Low molecular weight measles immunoglobulin M in subacute sclerosing panencephalitis and acute measles

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Summary: Thirty eight patients with subacute sclerosing panencephalitis (SSPE) were investigated. Five patients who previously had measles immunoglobulin M (IgM) detected in unfractionated serum and cerebrospinal fluid (CSF) had measles IgM exclusively in the low molecular weight (LMW) fractions of serum and CSF. Measles IgM had previously not been found in unfractionated serum from 33 patients but was detected exclusively in the LMW fractions of serum from 30 patients. Seven children with acute measles had the expected high molecular weight (HMW) measles IgM in serum but 5 also had LMW measles IgM. Four young adults who had had measles in childhood had neither HMW nor LMW measles IgM in their sera.

Introduction

The viral aetiology of subacute sclerosing panencephalitis (SSPE) was suggested by the finding of Cowdry type A inclusions in brain cells (Dawson, 1933) which were later shown to contain ribonucleic acid (Connolly *et al.*, 1968). Intranuclear tubular filaments were also seen electron-microscopically (Bouteille *et al.*, 1965). Measles antigen was found in the brain (Connolly *et al.*, 1967) and measles virus was isolated on co-cultivation of affected brain cells with susceptible cell cultures (Baublis & Payne, 1968; Horta-Barbosa *et al.*, 1969).

Measles antibody was found in serum and cerebrospinal fluid (CSF) of patients with SSPE (Connolly *et al.*, 1967). The antibody titres were higher than those found in childhood measles and titres increased during the illness in some cases. There was evidence that measles antibody was produced or released within the CNS (Connolly, 1968). Measles-specific IgM was found in unfractionated serum and CSF of 3 patients (Connolly *et al.*, 1971) but was not detected in the serum of 4 other patients (Thomson *et al.*, 1975).

IgM antibody circulating in the blood of healthy people is predominantly a pentamer of 5 subunits with a sedimentation coefficient of about 19 S and a molecular weight of about 900,000 (HMW). Monomeric low molecular weight (LMW) IgM consists of

individual subunits with a sedimentation coefficient of about 7–8 S and a molecular weight of 180,000–200,000.

Low molecular weight measles-specific IgM has not been described before and this investigation was initiated after finding it in the serum and CSF of a 21 year old Arab woman with SSPE in 1983. She was pregnant when her illness began 2 y previously and a healthy child was born. Thirty seven other patients from the UK with SSPE were retrospectively investigated and the findings are described.

Materials and methods

Serum and CSF from 5 SSPE patients, serum from a further 33 patients, convalescent sera from 7 children with acute measles and sera from 4 young adults who had had measles in childhood were fractionated on linear sucrose gradients (10% to 37% w/v) in 0.01 M phosphate buffered saline (PBS), pH 7.2. Undiluted CSF (0.2 ml) or serum (0.2 ml) diluted 1:1 in PBS was layered on top of gradient and centrifuged at 157,000 x g for 18 h in a swinging bucket rotor (Spinco 50.1, Beckman Instruments Inc.). After centrifugation, 0.4 ml fractions were collected dropwise through the tube bottom. Initially, fractions were dialysed against PBS overnight at +4°C to remove sucrose before testing but this was later found to be unnecessary.

All fractions were tested for measles-specific IgM or IgG on acetone-fixed measles infected HEP2 cell

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cultures on Flow 10 well Multitest slides using the indirect immunofluorescence test. Fluorescein isothiocyanate (FITC)-labelled anti-human IgG (sheep-Wellcome Reagents) and FITC labelled anti-human IgM from 3 manufacturers was used (sheep-Wellcome Reagents, goat-Atlantic Antibodies, goat-Otiz Behringwerke). Serum fractions were tested with anti-human IgM from 3 manufacturers in the Arab patient, from 2 manufacturers in an acute case of measles, and from one manufacturer (Wellcome Reagents) in all other cases.

Before testing for measles IgM, fractions were absorbed with RA latex (Hyland Laboratories) plus heat-aggregated human immunoglobulin (Lister Institute) as previously described (Shirodaria *et al.*, 1979), to exclude rheumatoid factor. CSF fractions before absorption had bovine albumin (Sigma Chemical Corp.), pH7, added to a final concentration of 2%. A serum containing rheumatoid factor (titre 512) was fractionated and tested for rheumatoid factor before and after absorption as above. In the Arab patient's serum, fractions 4, 5, 6 and 7 were absorbed as above and tested for rheumatoid factor using a mumps assay system.

