was seen. Significantly, only trace nucleocapsid activity was seen in this fraction. Weaker IgG bands, in the intermediate gamma region (fractions 9 and 10) carried some HLI activity, but low HI and neutralizing and no nucleocapsid CF activities.

The two materials tested display certain similarities. In both, the antibodies to nucleocapsids and virus hemolysin were found in the extremely basic gamma region. Antibodies to the hemagglutinin occupied basic to intermediate positions. In both cases, antibodies to nucleocapsids appeared to be carried by the dominant IgG band. The distribution of CF antibodies to whole virus material tended to follow that of nucleocapsid CF antibodies in both cases. In general, different antibody activity peaks were found to correspond to IgG maxima. An exception to this general tendency was seen in patient Lo (Fig. 3), where the fraction containing the highest content of IgG (fraction 4) did not give a clear-cut antibody peak, although HLI activity was relatively high. The slight elevation of CF activity to whole virus seen in this fraction, containing an apparently biclonal band of IgG, raises the question of whether other activities, e.g., to virus nonstructural proteins, are present in this IgG. The scarcity of material from this patient precluded further investigations into this problem. Results from antibody determinations of fractionated sera from the patients showed that titers were higher in the intermediate and basic gamma regions than in the fast gamma region. In addition, there were some differences in the distribution of antibodies with different specificities, to some extent similar to those found in the CSF from the same patients. The differences were, however, not pronounced and indicate the presence of diffuse or polyclonal measles virus antibodies, in addition to trace bands of homogeneous antibodies that presumably derive from the central nervous system.

## DISCUSSION

The results from the present study, using crossed immunoelectrophoresis, indicate that the electrophoretically homogeneous gammaglobulins in the CSF in patients with SSPE represent IgG populations with various degrees of restricted heterogeneity; this conclusion is supported also by criteria of light-chain determinants. In some instances, here best by illustrated by the CSF from patient Sa (Fig. 2), the homogeneity of kappa or lambda light-chain determinants of individual IgG bands was so pronounced that the term "monoclonal" immunoglobulins appears justified.

In evaluation of whether the IgG populations are also restricted in their antibody specificities, some limiting factors inherent in the methods used must be considered. Due to the close proximity of some IgG bands, some fractions contained protein from more than one band. The unavoidable diffusion of protein during the preparative procedures, as well as minor skewing of the electrophoretic runs, would further be expected to lead to contamination of neighboring bands in individual

fractions. Finally, the contribution of background IgG—presumably representing polyclonal antibodies—must be taken into account. In view of these factors, the purity of homogeneous IgG populations in the eluted fractions may have been considerably reduced. Some evidence for this reduction may be inferred from the fact that IgG content in the fractions did not reproduce the sharp peaks that might be expected from the electrophoretic patterns (Figs. 2 and 3). Despite these methodological limitations, the uneven distribution of different antibody specificities in both materials was pronounced, and corresponded well with the presence of IgG bands. The results give further support to data (15) from studies of oligoclonal IgG isolated from brain tissue of another patient with SSPE. In the present study, the most clear-cut results indicating restriction of antibody specificities within individual IgG bands were found in the CSF from patient Sa (Fig. 2). This was also the material in which the restriction of kappa and lambda light-chain determinants of the IgG bands was most pronounced.

In considering whether the titers found are compatible with homogeneous or monoclonal antibodies, the above-mentioned reservations about the purity of homogeneous protein in individual fractions must be taken into account. Further, some aggregation of IgG may have taken place during storage and repeated freezing and thawing. Even so, the titers appear high. For instance, recalculation to serum levels on the basis of IgG content yields peak HI titers in the range 8,000–16,000 and peak nucleocapsid CF titers in the range 3,000–5,000 in the two CSF materials. These are as high, or even higher, than the maximum titers of antibodies found in SSPE sera (10), in a study with the same serological systems as those used here.

The identification of a population of antibodies with HLI and neutralizing, but no HI activity, in one of the CSF samples (Fig. 2) is of interest, as it gives further support to the concept (11) that the hemolysin represents a separate entity in the virus envelope.

Antibodies against nucleocapsids dominate quantitatively among different virus-specific antibodies appearing after regular measles (11). The finding in both cases of antibodies to nucleocapsids in the dominant IgG bands was, therefore, not wholly unexpected. The findings may, however, also indicate that the nucleocapsids are produced in relative excess to other virus products in the (virus-infected) central nervous system of SSPE patients.

The oligoclonal IgG patterns found in the CSF in SSPE and certain other disorders are reminiscent of the antisera that can be produced under experimental conditions, e.g., by hyperimmunization of animals with certain bacterial antigens. Thus, rabbits hyperimmunized with streptococcal polysaccharide antigens produce antibody populations containing one or more electrophoretically distinct components (16). Recently presented results (17) demonstrate that a critical factor in eliciting this type of response is a repeated antigenic stimulation over long periods of time, rather than a particular genetic constitution of the animal. In SSPE, the protracted virus infection within the central nervous system may represent such a protracted antigenic stimulus, resulting in the local synthesis within the central nervous system of virus-specific antibodies of oligoclonal character. It seems to us that SSPE offers a unique possibility for analyzing the biological significance of homogeneous antibody response in humans. Apart from the restricted antibody specificities found here in individual IgG populations, the observed tendency for antibodies to certain virus products to display a preferential electrophoretic mobility is of interest. In experimental systems, the occurrence of an inverse relationship between the net charge of an antigen and the charge of the corresponding antibody has been observed (18). In case this would apply to SSPE, the markedly basic distribution of IgG antibody populations might indicate immunogenic virus products with pronounced negative charges. A similar basic distribution of IgG in the CSF of patients with multiple sclerosis has been observed (19). Antibody activities in the homogeneous IgG populations in patients with multiple sclerosis have not been defined. In certain cases of this disease, however, evidence for measles virus antibodies derived from the central nervous system has been found (10). In these cases, it should be of interest to analyze whether homogeneous IgG populations in the CSF represent antibodies against virus products and/or other types of antigens.

## ADDENDUM

After this paper was prepared for publication, a report on the distribution of measles virus antibody activity in IgG from SSPE materials appeared [Karcher, D., Mathyssens, G. & Lowenthal, A. (1972) *Immunology* **23**, 93–99]. These authors have, however, determined HI activity only. In agreement with our report, these authors found unevenly distributed HI activity in different IgG fractions, although their specific relation to homogeneous IgG bands was not stated.

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