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Introduction

MEASLES and rubella viruses cause chronic disease in the central nervous system (CNS). 1-5 Intralesional CNS antibody production has been demonstrated. 5-6 Mumps virus is a major cause of meningoencephalitis, 7 but has not been associated with any form of chronic infection in man. We report a patient with chronic encephalomyelitis with a specific increase in intrathecal antibodies to mumps virus.

Methods

Antibody Determinations

The complement fixation (CF) test was used to assay antibodies to adenovirus, coronavirus, Coxsackie B5, cytomegalovirus, hepatitis B, herpes simplex, influenza A and B, measles, mumps, parainfluenza 1 and 3, polio, respiratory syncytial, rotavirus, and varicella viruses and to Chlamydia group antigen, Mycoplasma pneumoniae, and Toxoplasma gondii. Single radial haemolysis (SRH) tests ('Orivir', Orion Diagnostica, Helsinki, Finland) were used to detect mumps, rubella, and influenza A (Victoria strain) viruses 8-11 and solid-phase enzyme-immunoassays (EIA) for mumps and cytomegalovirus antibodies. 12-14 In addition, patient's sera were absorbed with recombinant plasmid DNA for the identification and characterization of plasmid deoxyribonucleic acid.

Protein Determinations

Serum and CSF concentrations of IgG and albumin were simultaneously measured by automated fluoronephelometry (Technicon 'AutoAnalyzer'). The IgG index (CSF IgG/serum albumin/CSF albumin) was calculated according to Delpech and Lichtblau. 15 and de novo IgG synthesis in the CNS according to Tourtellotte et al. 16 Oligoclonal bands were determined as described. 12

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Determination of Antibody Specificity to Mumps Virus
Structural Proteins by Immunoprecipitation

Purified mumps virus (Enders strain; 1 mg) grown in chickens' eggs was radiolabelled with 125I (0-5 mCl) by the chloramine T method of Krohn et al. 18 Radioactive mumps virus (250 000 cpm/sample) was solubilised in 400 µl of 1% 'Triton' X-100 in 0-5 mol/l sodium chloride solution, and 20 µl of serum or 100 µl of CSF was added into the mixture. After 2 h of end-over-end mixing at 37°C, 100 µl of 10% protein A Sepharose (Pharmacia, Upsala, Sweden) in phosphate buffered saline was added for 1 h at 37°C. Sepharose was washed twice with the triton/saline liquid and once with sodium dodecyl sulphate (SDS) depleted electrophoretic buffer, always pelleting with an Eppendorf microcentrifuge between washings. Sepharose-bound proteins were solubilised in 50 µl of electrophoretic buffer in the presence of 10% mercaptoethanol and boiled for 3 min. The supernatant was run in polyacrylamide (10%) gel electrophoresis in the presence of SDS (SDS-PAGE), dried, and the precipitated virus proteins were detected as bands by autoradiography.

Case-report

In February, 1967, a 31-year-old, previously healthy man developed fever, headache, and vomiting followed by parasthesia, muscular weakness, disturbances of gait, and diplopia. Three weeks after the onset of symptoms spastic paresis of the legs, distal weakness of the arms, and vague sensory disturbances in the legs were noted. A positive Babinski sign appeared bilaterally, tendinous stretch reflexes were brisk, and abdominal reflexes were absent. In addition nystagmus, intention tremor and dysarthria were present and the patient seemed euphoric. In April his legs were paralysed. An electroencephalogram (EEG), first obtained in April, 1967, was normal, brain scan was also normal. An electroencephalogram (ENMG) revealed marked denervation in the legs suggesting an anterior-horn lesion similar to that in poliomyelitis. The patient was treated in hospital until October, 1967, and diagnosed as having acute encephalomyelitis.

He was rehabilitated, was able to walk in a year, received professional training as a technician, and worked until 1975. His condition deteriorated in 1975 and he had difficulty in walking and some spasticity. After a period of improvement lasting some years his symptoms increased again early in 1980, with positive Babinski sign, ankle clonus, and neuropsychological deficiencies. His condition has remained poor and he is unable to work.

Laboratory Findings

Hospital records showed that in 1967 serum mumps CF titre was high (1:128) without evidence of clinical parotitis or previous mumps vaccination. At that time the CSF was not tested for antibody and no virus was detected. CSF contained 321 leucocytes per µl, total protein was raised (1500 mg/1) (normal range 150-450 mg/1), and glucose concentration was normal. Serum mumps CF titre remained high and titres 1:4 in CSF and no other antibodies were detected in CSF. Thus the serum/CSF antibody ratio was 8, while the serum/CSF ratio for total IgG was normal at 252 (normal range 200-300). At this time total protein concentration in CSF was normal (353 mg/l) and there were no cells. This was also the situation in November, 1981. However, CSF IgG was slightly raised as was the IgG/albumin ratio (table I). The IgG index was raised (0-80 [normal range 0-34-0-58]), indicating intrathecal IgG production. De novo IgG synthesis in the CNS was 53-4 mg/day (normal range <3-3 mg/day).

