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CENTRAL NERVOUS SYSTEM VIRION DETECTION IN ACUTE MEASLES: HISTOPATHOLOGICAL, ULTRASTRUCTURAL AND PATHOGENETIC ASPECTS

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SUMMARY

Histopathological and ultrastructural studies of 23 patients who died with clinical diagnosis of measles were carried out. In 12 cases viral nucleocapsids were searched by electron microscopy and detected in 100% of the cases in the lungs and in 50% of the cases in the central nervous system. They were mostly intranuclear. Histopathological changes associated to neurological alterations and the detection of virion are discussed in relation to acute and delayed clinical manifestations.

KEYWORDS: Measles; Virus; Central Nervous System; Pathology; Ultrastructure; Pathogenesis.

INTRODUCTION

Neurological clinical signs in measles have been described due to either acute or delayed central nervous system involvement [8, 23, 24, 25, 42, 46]. Acute involvement has been described [3, 9, 18, 21, 35] and sequelae detected in 30-35% of the cases. Disseminated mild perivasculitis is most frequent in the early phases. Later demyelinization appears together with inflammatory changes [2, 3, 24, 26, 27, 35]. No virus was detected either in brain or cerebrospinal fluid in the acute phase. Late neurological complications of measles such as acute delayed encephalitis [4, 38] and subacute sclerosing panencephalitis [29, 31, 45, 46] have been reported. In cases of subacute sclerosing panencephalitis viral antigens were found by immunohistochemical methods [5, 6, 14, 29, 31] and virions were seen by electron microscopy in brain tissue [3, 40]. Recently, multiple sclerosis has also been related to measles [15, 28].

Neuropathological changes were described in acute measles, but no virus was detected in brain tissue neither by electron microscopy [24] nor immunohistochemistry [11, 18, 19, 21, 24, 36]. The pathogenesis of the brain lesions is not known [3, 9, 16, 18, 20, 21, 30, 43, 44].

MATERIAL AND METHODS

Brain, lung and lymph node tissue obtained from autopsies of 23 patients who died with clinical diagnosis of measles were collected for histopathological and ultrastructural studies.

In each case, the brain was sliced and 80 to 100 fragments from all cerebral regions and spinal cord

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were processed for light microscopy, stained with hematoxylin-eosin and Weil stain. Fragments of brain tissue were processed for electron microscopy counter stained with uranyl acetate 2% and with lead citrate observed through Phillips EM-301 electron microscope. The time between dead and necropsy did not exceed 3 hours.

Immunohistochemistry was carried out in all cases using antibodies anti-IgG, IgM, IgA and anti-Pan T cells (DAKO) by the avidine-biotine peroxidase anti peroxidase method.

The qui-square method was used for statistical evaluation of T cells and immunoglobulin values, T Student Test for parametric values and Kruskal-Wallis for semiquantitative non parametric values.

RESULTS

The age of the patients varied from 22 days to 10 years, 13 were male and 10 female. Malnutrition was present in 78.9% of the cases. The time from prodromal phase to death varied from 10 to 48 days. Neurological signs were present in 8 out of 23 patients and appeared between 2 and 21 days after the on set of the cutaneous rash (Table 1)

The lungs showed interstitial pneumonitis in 100% of the cases, epidermoid hyperplasia of tracheal and bronchial epithelium in 73.9%, syncytial multinucleated cells in 65.2%, hyaline membranes in 52.1%, necrotizing bronchiolitis in 47.8% and bronchopneumonia in 95.6% of the cases. The lymph nodes showed

TABLE 1

Measles: correlation of ages, neurological signs, histopathological features of brain, immunohistochemistry and electron microscopy of 23 cases

