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IN VITRO SYNTHESIS OF IgG BY CELLS FROM THE CEREBROSPINAL FLUID IN A PATIENT WITH MULTIPLE SCLEROSIS

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SUMMARY

Mononuclear cells of the cerebrospinal fluid from a patient with multiple sclerosis were maintained *in vitro* for 5 days. Synthesis of IgG occurred and was significantly higher during a relapse of the disease than during remissions. The electrophoretic IgG species that were found in the cerebrospinal fluid of the patient were also synthesized *in vitro*.

INTRODUCTION

Recently Cohen & Bannister (1967) reported *in vitro* synthesis of IgG and IgA globulins by mononuclear cells derived from the cerebrospinal fluid (CSF) of a patient with multiple sclerosis. They isolated proteins by specific immune precipitation at the end of an incubation period of 7 hr. In the present investigation mononuclear cells from CSF were maintained *in vitro* for 5 days in the presence of radioactive aminoacid. Proteins were isolated in the beginning and at the end of the period of incubation and *in vitro* formed IgG was studied with regard to electrophoretic mobility and compared with whole CSF IgG globulin.

MATERIALS AND METHODS

CSF was obtained from a patient suffering from multiple sclerosis. While the study was being undertaken, the patient suffered a relapse of short duration, during which she had a transient spastic paresis of her left leg. CSF was collected 5 days prior to this relapse, on the 3rd and 10th day of the relapse, and finally 1 and 3 months after it.

The patient was a 21-year-old female who had been well except for the present illness. Some years ago she had experienced numbness of both hands for a short period of time. A few months prior to hospitalization she had during several weeks repeated daily Jacksonian seizures affecting the right side of her body. Some time later she suffered a transient spastic paresis of her left leg and numbness of the right side of her face. On examination she was found to have diminished tactile discrimination on both legs and a spastic paresis of her left leg. Abdominal reflexes were diminished on the left side. Plantar reflexes were

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extensor. Routine laboratory examination was unremarkable. The cerebrospinal fluid contained no polynuclear cells but an excess of mononuclear cells ranging from 7 to 17 cells/mm^3. The total protein content was normal on each occasion, ranging from 24 to 36 mg/100 ml, with no variation correlated to the activity of the disease. On agar gel electrophoresis four bands were found in the γ-region. A pneumo-encephalogram was normal, as was a left side carotid angiogram.

Depending on cell count, between 15 and 25 ml of CSF was collected by lumbar puncture and immediately centrifuged at 250 *g* for 7 min at room temperature. The supernatant was carefully removed, and the sedimented cells were resuspended in 1 ml of Parker's culture medium supplemented with 15% newborn calf serum. The culture was transferred to a Carrel flask and flushed with a gas mixture consisting of 5% CO_2 and 95% O_2. The total cellnumber varied between $1{\cdot}9 \times 10^5$ and $2{\cdot}3 \times 10^5$ in the different experiments. [^{3}H]L-leucine (100 μCi), specific activity 1 mCi/ml (Radiochemical Centre, Amersham, England), was added and 200 μl of the culture was drawn immediately and frozen. The culture was incubated at 37°C and studied daily under the microscope. During the first 2 days the cells continually changed shape. Many small pseudopodia were put out and retracted from the cell surface, and the typical 'hand-mirror' appearance of the moving lymphocyte was seen. This activity diminished over the following days, and by the 5th day was no longer observed. The rest of the culture was then frozen.

The cells were disrupted by repeated thawing and freezing, and the cellular debris removed by centrifugation.

Protein was precipitated with 10% trichlor-acetic acid (TCA). The precipitates were washed twice with 10% TCA, once with equal parts of ethanol and ether, and once with ether. IgG was precipitated at equivalence with a specific rabbit anti-IgG serum after the addition of unlabelled IgG. Control experiments with transferrin–anti-transferrin were performed in an aliquot of the culture. All immune precipitates contained approximately equal amounts of total protein as measured by the Folin–Ciocalteau method (Lowry *et al.*, 1951). The immune precipitates were washed twice with phosphate buffered saline (PBS) with added unlabelled leucine. All precipitates were dried over night, solubilized in hyamine and standard volumes were pipetted into counting vials containing a toluene–PPO–POPOP scintillator. Radioactivity was determined on a Packard Tri Carb Liquid Scintillation Counter. Counting efficiency was determined by the addition of [^{3}H]toluene of known specific activity.

