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Multiple sclerosis in the Orkney and Shetland Islands

IV: Viral antibody titres and viral infections

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SUMMARY A controlled serologic survey of antibody titres to 17 viruses was undertaken in multiple sclerosis patients in the Orkney and Shetland Islands. No consistent pattern of elevated antibody titre levels or the presence or absence of antibody was noted in patients compared with two control groups. Because of the isolation of these islands, the mean age at acquisition of common childhood infections, including measles, was considerably older than that of inhabitants of Europe and the United States of America. The age of occurrence of varicella was consistently younger in patients than in controls. Of note is the fact that six patients had measles after the onset of MS. This study failed to incriminate any of the viruses tested in the aetiology of MS.

Epidemiologic studies of multiple sclerosis (MS) have provided evidence that the disease may well have a viral aetiology.[1] Serological investigations of MS patients and control groups have demonstrated elevated antibody levels in patients to a number of viruses including measles, adenovirus, mumps, varicella, respiratory syncytial virus, type C influenza, herpes simplex, parainfluenza 3, and Epstein-Barr virus.[2–5] In more than 30 investigations in which seroepidemiological studies have been performed, antibody titres to measles have been most consistently elevated in patients compared with controls. In one study, patients were compared with matched siblings of the same sex born within three years of the patient and childhood friend controls. Although the patients had higher titres to measles than their childhood friends, titres in patients and their siblings were similar.[4]

Detection of virus antibodies in cerebrospinal fluid by radioimmunoassay is more sensitive than the commonly used complement fixation or haemagglutination inhibition (HI) methods and has demonstrated elevated titres not only to measles but also to rubella, vaccinia, herpes simplex, and varicella in MS patients compared with controls.[6] The authors suggest that these findings may represent 'non-specific immunologic enhancement'.

In addition to serological studies, several investigators have recently reported the detection of parainfluenza viruses, measles virus or 'multiple sclerosis associated agents' in various tissues of MS patients.[7–11] Unfortunately, these reports have not been confirmed.[12–15] *In vitro* tests of cellular immune function to measles virus in MS patients have also been reported to be abnormal.[16 17] These studies have not always been confirmed by other investigators.[18 19]

Although the observations resulting from seroepidemologic surveys and elaborate laboratory experiments have produced tantalising clues, the perplexing array of unconfirmed or contradictory findings has made it impossible to identify measles or other conventional infections of childhood as the cause of MS. We report here a carefully controlled serological and epidemiological survey designed to reassess the possible role of childhood infections in the aetiology of MS.

In an area where the rates of MS are higher than any ever reported, the Orkney and Shetland Islands (309 and 184/100 000, respectively),[20] serologic studies for 17 viruses were carried out as part of a larger investigation of the disease.

Methods

Blood specimens were drawn from each of 81 patients found to have MS in the two islands, their spouses, siblings, children, and parents. In addition, samples were collected from two age- and sex-matched control groups—one selected from the same parish and a second from a parish discontiguous

to that of the patient. Specimens were obtained from the spouse and all available first-degree relatives of the parish controls. Over 700 specimens for serology were collected in the two islands. The methods of patient ascertainment and control selection are given in detail elsewhere.[21] A long questionnaire, discussed elsewhere, was administered to all participants aged over 10 and included sections on childhood illnesses and past medical history.[22]

The viruses tested and serological methods used are shown in Table 1. Blood specimens were stored at −20°C until testing was begun. All specimens were tested blindly.

STATISTICAL ANALYSIS

Both parametric and nonparametric procedures were used to analyse the antibody titres. Student's t test was restricted to cases where the distribution of titres was nearly normal. For several of the viruses in this study, this criterion was not met. The titre distributions for Coxsackie B3 and B4, echoviruses 4 and 9, varicella, and cytomegalovirus included a substantial proportion of specimens with undetectable or trace titres. The non-parametric procedures utilised included the χ^2 test for proportions of positive or negative titres among patients and controls, and either the Mann-Whitney U test or the Wilcoxon matched-pairs signed rank test for comparisons based on serial dilutions.

Titres were compared parametrically by transforming to $\log_2$ of the reciprocal of the dilutions. When two or three titre values were given for an individual specimen the lower value was used for calculations.

In a minor departure from previous studies of antibody titres, the titre distributions were used to estimate reasonable numerical values for trace and undetectable titres rather than arbitrarily assigning numbers to these two categories. This estimation was accomplished by extrapolating from the log normal distribution of detectable titre values and determining the proportion of specimens with either trace or undetectable titres.

