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Cerebrospinal Fluid Immunoglobulins in Sheep with Visna, A Slow Virus Infection of the Central Nervous System

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Summary

Icelandic sheep were injected intracerebrally with visna virus, which produces a persistent infection of the CNS accompanied by encephalomyelitis and focal demyelinating lesions. Studies were conducted on two groups of sheep, with short-term infections (25 sheep sampled 1–3 months after infection) and long-term infections (14 sheep sampled 5–6 years after infection). Quantitative determination of CSF immunoglobulin levels 5 years after infection indicated that IgM concentration was usually elevated, IgG2 was occasionally elevated and IgG1 was rarely elevated. CSF oligoclonal bands were seen in about half the sheep examined 5 years after infection. There was a correlation between high titers of CSF antiviral antibody and both elevated CSF IgM concentration and CSF oligoclonal bands. Serum/CSF IgG1 ratios indicated that the blood–brain barrier was apparently intact in long-term visna infection, consistent with intrathecal synthesis of IgM and of antiviral antibody. The alterations in CSF immunoglobulins in visna resemble those found in other persistent CNS virus infections and in multiple sclerosis.

Key words: *Central nervous system – Cerebrospinal fluid immunoglobulins – Encephalomyelitis – Sheep – Virus infection – Visna*

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Introduction

Visna is a chronic encephalomyelitis with occasional focal demyelinating lesions caused by a persistent infection by a naturally transmitted retrovirus of sheep (Gudnadottir 1974; Haase 1975; Narayan et al. 1977; Petursson et al. 1978; Nathanson et al. 1980). We have undertaken a series of studies (Georgsson et al. 1977, 1978; Petursson et al. 1976, 1978, 1980; Nathanson et al. 1979, 1980) of the pathogenesis of visna following intracerebral virus injection of Icelandic sheep, which are particularly susceptible to this disease.

The present report describes the level of immunoglobulin classes (IgM, IgA, IgG1, and IgG2) and the presence of oligoclonal bands in the cerebrospinal fluid (CSF) of sheep infected 1–3 months and 5 years previously and compares these findings with previously published data (Nathanson et al. 1979) on CSF antiviral antibodies and cell counts in the same animals.

Since visna represents a chronic CNS disease with a very irregular course and since the lesions are focal and may exhibit primary demyelination, the CSF findings provide an interesting comparison with CSF changes in multiple sclerosis (MS) and other chronic neurological diseases of humans.

Materials and Methods

The visna model

Animals and most procedures have been detailed in previous publications (Petursson et al. 1976; Georgsson et al. 1977; Nathanson et al. 1979) and will be only briefly noted here. Icelandic sheep are free of natural infection since visna virus has been eradicated from the whole country. The present report is based on two groups of sheep, both inoculated intracerebrally with 10^6 TCD50 of strain 1514 of visna virus. One group (25 sheep) was examined 1–3 months after infection and the other group (14 sheep) was examined 5–6 years after infection.

All virus-injected animals were infected as documented both by virus isolation from CSF or buffy coat (many animals) and by an evolving serum antibody response (all animals tested for at least 3 months). Relatively few animals in this series developed clinical paresis (cumulative 15% in 5 years), although histological examination of similar groups of sheep indicated that about 80% showed characteristic CNS lesions of variable severity (Petursson et al. 1976).

Immunoglobulin determinations

Freshly collected pairs of samples (serum and CSF on the same animal) were frozen and held at −70°C until tested. Samples of 25–100 µl of unconcentrated CSF were assayed. Laser nephelometry (Hyland Division, Travenol Laboratories Inc., Costa Mesa, CA) was used to determine the concentration of each immunoglobulin class, using subclass-specific rabbit or goat antisera described previously (Verdouw-Chamalaun et al. 1977; Goudswaard et al. 1980). The sensitivity of the method was (µg/ml): IgG1, 3; IgG2, 25; IgM, 3; IgA, 10.

Electrophoresis

Serum was tested neat but CSF samples were concentrated 50–100-fold by lyophilization. A commercial system (Panagel, Worthington, Freehold, NJ) was used. About 3 μl were applied and electrophoresis was run for about 90 min at 300 V. Gels were fixed, washed, and stained with Coomassie brilliant blue according to standard methods (Johnson and Nelson 1977).

Presentation of data

In presenting immunoglobulin levels, medians rather than means were used because some sets of determinations did not follow a normal distribution.