Nº Case	Age	NN	Time of Disease (days)	neuropathology	Virus - L	EM - CNS	Immunoh. - T.C	CNS - IgM+C
1	1y3m	-	10	Meningitis	+	+	3	2
2	1y	+	30	Perivasculitis	+	-	0	0
3	9m	-	20	-	+	+	0	0
4	7m	-	20	Meningitis	+	-	1	3
5	1y3m	-	10	-	+	-	0	0
6	1y	-	11	Perivasculitis	+	+	2	1
7	7m	-	24	-	+	-	0	0
8	4m	-	21	-	+	-	0	0
9	1y	-	16	Perivasculitis	+	+	0	2
10	4y1m	-	20	MEMR	+	-	3	2
11	1y10m	+	28	Meningitis	+	+	0	2
12	5y2m	+	30	Meningoencephalitis	+	+	1	2
13	9m	-	20	-	N	N	0	0
14	6m	-	16	-	N	N	0	0
15	1y	+	23	Meningoencephalitis	N	N	2	4
16	9m	-	16	Perivasculitis	N	N	0	0
17	10m	+	35	Meningoencephalitis	N	N	2	3
18	9m	-	16	Perivasculitis	N	N	0	1
19	22d	-	10	-	N	N	0	0
20	10y	+	48	Desmielinization	N	N	2	1
21	1y6m	+	25	Desmielinization	N	N	0	1
22	1y8M	+	40	Desmielinization	N	N	0	1
23	2y11m	-	12	Desmielinization	N	N	0	1

NN = Nº Neurological signs;
CNS = Central Nervous System;
IgM+C = IgM producers cells;
O to 3 = quantification of cells T;
VIRUS-EM = virus - electron microscopy;
IMMUNOH = Immunohistochemistry;
MEMR = meningoencephalorradiculitis;
0 to 4 = quantification of IgM producers cells
L = lung;
T.C. = T cells;
N = not done;

multinucleated giant cells in 20 patients. These findings were used as criteria for histopathological diagnosis of measles.

Gross examination of the brain showed edema in 9 out of 23 patients and subarachnoid hemorrhage in one case.

On histopathological examination, the cases were divided into 3 groups:

GO - no changes: 7 cases
GI - inflammatory changes only: 12 cases
G2 - degenerative and inflammatory changes: 4 cases

G1 - 12 patients showed meningeal and/or intraparenchymal perivascular infiltration by macrophages, lymphocytes and plasma cells. The perivasculitis was detected in the white matter of the centrum semiovale it was mild and scattered in the brain tissue (Fig. 1). They did not appear in all levels and mostly were one layer cell thick but more evident infiltrate was also found. Only in the leptomeninge inflammatory cells were detected in 3 cases. These changes were found in different brain regions as well as in spinal cord.

G2 - Degenerative changes were found together with the inflammatory infiltrate in 4 cases. Degenerative changes of the myelin, mostly surrounding small veins of the white matter (Fig. 2), were present in brain and medulla as well as demyelination in the oval center. In these cases perivasculitis was more intense, frequent and diffuse fibrillary astrogliosis was present mainly in the white matter. Many plasma cells were identified in the inflammatory infiltrate. In one case casual repaired demyelinated areas were detected.

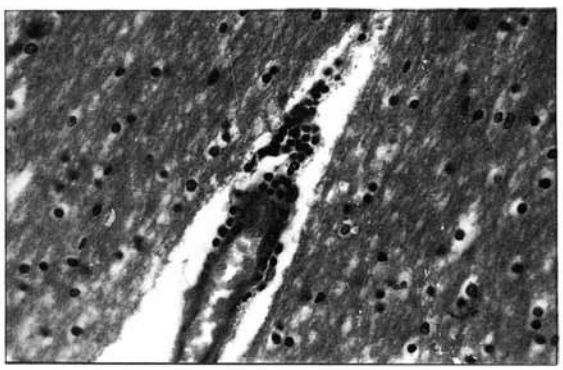

Fig. 1 - Group 1: Brain tissue with perivascular mononuclear cells infiltrate. HE. O.M. x 400.

Fig. 2 - Group 2: CNS - Degeneratives and inflammatories alterations. Weil stain. O.M. x200.

The electron microscopy was carried out in 12 cases and viral particles were detected in 100% of the cases in the lungs and in 50% of these cases in CNS. They were mostly intranuclear, and occupied almost all nuclei, with concentration of the nuclear chromatin in the nuclear membrane area. In some cells the nucleocapsids occupied only part of the nuclei (Fig. 3). The intranuclear granulomatous material described an viral particles is similar with those found in the cells of the lung with interstitial pneumonitis due to measles. Previous report on the ultrastructure of measles virus showed similar aspect [12, 37, 40]. We considered it as morphological demonstration of the virus. The viral particles were detected in astrocytes in the nuclei (Fig. 4) or in the nuclei and cytoplasm of cells such as