In an attempt to compare synthesized IgG with whole CSF IgG from the same patient, mixtures of culture supernatant obtained during the relapse and whole CSF were fractionated by preparative agar gel electrophoresis with Wieme-technique (1959). CSF was concentrated by ultrafiltration in collodion bags. A mixture of culture supernatant and ten-fold concentrated CSF was further concentrated in a Colover concentrating cell against a 30% polyethylene glycol solution in 0·9% sodium chloride so as to give an approximately 100-fold final concentration of CSF. The γ-region of the agar gel was cut into narrow segments of equal size and the gel fractions were eluted in standard amounts of PBS. The IgG content of each eluate was determined by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using a specific rabbit anti-IgG serum. The content of labelled IgG in the eluates was determined by counting the radioactivity of IgG–anti-IgG precipitates. In a control experiment aliquots of eluates were pooled and IgG–anti-IgG and transferrin–anti-transferrin precipitates were prepared from the pools.

RESULTS

Fig. 1 shows the results of two cultures obtained during a relapse. The results are given as increase in radioactivity of immunoprecipitates and TCA precipitates of 20 μl of culture supernatant. The TCA precipitates in both cases exhibit approximately twice the radioactivity of the IgG–anti-IgG precipitates, which again gave respectively three and thirty

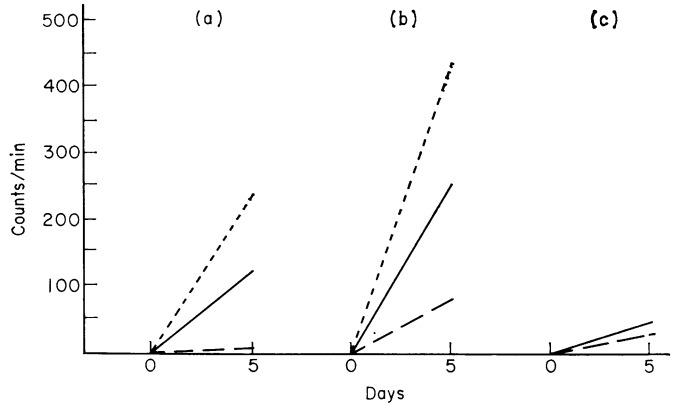

FIG. 1. Increase in radioactivity (counts/min) in immune precipitates and TCA-precipitates of 20 μl of culture supernatant from two cultures obtained during a relapse (a) and (b) and from the most active of the cultures obtained during remissions (c). The values for IgG–anti-IgG precipitates are averages of duplicates. The other values are based on single estimates. ——, IgG; - - - -, TCA; — —, transferrin.

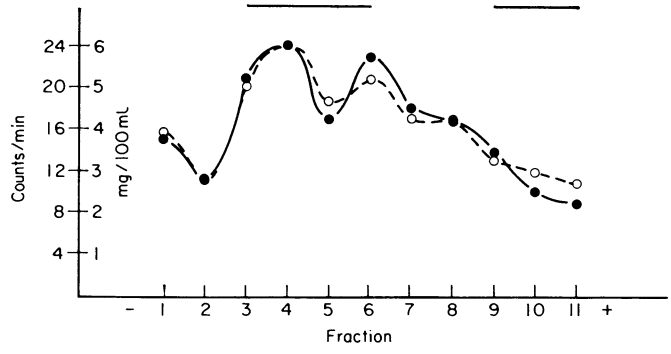

FIG. 2. Electrophoretic distribution of synthesized IgG (counts/min) and of total IgG (mg/100 ml) in CSF of the same patient. The heavy horizontal lines indicate the fractions from which two pools were prepared for one of the control experiments. ●, Counts/min; ○, mg/100 ml.

times more counts per minute than did the transferrin–anti-transferrin precipitates. Three cultures were obtained during remissions. In two cultures an increase in radioactivity of IgG precipitates could not be established with certainty, while in the third culture a small increase was found (Fig. 1).