Results

Immunoglobulin levels

Figure 1 shows immunoglobulin levels for 3 groups of Icelandic sheep: normal, infected 1–3 months, and infected 5–6 years. In normal serum, median levels were: IgG1, 20 mg/ml; IgG2, 5 mg/ml; IgM, 2.5 mg/ml; and IgA, 0.1 mg/ml. These values are consistent with those reported for other breeds of sheep (Verdouw-Chamalaun et al. 1977; Goudswaard et al. 1980). Overall, visna infection did not alter the levels of serum immunoglobulin, with the exception of a modest increase in IgG2 (10 mg/ml) seen in long-term infected sheep.

Median CSF immunoglobulin values in normal sheep were: IgG1, 45 μg/ml; IgG2, less than 25 μg/ml; IgM, less than 3 μg/ml. IgG1 provides a convenient measure of the ratios of serum to CSF immunoglobulin concentration. As seen in Table 1, this is about 400:1 in uninfected sheep, with a range of 280–780. A significant reduction in this value (less than 200) may reflect an alteration of the blood–brain barrier.

Infected sheep have median IgG1 concentrations in both serum and CSF which are similar to those in normal sheep. Furthermore, as shown in Table 1, the IgG1 serum/CSF ratio is not altered significantly in the two groups of visna-infected sheep, with the exception of a small number of samples (4/32 short-term infections; 1/24 long-term infections.) These findings reinforce other evidence that the blood–brain barrier remains intact during persistent visna infection (Griffin et al. 1978a; Nathanson et al. 1979).

The CSF IgG2 median value was unchanged in short-term infected sheep. There was a modest increase in median IgG2 in long-term infected sheep and 7/24 samples had values of 60 μg/ml or more, well above the maximum seen in normal sheep.

The most striking alteration in CSF immunoglobulin was the concentration of IgM. In sheep infected 1–3 months the median was 10 μg/ml and 25/32 (78%) of samples were above the maximum normal value. IgM was also elevated in sheep infected 5–6 years, but less markedly; 15/24 (62%) of samples were above the maximum normal value. Since normal CSF has an IgM concentration below that detectable in this system, it was not possible to quantitate the extent of the IgM

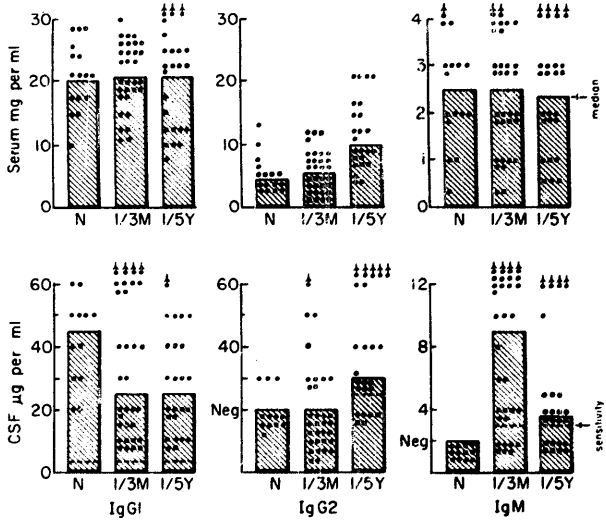

Fig. 1. Immunoglobulin levels in serum and CSF of normal and visna-infected Icelandic sheep. Each panel shows individual and median values (top of shaded bars) for 3 groups of animals: N, normal; 1/3M, infected 1–3 months; 1/5Y, infected 5–6 years. For CSF the lower limits of sensitivity are indicated by broken lines.

TABLE 1

RATIO OF IgG1 CONCENTRATIONS IN SERUM AND CSF OF NORMAL AND VISNA-INFECTED ICELANDIC SHEEP[a]

Group	No. of samples pairs	Median (µg/ml)		Serum/CSF median ratio[a]	Ratios <200[a]
		Serum	CSF		
Normal	12	20,000	44	450	0/12
Infected 1–3 months	32	22,000	25	1,000	4/32
Infected 5–6 years	24	22,000	26	700	1/24

[a] Ratios were calculated for each serum–CSF pair and median values then determined. Based on normal ratios (280–780); ratios less than 200 were considered abnormal.