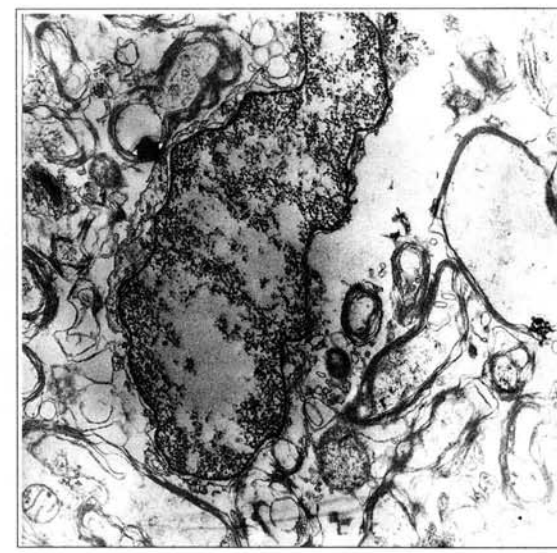

Fig. 3 - Intranuclear nucleocapsid filling only part of the nucleus. EM. x28.500.

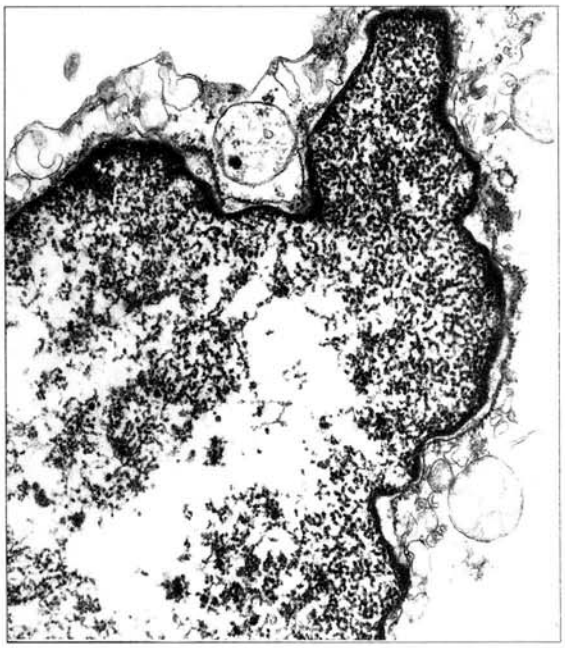

Fig. 4 - Astrocytes with intranuclear nucleocapsid. EM. x36.000

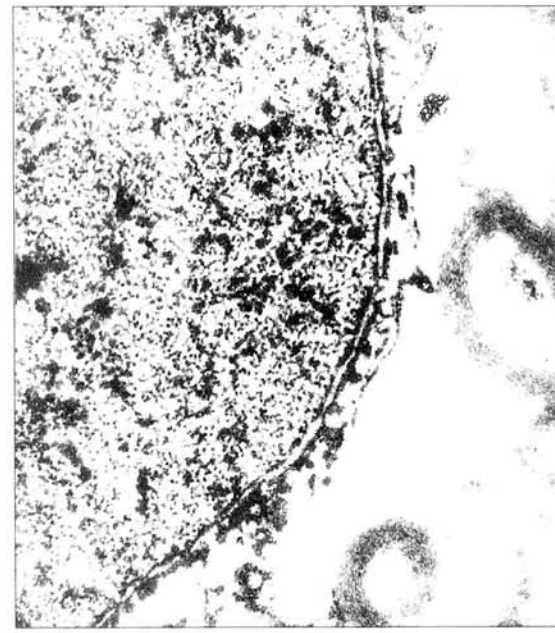

Fig. 6 - CNS cell with intranuclear nucleocapsid. EM. x36.000

microglia (Fig. 5). The Fig. 6 shows aspect of viral particles. No virus was detected in nervous. Immunopathological detection was tried but the policlonal antibodies produced in our laboratory from vaccine virus was not suitable for test in tissue. We are typing to get monoclonal antibodies for the next step of this work. The appearance of the nucleocapsids found in brain tissue was similar to those found in giant cells of the lung in cases with interstitial pneumonitis due to measles.