Controls for the ultracentrifugation consisted of (a) testing serum fractions from an acute case of mumps to show that mumps IgM was in the HMW peak and (b) assaying total serum IgM and IgG in fractions from the Arab patient using an enzyme immunoassay consisting of a double antibody sandwich with the primary antiglobulin on the solid phase (Dynatech microtiter wells).

Controls for viral specificity consisted of testing LMW IgM fractions on uninfected HEp2 cells and VERO cells infected with mumps virus.

Some sera and CSF used in this study had been stored at -20°C for up to 18 y.

Results

Measles-specific IgM was found in unfractionated serum and CSF of 5 SSPE patients. This included the Arab patient and 3 patients previously described (Thomson *et al.*, 1975). The peak of measles IgM was found exclusively in the LMW fraction (4,5,6) of serum and CSF. All 3 preparations of FITC labelled anti-human IgM stained the LMW measles IgM equally well in the Arab patient. HMW measles IgM was not found in fractions 1 and 2. The peak of measles IgG was found in fractions 4, 5, 6 and 7 of serum and CSF. The LMW measles IgM in serum was not removed by RA latex plus heat-aggregated human immunoglobulin absorption but was removed from CSF. The addition of bovine albumin to a final concentration of 2% to CSF prevented the removal of LMW measles IgM.

Rheumatoid factor was not detected in the Arab patient's serum fractions or in the serum fractions from a patient with a known rheumatoid factor titre of 1:512 after absorption with RA latex plus heat-aggregated human immunoglobulin.

Total serum IgM in the Arab patient was predominantly in fractions 1 and 2 (HMW) and total serum IgG was in fractions 4, 5 and 6. Serum fractions from a patient with acute mumps showed that the peak of mumps IgM was exclusively in HMW fractions 2 and 3.

Fractions containing LMW measles IgM did not stain uninfected HEp2 cells or mumps-infected VERO cells.

The fractionated sera from a further 33 SSPE patients showed that the peak of measles IgM was exclusively found in the LMW fractions 4, 5 and 6 of 30 patients, and was totally absent in all fractions from 3 patients. The peak of measles IgG was in fractions 4, 5, 6 and 7 but faint staining was also found in fractions 3, 8, 9 and 10 in some sera. One patient with SSPE who survived for over 6 y, had serum samples taken 6 y apart, and measles IgM was present exclusively in the LMW fractions of both sera.

Seven children with acute measles who had greater than 4-fold rises of measles complement fixing antibody had their convalescent sera fractionated. Measles IgM was found in the HMW fractions in all 7 children but was also present in the LMW fractions in 5 children. Two preparations of FITC-labelled anti-human IgM stained the HMW and LMW measles IgM equally well. The 4 healthy young adults who were known to have had measles in childhood and had measles complement fixing antibody in their sera had neither HMW nor LMW measles IgM in their serum fractions.

Discussion

Measles IgM was found in unfractionated serum and CSF of 5 SSPE patients but was not detected in unfractionated serum of a further 33 patients. Measles IgM has previously been described in unfractionated serum and CSF of 3 patients with SSPE, but was not detected in 4 other patients (Thomson *et al.*, 1975) using the indirect immunofluorescence method. Kiesling *et al.* (1977) reported 20 patients with SSPE where measles IgM was present in unfractionated sera and CSF using an indirect radioimmunoassay, while Mehta *et al.* (1977) found measles IgM in 3 out of 6 SSPE patients using radial immunodiffusion.

Our failure to detect measles IgM in 33 SSPE patients led us to fractionate serum and CSF of the recent Arab patient. This led to a retrospective study which revealed that LMW measles IgM was present in serum of 35 SSPE patients and in CSF of 5 patients

who were tested. Fractionation may separate out substances which mask or inhibit measles IgM detection while the specific anti-human IgM and IgG used in the immunofluorescence assay would detect the globulins in the various fractions separated according to molecular size and weight. Since the LMW measles IgM fractions also contained the bulk of measles IgG it is difficult to postulate blocking by IgG as the cause of the previous failure to detect measles IgM. It would seem more likely that the 'total' serum IgM in the HMW fractions (which did not contain measles IgM) may have blocked the detection of LMW measles IgM in unfractionated sera.

Our studies show that the HMW and LMW measles IgM found in acute measles is not detectable in young healthy adults who have had measles in childhood. Whether the LMW measles IgM persists in those individuals who later develop SSPE or it arises *de novo* when SSPE develops is unknown. However, it is interesting that LMW measles IgM persisted for 6 y in a patient with SSPE.