TABLE 2

OLIGOCLONAL BANDS IN SERUM AND CSF OF ICELANDIC SHEEP 5–6 YEARS AFTER INFECTION WITH VISNA VIRUS [a]

Bands in	Location of bands in electropherogram	No. of sheep [a]
Serum only		6
Serum and CSF	Similar	7
Serum and CSF	Dissimilar	1
Totals		14

[a] Serum and CSF pairs collected and tested simultaneously. In many instances several pairs of specimens from a single sheep were tested.

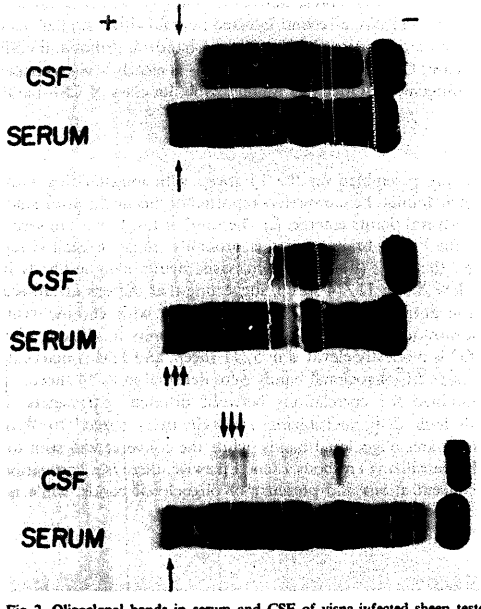

Fig. 2. Oligoclonal bands in serum and CSF of visna-infected sheep tested 5–6 years after infection. Serum and CSF obtained and run simultaneously. *Upper panel*: Similar band in serum and CSF (1557); *Middle panel*: Bands in serum, no bands in CSF (1551); *Lower panel*: Bands in both serum and CSF, but different pattern (1525). Arrows mark bands.

elevations. In humans, normal CSF has an IgM concentration of about 0.05 μg/ml (Nerenberg et al. 1978; Williams et al. 1978). This suggests that the considerable proportion of values of 3 μg/ml or greater (25/32 short-term and 15/24 long-term infected sheep) represent massive elevations of CSF IgM concentration.

Oligoclonal bands

A group of 14 sheep, infected 5–6 years previously by intracerebral inoculation of visna virus, were examined for evidence of oligoclonal bands in serum and CSF. Unexpectedly, serum of all these sheep showed bands (Table 2 and Fig. 2). However, a comparison with normal Icelandic sheep showed that serum bands were present in most uninfected animals over 5 years of age but absent in sera of young uninfected animals (6 months of age). Therefore, serum bands cannot be considered a visna-specific phenomenon.

CSF electrophoresis showed evidence of bands in 8 of 14 animals infected 5 years previously (Table 2). Figure 2 portrays representative examples, showing one infected animal with bands confined to serum, a second infected animal with a similar band in both CSF and serum, and a third animal with different bands in serum and CSF. Since bands are never seen in CSF from normal sheep, this is clearly a visna-associated phenomenon. The antigenic specificity or immunoglobulin class of CSF bands was not determined.

Correlations

In Table 3, CSF data are assembled on the 14 sheep with longstanding visna infections, to show for each animal the parameters reported in this and a prior study (Nathanson et al. 1979). Several points emerge, (i) The ratio of IgG1 levels in serum and CSF indicates that the blood–brain barrier is probably intact in each sheep, since all ratios are greater than 200. (ii) The ratio of visna neutralizing antibody in serum and CSF is far below 200 in 11/14 sheep, suggesting that there is intrathecal synthesis of virus-specific antibody in most Icelandic sheep with chronic visna infection. (iii) CSF concentration of IgG1 is within normal limits in all long-term infected sheep, while IgG2 is modestly elevated in 5/11 sheep, and IgM is markedly elevated in 8/11 sheep. (iv) CSF oligoclonal bands were detected in 8/14 sheep.

When Table 3 is examined for correlations between different parameters, it appears that sheep with high CSF neutralizing antibody titers tended to show elevated IgM concentration and oligoclonal bands, while the converse was seen for sheep with minimal CSF neutralizing antibody titers. Likewise, there is a correlation between elevated IgM concentrations and presence of oligoclonal bands, while no bands were seen in the 3 sheep with lowest IgM levels.

Discussion

CSF immunoglobulins in visna

From our studies (Petursson et al. 1976; Georgsson et al. 1977; Nathanson et al. 1979) and those of Griffin, Narayan and colleagues (Griffin et al. 1978a, b), several

TABLE 3

CSF IMMUNOGLOBULIN CONCENTRATION, OLIGOCLONAL BANDS, NEUTRALIZING ANTIBODY TITERS, AND CELL COUNTS IN ICELANDIC SHEEP 5–6 YEARS AFTER VISNA INFECTION

Sheep number	CSF cells/μl	IgG1 serum/CSF ratio	Neutralizing antibody[c]		Antibody serum/CSF ratio	CSF Ig (μg/ml)[d]			CSF oligoclonal bands
			Serum	CSF		IgG1	IgG2	IgM	
1518	N[a]		*250*	<4	>64				0
1521	N	880[b]	*500*	*250*	*2*	14	*37*	*3*	*5*
1523	N	550	*250*	*50*	*5*	44	30	*3*	*1*
1524	*E*	320	*24*	*8*	*3*	36	31	*4*	*1*
1525	N		*64*	*12*	*5*				*3*
1527	*E*	220	*64*	*16*	*4*	45	*36*	<3	0
1548	*E*	600	*250*	*64*	*4*	50	*36*	*3*	0
1549	*E*		*1000*	*1500*	<1				*2*
1551	N	1300	*16*	<4	>4	15	30	<3	0
1553	*E*	5200	*250*	*125*	*2*	5	*68*	*28*	0
1554	N	820	*100*	<4	>25	35	30	<3	0
1555	*E*	1360	*125*	*64*	*2*	18	*40*	*38*	*2*
1556	N	900	*100*	*4*	*25*	38	10	*5*	*3*
1557	N	550	*64*	*8*	*8*	24	14	*2*	*1*
Normal	N	>200	<4	<4		20–60	<30	<3	0
Proportion abnormal[e]	6/14	0/11	14/14	11/14	11/14	0/11	5/11	8/11	8/14

[a] Cell counts: N=normal (less than 20 cells/μl); E=elevated (50–250 cells/μl).

[b] IgG1, ratio calculated from determinations on paired serum and CSF samples. Normal range, 280–780; values below 200 considered abnormal.

[c] Neutralizing antibody data from Nathanson et al. (1979). Titers against virus strain 1514 which was used to infect sheep.

[d] CSF immunoglobulin concentrations based on median of 2–3 samples.

[e] Abnormal values in italics.

general points emerge in regard to the CSF in this persistent virus infection of the CNS.

(a) The blood–brain barrier apparently remains intact even though there is a chronic focal inflammatory process in the neuroparenchyma and a modest elevation in CSF cell counts.

(b) CSF titers of antiviral antibody are elevated far above the levels which would be generated by passive diffusion of serum antibody in animals with an intact blood–brain barrier.

(c) CSF immunoglobulins show a class-specific elevation, the most striking being the early and persistent elevation of IgM. IgG1 (the dominant subclass in serum and in normal CSF) is rarely elevated, but there is a modest increase in IgG2 in some long-term infected sheep. These findings differ somewhat from those of Griffin et al. (1978a) who observed a persistent elevation of IgG (subclass not determined) and a transient elevation of IgM, reminiscent of the changes seen in an acute encephalitis, such as louping ill (Reid et al. 1971).

(d) These data are consistent with the chronic intrathecal synthesis of antiviral antibody and of IgM antibody. The presumed source of intrathecal antibody synthesis in visna-infected animals is the plasma cells which are prominently represented in the inflammatory CNS lesions of visna (Georgsson et al. 1977; Nathanson et al. 1979). The present elevation of CSF IgM concentration suggests a chronic antigenic stimulus, and this is consistent with the persistence of visna in the CNS throughout the lifelong infection (Petursson et al. 1976; Petursson et al. 1978; Petursson et al. 1980).

(e) Marked variation between sheep is seen in all CSF parameters even though the animals were infected with an identical dose of a single strain of virus. This is pertinent in evaluating patient-to-patient variation in multiple sclerosis and other chronic neurological diseases of unknown etiology.

Questions which require further study include the immunoglobulin class-specific titer of visna antibodies and the immunoglobulin class and antigenic specificity of oligoclonal bands.