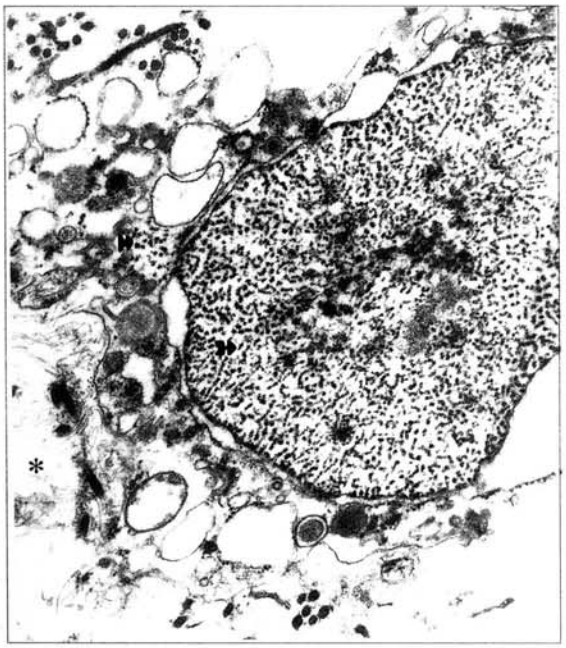

Fig. 5 - Microglia with nucleocapsid in the nucleus and in the cytoplasm (➽) Astrocyte cytoplasmic processes (*). EM. x36.000.

There was correlation between immunological signs and lesion intensity but even cases with no clinical signs showed mild lesions. Myelin degenerative changes found in G2 appeared latter than the inflammatory infiltrate. The immunohistochemistry of the infiltrate showed predominance of T cells and IgM producing plasma cells. Close correlation ($p=0.043$) was detected between age and time of disease which could be related to maturity of the immune system. Correlation between syncytial giant cells in lung and lymphoid organs and neuropathological lesions was also detected.

DISCUSSION

In acute measles, electroencephalographic and cerebrospinal fluid changes have been reported [13, 34, 39, 41]. Neuropathological lesions have also been detected in post-mortem examination of patients who died with clinical diagnosis of measles [23]. However, no viral particles were found, and authors have proposed that these lesions would be due to an autoimmune reaction

based on similarities of the inflammatory infiltrate to that of experimental allergic encephalitis [3, 11, 18, 19, 21, 22].

The detection of viral nucleocapsids in the CNS in 50% of the cases with or without clinical manifestations suggests that the virus itself was related to the neuropathological changes. When only intranuclear viral inclusions were found, it was thought to be a way of viral persistence [10, 37] which would be related to later clinical manifestations. In these cases the viral antigens would not be expressed on the cell surface, no antibodies would be produced and the host cell would not be destroyed by the immune response. The supposedly defective virus would persist in the CNS and this could explain late neurological involvement. Viral persistence in CNS would be facilitated by the heterogeneous cellular population where the virus is sequestrated and kept away from the immunological defenses of the host for a long time [1, 7, 17, 32, 33].

We found one case where encephalitis was present and virions were not detected and another where virions were detected but there was no encephalitis. It could mean that the disease has two poles, one where the inflammatory reaction is effective to destroy the virus and another where the virus does not stimulate effective inflammatory reaction leading to a persistence of the virus within the host cells for long time explaining latter appearence of lesions.

The measles virus usually reaches the CNS in the acute phase with few clinical manifestations but with persistence and replication leading to production of defective virions responsable for slow inflammatory reaction producing chronic neurological disease like immunosuppressive delayed encephalitis or subacute sclerosing panencephalitis and perhaps related with multiple sclerosis in some cases.

The incidence of measles decreased in the last years due to vaccination. The vaccine made of attenuated virus leads to viremia which also could develop an immunological response that could represent the beginning of the late neurological lesions found in measles. It means that the virus either wild or vaccine, reaching the CNS will find host cells in which they could persist for long time and develop late lesions related to measles. It is important to have in mind that there is also the possibility of vaccine virus, during the viremia, starting immunological response similar to the wild virus.

Further studies are being developed to better understand the immunopathology of acute measles encephalitis. Immunopathological identification of the virus using monoclonal antibodies, "in situ" hybridization and PCR will be carried out shortly.

The ultrastructural aspect of the viral nucleocapsids is similar to that found in giant cells of the interstitial pneumonitis usually present in measles with lung involvement.

RESUMO

Detecção de partículas virais no SNC no sarampo agudo: aspectos histopatológicos, ultraestruturais e patogenéticos.

Foram realizados estudos histopatológicos e ultraestruturais de 23 pacientes que morreram com diagnóstico clínico de sarampo. Presença de nucleocapsides virais foi pesquisada em 12 casos e detectada em 50% destes casos no SNC. