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# A study of immunoglobulin M antibody to measles, canine distemper, and rinderpest viruses in sera of patients with subacute sclerosing panencephalitis

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SYNOPSIS Seven boys were studied who had the clinical features of subacute sclerosing panencephalitis (SSPE) and whose brain histology was consistent with SSPE. Measles antigen was detected in the seven brains by the direct fluorescent antibody method. Three out of the seven boys had in their sera measles specific immunoglobulin M (IgM) which was detected by the indirect fluorescent antibody method, and the cell receptors for it were acetone stable. A prozone effect was noted in the sera of two patients. The absorption of one patient's serum with *Staphylococcus aureus* to reduce the titre of immunoglobulin G (IgG) removed the prozone effect. Two of the boys who had high titres of measles specific IgM also had serum IgM which reacted with canine distemper virus antigen but the titres were eightfold lower. None of the boys had detectable rinderpest specific IgM in their sera.

In a previous study (Connolly, Allen, Hurwitz, and Millar, 1967) three patients with subacute sclerosing panencephalitis (SSPE) were shown to have measles antibody in their serum and cerebrospinal fluids (CSF). High titre measles antibody was present in the serum of two patients, and in one of them the titre increased 16-fold during the course of the illness. Measles, but not canine distemper antigen, was present in the brain cells of the three patients (Connolly, 1968). Later work using the fluorescent antibody technique showed that following childhood measles specific IgM is undetectable after about six weeks whereas measles specific IgG persists for years. In contrast, both measles specific IgM and IgG were present in the serum and CSF of the three SSPE patients, and it was concluded that these antibodies were produced within the central nervous system in SSPE (Connolly, Haire, and Hadden, 1971). In view of the failure of Najera, Gracia Saiz, Herrera, and Valenciano (1972) to find measles specific IgM in SSPE sera by sucrose gradient centrifugation, we have re-examined, by modified methods, the sera from our original study together with sera from another four patients and have also looked for reactivity with canine distemper and rinderpest virus antigens in addition to measles virus antigen. All the seven patients fulfilled the clinical criteria for SSPE, and the histology of their brains on biopsy or at necropsy was consistent with SSPE (Connolly, Allen, Hurwitz, and Millar, 1968).

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## Material and Methods

The direct fluorescent antibody method for the detection of measles antigen in brain sections or smears has been described (Connolly *et al*, 1967). Viral specific IgM or IgG in sera was measured by the indirect fluorescent antibody technique. Unfixed living Vero cells well spaced out on coverslips and infected with either measles, canine distemper or rinderpest viruses were used since it has been shown that cell surface receptors for some IgMs are acetone labile (Fraser, Haire, Shirodaria, and Millar, 1973). In further experiments measles specific IgM was also titrated on Vero cells fixed in acetone for 10 min at 22°C. The sera were inactivated at 56°C for 30 min and absorbed with Vero cells. Although the sera from the seven patients had no detectable rheumatoid factor when tested at a dilution of 1:20 with the Hyland latex-globulin reagent, this test does not detect all rheumatoid factors,

which, if present, would give false positive viral IgM fluorescent staining (Shirodaria, Fraser, and Stanford, 1973). Consequently all the sera were absorbed with heat aggregated human immunoglobulin to remove any rheumatoid factor not detected by the test. The method used in this work, therefore, differs from that used in the original report (Connolly *et al*, 1971) of measles specific IgM in SSPE where acetone fixed HEp2 cells were used and the sera were not absorbed with heat aggregated human immunoglobulin.

Sufficient serum from patients 1, 4, 6, and 7 was available to absorb with a suspension of *Staphylococcus aureus* (NCTC 8530) as described by Skaug and Tjøtta (1974) to reduce the concentration of IgG.

## Results

Some epidemiological factors relating to the patients' illnesses are shown in table I. All the patients were male. The onset of illness in patients 1-3 was nine years ago whereas patients 4-7 showed symptoms from three years ago up to the present. The first three boys were, on average, twice as old as boys 4-7, and also the latent period between their childhood measles and the onset of SSPE was 11-13 years in the first three boys but only one to six years in boys 4-7.

| *Patient No.* | *Year of Onset of SSPE* | *Years* | | |
|---|---|---|---|---|
| | | *Age at Onset of SSPE* | *Childhood Measles at Age* | *Latent Period* |
| 1 | 1965 | 12 | <1 | 11 |
| 2 | 1965 | 15 | 3 | 12 |
| 3 | 1965 | 17 | 4 | 13 |
| 4 | 1971 | 8 | 4 | 4 |
| 5 | 1971 | 5 | 4 | 1 |
| 6 | 1972 | 8 | 2 | 6 |
| 7 | 1973 | 7 | 2 | 5 |