Table 1 *Serologic testing in the Orkney and Shetland Islands: viruses tested and methods used*

Haemagglutination inhibition (HI)
Measles
Rubella
Complement fixation (CF)
Varicella
Parainfluenza A
Mumps
Coxsackie B3 and B4
ECHO 4 and 9
Neutralisation (NT)
Polio 1, 2, and 3
Flourescence
Epstein-Barr (VCA)
Indirect haemagglutination (IHA)
Herpes simplex 1 and 2
Cytomegalovirus
Enzyme-linked immunosorbent assay (ELISA)
Measles
Canine distemper

Results

The geometric mean titres (GMT) for seven viruses are shown for patients and both sets of controls in the two island groups in Tables 2 and 3, and discussed by individual disease.

MEASLES

The titre distributions for measles virus in MS patients and controls in Orkney and Shetland are similar, as shown in Table 4. No significant differences were found when patients, parish controls, and discontiguous controls were compared overall or on a matched pair basis. No difference in titres was seen when the siblings, spouses, parents, and children of patients and of controls were compared. When separated by sex, both female patients and controls had a higher GMT than male patients and controls (Tables 2 and 3). In Orkney, female patients had significantly higher measles titres than male patients.

On questionnaires, at least 90% of the MS patients and controls in Orkney and Shetland reported having clinical measles (Tables 5 and 6), although only 75% had positive titres. This result probably reflects a lack of sensitivity of the HI test.

The mean age at infection of measles for all of the Orkney and Shetland respondents was 13·6 and 13·9 years respectively, considerably older than the age at which persons contract the illness in Europe and the United States of America.[23] However, no consistent difference in age of infection was found between patients and controls, although patients tended toward a lower mean age for measles when compared with parish and discontiguous controls; in Orkney, patients had a significantly lower mean age for measles than parish controls. Both the patients and controls were likely to contract measles at the same time as their siblings.

When the age of measles infection was plotted over time, there was a decline in mean age of infection with measles in both Orkney and Shetland from age 20 in 1900–1909 to the present mean age of 13·7. The change in age of infection was precipitous during the second world war with the influx of many outside service personnel to the islands and to the major naval base at Scapa Flow, in particular. Increased contact with outsiders and greater mobility within the islands changed the epidemiology of measles.

Table 2 *Serum antibodies to seven viruses of patients and controls by sex*

ORKNEY	*GEOMETRIC MEAN TITRES*					
	Patients		*Parish controls*		*Discontiguous controls*	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Measles	5·6*	11·8	8·3	11·2	11·2	11·5
Rubella	16·6	35·5	16·6	25·7	26·9	19·5
Herpes 1	113·8	75·8	69·2	64·6	53·7	40·7
Herpes 2	34·7	21·9	27·5	22·4	19·1	11·2
Cytomegalovirus	28·2	49·0	8·1	24·6	5·4	14·2
Mumps	2·0	3·2	3·0	4·7	4·0	2·5
Parainfluenza A	8·5	9·6	10·2	8·7	10·2	11·0

*Reciprocal of mean dilution (for example, 1/5·6)

Table 3 *Serum antibodies to seven viruses of patients and controls by sex*

SHETLAND	*GEOMETRIC MEAN TITRES*					
	Patients		*Parish controls*		*Discontiguous controls*	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Measles	10·0*	11·7	7·8	11·2	8·7	12·3
Rubella	31·6	23·4	33·9	27·5	35·5	23·4
Herpes 1	37·2	85·3	109·6	104·7	104·7	89·1
Herpes 2	22·9	34·7	33·9	33·1	40·7	30·9
Cytomegalovirus	20·9	38·9	20·9	66·1	33·1	41·7
Mumps	5·5	3·2	4·7	4·5	5·1	7·4
Parainfluenza A	20·0	14·8	15·1	15·1	15·1	15·5

*Reciprocal of mean dilution (for example, 1/10·0)

Table 4 *Measles virus—serum antibody titres Orkney and Shetland multiple sclerosis patients and controls*

	ORKNEY			*SHETLAND*		
Reciprocal of dilution	*MS patients*	*Parish controls*	*Discontiguous controls*	*MS patients*	*Parish controls*	*Discontiguous controls*
Undetectable	16	11	8	6	4	4
Trace	2	3	1	3	4	2
8	12	12	11	11	11	13
16	9	6	11	5	7	6
32	6	1	8	3	5	4
64	3	9	3	4	0	2
128	0	0	0	1	0	0
Total	48	42	42	33	31	31
Geometric mean titre	7·6	9·4	11·0	10·3	8·9	10·0

Table 5 *Frequency and age at aquisition of some common infections of childhood in patients and controls in the Orkney Islands*

	PATIENTS		*PARISH CONTROLS*		*DISCONTIGUOUS CONTROLS*	
	Freq (%)	*Mean age*	*Freq (%)*	*Mean age*	*Freq (%)*	*Mean age*
Measles	97	12·4	93	13·7	90	16·0
Rubella	45	14·2	68	19·4	60	15·4
Mumps	68	15·0	76	17·5	81	17·6
Varicella	69	7·1	91	13·0	74	11·7
Pertussis	70	7·2	84	9·1	92	9·3