It can be seen in Fig. 2 that the electrophoretic distribution of immunoprecipitable radioactivity from the cultures obtained during a relapse and that of total IgG concentration

parallel each other. This was confirmed in duplicate experiments. Cathodic γ-globulin had to be run out of the plate in order to achieve maximum resolution of the more anodic γ-globulin region. After less extensive electrophoretic separation of the same material, the anodic parts of the total IgG and radioactivity profiles were roughly parallel (Fig. 3). In the cathodic region, however, there appeared a major peak of radioactivity which did not correspond to any definite total IgG peak. This finding was again confirmed in duplicate experiments performed on different occasions with the same material.

In a control experiment aliquots of eluates from two different parts of the agar gel were pooled, and immune precipitation was carried out on the two pools (Fig. 2). The radioactivity of the transferrin–anti-transferrin precipitates did not differ significantly between the pools, whereas IgG–anti-IgG precipitates from fractions with high radioactivity gave significantly higher counts/min than did precipitates from fractions with low radioactivity. This result proves the specificity of the IgG–anti-IgG precipitating system. There was not enough material to carry out the same control experiment on the cathodic radioactive material shown in Fig. 3.

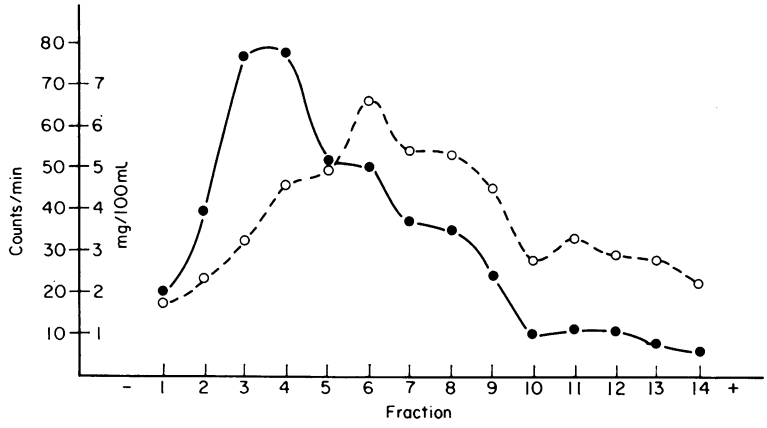

FIG. 3. Distribution of synthesized IgG (counts/min) and of total IgG (mg/100 ml) after less extensive electrophoretic separation. ●, Counts/min; ○, mg/100 ml.

In order to show that isotope did not adhere passively to the added CSF IgG the following control experiment was carried out. Culture supernatant was mixed with CSF with a different electrophoretic γ-globulin pattern, i.e. CSF from a patient with neurosyphilis. The radioactivity profile resembled those from the previous electrophoretic runs of the same length, while the profile of the total IgG in this case differed distinctly both from the total IgG profiles earlier obtained and from the radioactivity profile.

DISCUSSION

There seem to be no established methods for the culture of CSF cells. It has been possible to maintain CSF mononuclear cells *in vitro* for a few days, but after this time they undergo lysis despite an abundance of nutrients. The reasons for this are not known. A case of multiple sclerosis was chosen because of its moderate increase in CSF mononuclear cells and because it may be supposed that these cells are antigenically stimulated.

Using specific immune precipitation on culture material obtained during a relapse of multiple sclerosis more radioactivity was precipitated with anti-IgG than with anti-transferrin, suggesting that synthesis of IgG had occurred during the 5 days. In comparison cultures obtained during remissions showed little if any synthesis. The reasons for this are not known. It is conceivable, however, that it reflects differences between the cell materials, i.e. activity of the disease would be associated with IgG synthesis.

Also of interest are the profiles obtained when fractions from agar gel electrophoreses of culture material were immunoprecipitated. The control experiments showed that the isotope did not passively adhere to the IgG precipitates. The amount of radioactivity, i.e. *in vitro* synthesized IgG, in the different fractions closely follows the pattern of the total CSF IgG with the exception of the extreme cathodic fractions. It may be concluded that most of the electrophoretic species of IgG that are found in the CSF of the patient are also synthesized *in vitro*. The strongly radioactive peak in the cathodic electrophoretic region had no obvious counterpart in the patient's CSF IgG. The nature of it is uncertain. It does not seem to represent impurities in the isotope preparation. If it represents *in vitro* synthesized IgG it is either produced in excess, or it is a material rich in leucine. Enough material was not available to establish unequivocally its nature as IgG.

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