Table I *Some epidemiological data on seven boys with SSPE*

The measles antigen and antibody results in the seven patients are shown in table II. Measles antigen was detected in the brain cells of all seven patients in configurations already described (Connolly *et al*, 1967, 1968). Measles specific IgG was found in the sera of all patients, and high titres were present in patients 1, 2, 6, and 7. Measles specific IgM was found in the sera of patients 1, 2, and 3, and high titres were present in patients 1 and 2. These titres were identical when either acetone fixed or unfixed measles infected Vero cells were used. Patients 1 and 2 had canine distemper specific IgM at an eightfold lower titre than the measles specific IgM. Rinderpest specific IgM was not detected. A prozone-like effect was observed in the sera of patients 1 and 2 where measles specific IgM staining could not be found up to a titre of 1:20. Absorption of serum from patient 1 with the staphylococcal suspension resulted in a 16-fold reduction of the measles specific IgG titre without a corresponding reduction in measles specific IgM (table III) which could now be detected at the starting dilution of 1:5.

| *Patient No.* | *Measles Antigen in Brain* | *Reciprocal of Serum Antibody Titre* | | | |
|---|---|---|---|---|---|
| | | *Measles IgG* | *Measles IgM* | *Canine Distemper IgM* | *Rinderpest IgM* |
| 1 | ··· | 1280 | 320 | 40 | < 5 |
| 2 | ··· | 1280 | 160 | 20 | <10 |
| 3 | ·:· | 160 | 20 | <10 | <10 |
| 4 | · | 160 | <5 | < 5 | < 5 |
| 5 | - | 160 | <5 | < 5 | < 5 |
| 6 | · | 1280 | <5 | < 5 | < 5 |
| 7 | ·:· | 1280 | <5 | < 5 | < 5 |

Table II *Measles antigen in brain, and serum measles, canine distemper and rinderpest IgM titres in seven patients with SSPE*

| *Patient No.* | *Reciprocal of Serum Antibody Titre* | | | |
|---|---|---|---|---|
| | *Measles IgG* | | *Measles IgM* | |
| | *Before Absorption* | *After Absorption* | *Before Absorption* | *After Absorption* |
| 1 | 1280 | 80 | 320 | 320 |
| 4 | 320 | 80 | <5 | <5 |
| 6 | 320 | 80 | <5 | <5 |
| 7 | 1280 | 80 | <5 | <5 |

Table III *Measles IgG and IgM titres in four patients with SSPE before and after absorption of the sera with* Staphylococcus aureus (*NCTC 8530*). *The serum samples tested were different from those shown in table II*

Patients 4-7 had no detectable measles, distemper or rinderpest specific IgM in their sera although they had measles specific IgG titres comparable to those of patients 1-3. It is worth noting that the sera of patients 4-7 produced some positive IgM staining on measles infected cells but this staining was completely removed by absorption of the sera by heat aggregated immunoglobulin. Measles specific IgM was not detected in sera from patients 4, 6, and 7 after staphylococcal absorption (table III).

It was noted that absorption of other sera with a staphylococcal suspension also markedly reduced or removed secondary IgM staining due to rheumatoid factor even in sera with known high titres of rheumatoid factor.

Other possible inhibitors which might prevent the detection of measles specific IgM in patients 4-7 were looked for by mixing equal volumes of serum from patient 7 (measles specific IgM < 1:5) with that of patient 1 (measles specific IgM 1:320) for one hour at 37°C. The measles specific IgM titre of patient 1 was not reduced.

## Discussion

The results in table II show that the titres of measles specific IgG were about equal in the two groups of patients. However, measles specific IgM was found only in the older patients with a long period between infection with measles virus and the onset of SSPE. The receptors for SSPE measles specific IgM were not acetone sensitive since no reduction in titre was obtained when acetone-fixed, measles-infected Vero cells were used, unlike the receptors for measles specific IgM in the sera of patients with multiple sclerosis (Fraser *et al*, 1973).

The failure to detect measles specific IgM in the sera of patients 4-7 may be due to several factors. Measles specific IgM may be absent in some cases of SSPE, and the previous finding that persisting measles specific IgM in the patients' serum was associated with persisting measles antigen in the brain (Connolly *et al*, 1971) may not apply in all cases. Differences in the avidity of either the measles specific IgM or IgG for measles antigen could also markedly influence the amount of measles specific IgM detected. It is possible that some measles specific IgM may be removed non-specifically during the absorption of the sera with heat aggregated immunoglobulin.