Table 6 *Frequency and age at aquisition of some common infections of childhood in patients and controls in the Shetland Islands*

	PATIENTS		*PARISH CONTROLS*		*DISCONTIGUOUS CONTROLS*	
	Freq (%)	*Mean age*	*Freq (%)*	*Mean age*	*Freq (%)*	*Mean age*
Measles	90	11·6	93	15·8	90	14·0
Rubella	42	15·0	55	15·0	61	20·0
Mumps	64	16·7	68	12·4	87	16·4
Varicella	75	7·6	74	14·3	74	9·5
Pertussis	84	9·8	75	8·7	92	7·9

RUBELLA

There was no evidence of significant differences in antibody levels in the patient or control groups. Only 45% of Orkney patients and 42% of Shetland patients reported having rubella. These observations contrast with the fact that over 90% of both populations had positive titres for the rubella virus. This inconsistent result probably stems from the lack of recognition of rubella as a separate disease entity compared with the five or six similar illnesses with which it is confused, or because of the relatively minor nature of rubella as an illness.

MUMPS

No consistent differences in titres between patients and controls were demonstrated for mumps. The mean age of acquisition of mumps for all respondents was remarkably high, 17·3 years in Shetland and 16·3 years in Orkney.

VARICELLA

No significant differences in titres or the presence or absence of antibody were found for varicella. Over 33% of the varicella titres were undetectable, which is not unusual with the complement fixation method. In both island groups, patients acquired chickenpox at a younger age than their controls. For patients, the mean age at acquisition of varicella was 7·6 years in Shetland and 7·1 years in Orkney; for parish controls, it was 14·3 years in Shetland and 13·0 in Orkney. However, the patient's age of acquisition was not significantly different from that of their siblings nor of the siblings of controls.

Of some interest was the frequency of reporting of herpes zoster by individuals at remarkably young ages. Ten Orkney individuals, including two patients, reported shingles at ages 16, 19, 20, 27, 31, 39, 48, 52, 55, and 63.

HERPES SIMPLEX 1 AND 2 AND CYTOMEGALOVIRUS

Antibodies against herpes 1 were prevalent in both islands, appearing in about 85% of tested specimens, and increased with age. In the Orkney Islands, elevated titres to herpes 1, herpes 2, and cytomegalovirus were found in patients compared with discontiguous controls. As the two groups were matched for age and sex but not for parish of birth, the results may have been related to differences in geographical exposure. The trend was not seen when patients were compared with their parish controls; furthermore, the trend was not observed in the Shetland Islands.

As with herpes simplex 1, the antibody levels of herpes simplex 2 increased with age. No differences in titre levels or presence of antibody were demonstrated between patients and the control groups.

PARAINFLUENZA

Over 95% of the sera tested were positive for parainfluenza A, but no significant differences in titre levels or presence of antibody were demonstrated between groups.

COXSACKIE B3 AND B4

Two viruses were arbitrarily selected from the Coxsackie group for testing. With the complement fixation method, these antigens are broadly reactive and generally detect antibody to all six Coxsackie B viruses. Seventy-five per cent of the Coxsackie B3 titres and 90% of the Coxsackie B4 titres were either trace or undetectable. Because of the titre distribution, parametric testing was inappropriate; non-parametric testing failed to demonstrate any significant differences between patient and control groups.

Random testing of other viruses

Because of the limited availability of sera, 20 sample pairs of patients and parish controls were selected at random for testing of the following viruses.

POLIOMYELITIS 1, 2, AND 3

No significant differences in antibody titre levels were shown between patients and parish controls by neutralisation tests for polio 1, 2, or 3 viruses. All patients and controls had a positive titre to at least one of the polioviruses. Immunisation with killed active vaccine has been used in the islands since 1959.

ECHOVIRUSES 4 AND 9

Types 4 and 9 of the Echovirus series were arbitrarily selected for testing as representative of relatively common ECHO antigens which are broadly reactive when tested with the complement fixation method. Antibody levels showed little activity; almost all samples were undetectable or trace.

EPSTEIN-BARR VIRUS

No significant antibody differences were shown to Epstein-Barr virus in patients and controls. Of peripheral interest was the finding that four patients had titres of 1:640 while the titres in controls did not exceed 1:160. All but one individual had antibody to the Epstein-Barr virus.

CANINE DISTEMPER VIRUS

Antibodies to canine distemper virus and measles virus were measured by the enzyme-linked immunosorbent assay (ELISA), which is 200 times more sensitive than conventional methods.[24]

Determinations were performed for patients and controls; in addition, some family members with a negative history of measles infection were tested. The majority of patients, controls, and family members had antibody to both measles and distemper. This finding is not unexpected as distemper virus elicits antibody to distemper only, while measles virus elicits antibody to both measles and distemper. Of note is the fact that 50% of those individuals who reported no prior infection with measles did, in fact, have detectable antibody to measles.