The specificity of the measles IgM assay was shown by several controls. Firstly it was important to exclude rheumatoid factor which can cause non-specific IgM staining in the indirect immunofluorescence test (Shirodaria *et al.*, 1973). Rheumatoid factor was not detected in the Arab patient's LMW fractions containing measles IgM or in serum fractions from a known rheumatoid-factor positive patient after absorption with RA latex plus heat-aggregated human immunoglobulin. This method has previously been shown to remove the rheumatoid factor from sera (Shirodaria *et al.*, 1979). The addition of bovine albumin to CSF preserved the LMW measles IgM. The removal of LMW measles IgM after absorption of untreated CSF for rheumatoid factor was probably caused by non-specific adsorption of the small amount of LMW measles IgM to the particulate reagents in a fluid with a very low protein content. Secondly, the specificity of the anti-human IgM used in the assays is important. Anti-human IgM from 3 different manufacturers was used and all gave the same results in the Arab patient. HMW measles IgM was detected in acute measles and HMW mumps IgM in acute mumps as expected in addition to the LMW measles IgM in acute measles and in SSPE. Measles IgG was only found in the fractions expected which overlapped with those containing LMW measles IgM. Thirdly, it was shown that the sucrose density centrifugation had worked correctly and had fractionated the Arab patient's sera in the expected manner in that most of the HMW 'total' IgM was present in fractions 1 and 2 as expected whereas the measles specific LMW IgM was present in fractions 4, 5 and 6. Obviously if only the fractions containing normal HMW IgM were assayed, measles IgM would not have been detected in them. Again HMW measles IgM and HMW mumps IgM as well as

measles IgG were found in the expected fractions from acute cases. Fourthly, it was important to consider the stability of the measles IgM with storage and freeze-thawing. Some of the sera and CSFs examined were stored at -20°C for up to 18 y, although most were of more recent origin. Stobo & Tomasi (1967) subjected patients' sera to repeated freezing and thawing, prolonged storage at $+4^{\circ}\text{C}$, and incubation at $+37^{\circ}\text{C}$ for 4 d but 7S IgM was not produced from breakdown of 19S IgM. Dammacco *et al.* (1970) also subjected sera without 7S IgM to similar tests but 19S IgM was not degraded to 7S IgM and Nagington *et al.* (1982) also confirmed that repeated freeze-thawing of sera did not produce 7S IgM from 19S IgM.

LMW IgM has been found in lower vertebrates and in disease states associated with abnormalities of serum immunoglobulins such as Waldenström's macroglobulinaemia, multiple myeloma, lepromatous leprosy, rheumatoid arthritis, systemic lupus erythematosus, hereditary ataxia telangiectasia (Stobo & Tomasi, 1967, Dammacco *et al.*, 1970) and in primary biliary cirrhosis (Fakunle *et al.*, 1979). It has also been found in parasitic infections such as trypanosomiasis and filariasis and in syphilitic meningo-encephalitis (Klein *et al.*, 1967).

Viral specific LMW IgM has been found exclusively in the 7S fraction in patients with influenza and in recipients of live attenuated or killed influenza virus vaccines (Brown & O'Leary, 1973). Low molecular weight cytomegalovirus IgM has been found in patients with acute cytomegalovirus infections and in some patients with ischaemic heart disease or cardiomyopathy (Nagington *et al.*, 1982). In another study of persistent hepatitis B carriers with chronic active hepatitis, anti-hepatitis B core antigen IgM was predominantly in the LMW 7–8S fractions with smaller amounts in the HMW 19S fraction (Sjogren & Lemon, 1983).

The presence of LMW measles IgM in SSPE patients or in children with acute measles, and the absence of LMW measles IgM in young adults who had had measles in childhood suggested that LMW measles IgM is associated with viral replication. In the studies of cytomegalovirus infection and hepatitis B carriers with chronic active hepatitis, the presence of viral specific LMW IgM is associated with viral replication and persistence, although this was not the case in the studies on acute influenza and influenza vaccines.

Many of the diseases associated with LMW IgM involve abnormalities of B lymphocytes or disordered immunoregulation. It is therefore of interest that suppressed measles virus has been isolated from lymph nodes of 2 acute cases of SSPE (Horta-Barbosa *et al.*, 1971) and measles virus antigen was found in phytohaemagglutinin-stimulated T lymphocytes from peripheral blood of 5 SSPE patients (Wrzos *et al.*, 1979).

In SSPE patients it would seem that the presence of measles IgM exclusively in the LMW fractions of serum and CSF is a useful and more precise diagnostic test than the measles antibody tests previously available.

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