Alternatively, measles specific IgG may interfere with measles specific IgM combining with measles antigen. The prozone effect noted in the measles specific IgM titrations in patients 1 and 2 was shown in patient 1 to be due to competition with high titre measles specific IgG and could be removed by reducing the titre of measles specific IgG with staphylococcal absorption. However, reducing the concentration of IgG did not enhance the titre of measles specific IgM in patient 1 or reveal its presence in patients 4, 6, and 7 (table III). Other investigators have found that high titres of antigen specific IgG interfere with the detection of antigen specific IgM in gonococcal (Cohen, Norins, and Julian, 1967), chlamydial (Juchau, Linscott, Schachter and Jawetz, 1972), rubella virus (Cradock-Watson, Bourne, and Vandervelde, 1972; Hornsleth, Leerhøy, Grauballe, and Spanggaard, 1974), Epstein-Barr virus (Banatvala, Best, and Waller, 1972), cytomegalovirus (Schmitz and Haas, 1972) and herpes simplex virus (Kurtz, 1974; Skaug and Tjøtta, 1974) infections and that removal or reduction of IgG from sera permitted detection or enhancement of antigen specific IgM when using the indirect fluorescent antibody method.

It is possible that inhibitors other than IgG may have prevented the detection of measles specific IgM in patients 4-7. However, the results obtained when sera from patients 1 and 7 were mixed would indicate that no other inhibitor was present in a measles specific IgM negative serum.

Since measles, canine distemper, and rinderpest viruses are serologically and biologically related (Imagawa, 1968), the higher titres of viral specific IgM obtained against measles virus in patients 1-3 implicate measles virus as the infecting agent rather than canine distemper or rinderpest viruses. This is in accordance with the detection of measles virus antigen in the brain of all the patients investigated.

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## References

Banatvala, J. E., Best, J. M., and Waller, D. K. (1972). Epstein-Barr virus-specific IgM in infectious mononucleosis, Burkitt lymphoma, and nasopharyngeal carcinoma. *Lancet*, **1**, 1205-1208.

Cohen, I. R., Norins, L. C., and Julian, A. J. (1967). Competition between, and effectiveness of, IgG and IgM antibodies in indirect fluorescent antibody and other tests. *J. Immunol.*, **98**, 143-149.

Connolly, J. H. (1968). Additional data on measles virus antibody and antigen in subacute sclerosing panencephalitis. *Neurology (Minneap.)*, **18**, Part 2, 87-89.

Connolly, J. H., Allen, I. V., Hurwitz, L. J., and Millar, J. H. D. (1967). Measles-virus antibody and antigen in subacute sclerosing panencephalitis. *Lancet*, **1**, 542-544.

Connolly, J. H., Allen, I. V., Hurwitz, L. J., and Millar, J. H. D. (1968). Subacute sclerosing panencephalitis: clinical pathological, epidemiological, and virological findings in three patients. *Quart. J. Med.*, **37**, 625-644.

Connolly, J. H., Haire, M., and Hadden, D. S. M. (1971). Measles immunoglobulins in subacute sclerosing panencephalitis. *Brit. med. J.*, **1**, 23-25.

Cradock-Watson, J. E., Bourne, M. S., and Vandervelde, E. M. (1972). IgG, IgA and IgM responses in acute rubella determined by the immunofluorescent technique. *J. Hyg. (Lond.)*, **70**, 473-485.

Fraser, K. B., Haire, M., Shirodaria, P. V., and Millar, J. H. D. (1973). Multiple sclerosis and virus antigens. (Letter). *Lancet*, **2**, 1271.

Hornsleth, A., Leerhøy, J., Grauballe, P., and Spanggaard, H. (1974). Rubellavirus-specific IgM- and IgA-antibodies The indirect immunofluorescence (IF)-technique applied

to sera with reduced IgG-concentration. *Acta path. microbiol. scand.*, Sect. B., **82**, 742-744.
Imagawa, D. T. (1968). Relationships among measles, canine distemper and rinderpest viruses. *Progr. med. Virol.*, **10**, 160-193.
Juchau, S. V., Linscott, W. D., Schachter, J., and Jawetz, E. (1972). Inhibition of antichlamydial IgM antibody by IgG antibody in immunofluorescence tests. *J. Immunol.*, **108**, 1563-1569.
Kurtz, J. B. (1974). Specific IgG and IgM antibody responses in herpes-simplex-virus infections. *J. med. Microbiol.*, **7**, 333-341.
Najera, R., Gracia Saiz, A., Herrera, I., and Valenciano, L. (1972). Serological and tissue culture observations from cases of subacute sclerosing panencephalitis. *Ann. Inst. Pasteur*, **123**, 565-570.
Schmitz, H. and Haas, R. (1972). Determination of different cytomegalovirus immunoglobulins (IgG, IgA, IgM) by immunofluorescence. *Arch. ges. VirusForsch.*, **37**, 131-140.
Shirodaria, P. V., Fraser, K. B., and Stanford, F. (1973). Secondary fluorescent staining of virus antigens by rheumatoid factor and fluorescein-conjugated anti-IgM. *Ann. rheum. Dis.*, **32**, 53-57.
Skaug, K. and Tjøtta, E. (1974). Diagnosis of recent herpes simplex infections: a modified immunofluorescent test for the detection of specific herpes simplex IgM antibodies after staphylococcal adsorption of IgG. *Acta path. microbiol. scand.*, Sect. B. **82**, 323-328.

# The June 1975 Issue

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