INFECTIOUS HEPATITIS

Although no serological tests were carried out for infectious hepatitis, reporting of this illness failed to demonstrate any difference in the occurrence of infectious hepatitis in patients, controls, and discontiguous controls. Seven patients and 19 controls reported having the illness in the two islands. Among all respondents, hepatitis was reported in 11% in Orkney and 8% in Shetland. Table 7 demostrates the comparison of mean age at hepatitis infection and MS between the two islands. Infection with hepatitis and mean age at onset of MS are both lower in Shetland.

Discussion

For many years, it has been recognised by general practitioners in Orkney and Shetland that the behaviour of common infectious diseases in the two islands differs from that in less isolated populations such as the mainland of Great Britain. This is borne out in the present study by the relatively late onset of measles and mumps, particularly among the older respondents.

Measles has been recognised as a dangerous disease in the islands because of its occurrence in adults who escaped infection in childhood due to isolation. The fact that there has been an insufficient number of susceptible individuals to maintain measles in the typical two-to-three-year epidemic cycle has also contributed to the unconventional pattern of measles infection in these islands. The illness was considered to be so severe that if an epidemic of measles occurred on the Mainland (the name of the central and largest island of Orkney and of Shetland) children attending school there were not permitted to return to the outlying islands until the epidemic ran its course. In addition, visiting ships were frequently turned away from outlying islands when measles was epidemic. If they were allowed to dock, the crews were kept on board to prevent the spread of measles (Mears, Peace, and Haddow, personal communication).

Of particular interest in the present study is the fact that five patients in Orkney and one in Shetland with probable MS had measles from two to 14 years *after* onset of MS (Table 8). It can be argued that the disease which occurred in the third and fourth decades of life was not, in fact, measles. In five of the six cases, however, the diagnosis was made by a local general practitioner. In all cases, children or siblings in the patients' families who were residing in the same household as the patient acquired measles at the same time. Furthermore, measles infection corresponded to an epidemic year of measles in the islands.

A second attack of measles in individuals previously infected in childhood might have occurred in these patients, without recollection of the initial event. Recurrent clinical measles is rare, but not

Table 7 *Mean age at infection with hepatitis (all respondents) and mean age at onset of multiple sclerosis in the Orkney and Shetland Islands*

	Mean age at hepatitis infection			*Mean age at onset of multiple sclerosis*		
	Males	*Females*	*Total*	*Males*	*Females*	*Total*
Orkney	23·6	19·8	21·5	35·5	31·1	33·6
Shetland	20·4	16·6	18·0	30·5	26·8	29·0

Table 8 *Multiple sclerosis preceding measles infection in the Orkney and Shetland Islands*

ORKNEY *Patient Number*	*Present Age (1974)*	*Age at onset of multiple sclerosis*	*Year of measles*	*Age at measles*
0006	53	22	1956	30's
0008	43	11	1944	13
0014	60	33	1963	39
0115	51	17	1945	22
1001	60	31	1947	33
SHETLAND 1008	44	22	1959	29

unknown. In some instances, it was possible to examine the log books of the public schools retained in the public libraries of the two areas for the period during which the individuals who had adult-onset measles attended school. Not only was no evidence found of absence from school because of measles, but in none of the schools were large measles outbreaks recorded while the individuals were in attendance. No prior recollection of measles could be elicited from the patients or in three instances from their mothers. Each of the patients had cough, coryza, and conjunctivitis preceding the classic rash of measles.

If a prior infection with measles was to be postulated as an aetiological cause of MS, it seems highly unlikely that such patients would be susceptible to reinfection with clinical measles while harbouring a 'latent infection' with measles such as that which is present in subacute sclerosing panencephalitis. Furthermore, the occurrence of six cases of recurrent measles among less than 100 cases of MS would certainly constitute a very high rate of a second clinical infection with measles.

Recent reports by Cook and his colleagues have suggested that exposure to small dogs and consequent infection with the virus of canine distemper may play a role in the aetiology of MS.[25 26] There is no evidence from the present study to incriminate canine distemper virus, based on the presence of antibody in both patients and controls. Ownership of dogs is widespread in the Orkney and Shetland Islands and canine distemper is endemic in the dog population. No difference in degree of exposure to house pets was noted between patients and controls.[22]

The present study failed to demonstrate a consistent pattern of elevated antibody titre levels or the presence or absence of antibody for any of 17 viruses tested in patients compared with two control groups and family members. Age, sex, and geographic location are among the factors which influence viral titres; of these, the latter is the most important in explaining the findings in the present study.

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