An altered serum/CSF ratio for mumps antibodies was also detected by both EIA for IgG and SRH (table I). No mumps-specific IgM or IgA antibodies were found by EIA. The patient also had detectable amounts of CF antibodies to rubella, influenza A, cytomegalovirus, adenovirus, and Coxsackie B viruses in his serum but we found no antibodies to these viruses or to 12 other microbes tested in the CSF (table I). After absorption of CSF with mumps virus, the SRH value for mumps fell from 5 to <3.

| TABLE I—DETERMINATION OF PROTEINS AND ANTIBODIES IN SERUM AND CSF SAMPLES |
|---------------------------|-----------|-----------|-----------|
| Protein                  | Serum     | CSF       | Serum/CSF |
| IgG (g/l) (serum 8-0-19-9; CSF 0-04-0-38) | 10:6      | 0:042     | 252       |
| Albumin (g/l) (serum 35-55; CSF 0-082-0-290) | 38:0      | 0:187     | 203       |
| IgG/Albumin ratio (serum 0-15-0-57; CSF 0-08-0-20) | 0:28      | 0:22      | 1:27      |

Mumps antibodies:

| CF titre | Serum | CSF | Serum/CSF |
|-----------|-------|-----|-----------|
| 1:32      | 0:8   | 0:8 |
| 1:4       | 1:6   | 1:6 |

SRH (mm): 10

| IgG A105 (A105) | 1:082 | 0-372 |
| IgG A500 (A500) | 0:555 | 0-178 |
| IgG A500 (A500) | 0:201 | 0-025 |
| IgG A500 (A500) | 0:127 | 0-016 |
| IgG A500 (A500) | 0:014 | 0-002 |

Other antibodies:

| Rubella SRH (mm) | 8     | <3   | >30 |
| Influenza A CF titre | 1:16 | 1:22 | >8  |
| Influenza SRH (mm) | 8     | <3   | >20 |
| Cortyomelitis CF titre | 1:16 | 1:22 | >8  |
| Cortyomelitis SRH (mm) | 0:540 | 0-011 | >50 |
| Cortyomelitis SRH (mm) | 0:171 | 0-005 |
| Adenovirus CF titre | 1:8   | 1:22 | >4  |
| Coxsackie B CF titre | 1:8   | 1:22 | >4  |
| Other CF titre | 1:8   | 1:22 |

*Reference ranges are given in parentheses.
†Ratio is calculated from standard curve with serial dilutions of positive sera diluted with a negative (SRH<3 mm) serum.
‡Ratio is calculated from interpolated dilutions corresponding to A405=0-200.
§Herpes simplex, varicella zoster, influenza B, parainfluenza 1 and 3, respiratory syncytial, measles, mumps and hepatitis A viruses, Chlamydia group antigens, Mycoplasma pneumoniae, and Toxoplasma gondii.

Symbols: CF=complement fixing; EIA=enzyme immunoassay; A405=absorbance values in EIA; SRH=single radial haemolysis; ND=not done. In EIA absorbance values of A405<0.200 are regarded as not significant.

The specificity of the CSF mumps antibody finding was further assessed by determination of mumps antibodies from 22 control patients with serum mumps antibodies and 35 seronegative control patients with various neurological symptoms. None of the 57 controls had mumps antibodies in the CSF specimen tested at a 1:50 dilution (table II).

Since oligoclonal CSF IgG bands have been described in...
acute as well as in chronic infections of the central nervous system, the patient's CSF specimen was further analysed by the SDS-PAGE procedure. We found a moderate increase in the IgG region (fig. 1).

Specificity of Antibody Response to Mumps Virus Structural Proteins

The patient's antibody response to mumps virion proteins was investigated by immunoprecipitation. The precipitated protein bands indicate the presence of antibodies. We found antibodies to envelope glycoproteins haemagglutinin-neuraminidase (HN, 75K), fusion protein (F1, 58K), viral membrane protein (M, 39K), and various nucleocapsid proteins (200K, 68K, 45K and 42K) (fig. 2). Our lysis buffer leaves the nucleocapsid intact and possibly different nucleocapsid proteins are precipitated together. The antibody patterns in serum and CSF were similar and resemble the antibody response in serum of patients with acute mumps infection (Julkunen I, unpublished observations). Control sera and CSF with no mumps antibodies did not precipitate any virion proteins (fig. 2).

Discussion

This patient had chronic encephalomyelitis with a slowly progressive course and a specific increase in mumps antibodies in the CSF and intrathecal IgG production with an oligoclonal pattern. Intrathecal antibody production has been reported in chronic CNS infections caused by measles and rubella, and probably herpes viruses. In acute mumps virus meningitis CNS antibody production has been detected. Vandvik et al. reported prolonged pleocytosis in CSF as late as a year after mumps meningitis, suggesting persistence of virus infection in the CNS. An earlier paper reported a case of aqueductal stenosis two years after mumps encephalitis but with no serological follow-up in the CSF or other evidence of ongoing mumps infection. We know of no other reports of prolonged disease or antibody production caused by mumps virus. Mumps virus can cause spinal-cord as well as pontine and cerebellar lesions. Our patient displayed these features but a chronic progressive course also developed. In addition, some cerebral signs such as neuropsychological deficiencies were observed.

Increased titres to measles virus and to several other viruses including mumps virus have been found in multiple sclerosis without evidence of viral antigen in CNS. It has been suggested that the broad-spectrum viral antibody response in the CNS in this disorder results from a polyclonal B cell activation. In the present case only antibodies to mumps virus were increased in the CSF, and we were able to absorb the antibodies with purified mumps virus.

By immunoprecipitation we demonstrated that the antibody response to mumps virus was similar in the patient's serum and CSF and was directed against nearly all structural proteins including M-protein. In chronic measles-virus encephalitis a lack of antibodies against M-protein has been
The present case could represent a chronic form of mumps virus infection in the CNS. This can only be confirmed by virus isolation or demonstration of antigen within brain tissue. Mumps virus has a remarkable tendency to produce chronic infections in cell cultures, but has not previously been associated with chronic human infection.

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