CSF immunoglobulins in other viral infections of the CNS

More attention has been given to the CSF in subacute sclerosing panencephalitis (SSPE) than to any other persistent viral infection of the CNS. In SSPE the blood–brain barrier remains intact (Cutler et al. 1970), and the CSF measles antibody titer is high relative to serum antibody (Connally 1972; Mehta et al. 1977) indicating intrathecal synthesis of antibody. CSF immunoglobulin quantitation (Cutler et al. 1970; Mingioli et al. 1978) usually shows an elevation of total IgG (often 4–8-fold normal) but not of total IgM, in contrast to visna. Oligoclonal immunoglobulin bands are common in the CSF in cases of SSPE and the majority of these can be absorbed with measles antigens (Vandvik et al. 1976; Mehta et al. 1978).

From studies of visna, SSPE, and other viral infections of the CNS (Morgan 1947; McFarland et al. 1972; Gerhard and Koprowski 1977), a general picture emerges of which the most important features appear to be migration into the CNS

of B cells responsive to antigens of the infecting virus whilst the blood–brain barrier retains its integrity to serum proteins.

CSF immunoglobulins in multiple sclerosis (MS)

The CSF findings in visna show a number of parallels with the findings in MS (Johnson and Nelson 1977).

(a) In MS there may be an elevation of any immunoglobulin class, and IgM concentration is often high (Thompson 1977; Mingioli et al. 1978; Nenenberg et al. 1978; Williams et al. 1978).

(b) In MS there are plasma cells in focal lesions and evidence of local immunoglobulin synthesis (Sandberg-Wollheim 1974; Williams et al. 1978).

(c) In MS over 80% of patients show oligoclonal bands (Williams et al. 1978; Olsson and Nilsson 1979).

These findings have been construed as consistent with the hypothesis that MS is associated with a persistent CNS infection (Johnson 1975). The parallels between the CSF changes in visna and those in MS bolster this line of reasoning and constitute an important reason for detailed study of this slow viral disease of sheep. Such comparisons could help to understand confusing phenomena in MS such as the occurrence of electrophoretically restricted antibodies against a number of human viruses (Norrby et al. 1974; Nordal et al. 1979).

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References

Connally, J.H., Subacute sclerosing panencephalitis, J. Clin. Path., 25 (S6) (1972) 73–77.

Cutler, R.W.P., G.V. Watter and J.P. Hammerstad, The origin and turnover rates of cerebrospinal fluid albumin and gamma-globulin in man, J. Neurol. Sci., 10 (1970) 259–268.

Georgsson, G., P.A. Palsson, H. Panitch et al., Ultrastructure of early visna lesions, Acta Neuropath. (Berl.), 37 (1977) 125–135.

Georgsson, G., G. Petursson, P.A. Palsson et al., Experimental visna in fetal Icelandic sheep, J. Comp. Path., 88 (1978) 599–605.

Gerhard, W. and H. Koprowski, Persistence of virus-specific memory B cells in mice CNS, Nature (Lond.), 266 (1977) 360–361.

Goudswaard, J., C.V.M. Verdouw-Chamalaun and A. Noordzij, Quantitation of immunoglobulins in ovine sera and secretions by laser nephelometry — Comparison with radial immunodiffusion (RID) technique, Vet. Immunol. Immunopath., 1 (1980) 163–177.

Griffin, D.E., O. Narayan, J.F. Bukowski et al., The cerebrospinal fluid in visna, a slow viral disease of sheep, Ann. Neurol., 4 (1978a) 212–218.

Griffin, D.E., O. Narayan, and R.J. Adams, Early immune responses in visna, a slow viral disease of sheep, J. Inf. Dis., 138 (1978b) 340–350.

Gudnadottir, M., Visna-maedi in sheep, Progr. Med. Virol., 18 (1974) 336–349.

Haase, A.T., The slow infection caused by visna virus, Curr. Topics Microbiol. Immunol., 72 (1975) 101-156.

Johnson, K.P. and B.J. Nelson, Multiple sclerosis – Diagnostic usefulness of cerebrospinal fluid, Ann. Neurol., 2 (1977) 425-431.

Johnson, R.T., The possible viral etiology of multiple sclerosis, Adv. Neurol., 13 (1975) 1-46.

McFarland, H.F., D.E. Griffin and R.T. Johnson, Specificity of the inflammatory response in viral encephalitis, Part 1 (Adoptive immunization of immunosuppressed mice infected with Sindbis virus), J. Exp. Med., 136 (1972) 216-226.

Mehta, P.D., A. Kane and H. Thormar, Quantitation of measles virus-specific immunoglobulins in serum, CSF, and brain extract from patients with subacute sclerosing panencephalitis, J. Immunol., 118 (1977) 2254-2261.

Mehta, P.D., A. Kane and H. Thormar, Relationship between homogenous IgG fractions and measles virus antibody activities in subacute sclerosing panencephalitis brain, J. Immunol., 117 (1978) 2053-2060.

Mingioli, E.S., W. Strober, W.W. Tourtellotte et al., Quantitation of IgG, IgA and IgM in the CSF by radioimmunoassay, Neurology (Minneap.), 28 (1978) 991-995.

Morgan, I.M., The role of antibody in experimental poliomyelitis, Part 3 (Distribution of antibody in and out of the central nervous system in paralyzed monkeys), Amer. J. Hyg., 45 (1947) 390-400.

Narayan, O., D.E. Griffin and A.M. Siverstein, Slow virus infection — Replication and mechanisms of persistence of visna virus in sheep, J. Inf. Dis., 135 (1977) 800-806.

Nathanson, N., G. Petursson, G. Georgsson et al., Pathogenesis of visna, Part 4 (Spinal fluid studies), J. Neuropath. Exp. Neurol., 38 (1979) 197-208.

Nathanson, N., G. Petursson, J. Martin et al., Pathogenesis of visna. In: A. Boese (Ed.), First International Symposium of the Hertie Foundation, Verlag Chemie, Weinheim (F.R.G.), 1980, pp. 184-188.

Norenberg, S.T., R. Prasad and M.E. Rothman, Cerebrospinal fluid IgG, IgA, IgM, IgD, and IgE levels in central nervous system disorders, Neurology (Minneap.), 28 (1978) 980-990.

Nordal, H.J., B. Vandvik and E. Norrby, Multiple sclerosis – Local synthesis of electrophoretically restricted measles, rubella, mumps, and herpes simplex virus antibodies in the central nervous system, Scand. J. Immunol., 7 (1979) 473-478.

Norrby, E., H. Link, J.E. Olsson et al., Comparison of antibodies against different viruses in cerebrospinal fluid and serum samples from patients with multiple sclerosis, Inf. Immunol., 10 (1974) 688-894.

Olsson, J.E. and K. Nilsson, Gamma globulins of CSF and serum in multiple sclerosis — Isoelectric focusing on polyacrylamide gel and agar gel electrophoresis, Neurology (Minneap.), 29 (1979) 1383-1391.

Petursson, G., N. Nathanson, G. Georgsson et al., Pathogenesis of visna, Part 1 (Sequential virologic, serologic, and pathologic studies), Lab. Invest., 35 (1976) 402-412.

Petursson, G., N. Nathanson, P.A. Palsson et al., Immunopathogenesis of visna, a slow virus disease of the central nervous system, Acta Neurol. Scand., 57(S67) (1978) 205-219.

Petursson, G. Martin, G. Georgsson et al., Visna — The biology of the agent and the disease. In: D.A.J. Tyrell (Ed.), The Biology of Some Slow and Persistent Infections, Martinus Nijhoff, The Hague, 1980, pp. 165-197.

Reid, H.W., P.C. Doherty and A.M. Dawson, Louping ill encephalomyelitis in the sheep, Part 3 (Immunoglobulins in cerebrospinal fluid), J. Comp. Path., 81 (1971) 537-543.

Sandberg-Wollheim, M., Immunoglobulin synthesis in vitro by cerebrospinal fluid cells in patients with multiple sclerosis, Scand. J. Immunol., 3 (1974) 717-730.

Thompson, E.J., Laboratory diagnosis of multiple sclerosis — Immunological and biochemical aspects, Brit. Med. Bull., 33 (1977) 28-33.

Vandvik, B., E. Norrby, H.J. Nordal et al., Oligoclonal measles virus-specific IgG antibodies isolated from cerebrospinal fluids, brain extracts, and sera from patients with subacute sclerosing panencephalitis and multiple sclerosis, Scand. J. Immunol., 5 (1976) 979-992.

Verdouw-Chamalaun, C.V.M., A. Noordzij and J. Goudswaard, Quantitative studies on the immunoglobulins of adult Texel sheep and lambs during the first weeks of life, Zbl. Vet. Med., B24 (1977) 358-366.

Williams, A.C., E.S. Mingioli, H.W. McFarland et al., Increased CSF IgM in multiple sclerosis, Neurology (Minneap.), 28 (1978) 996-998.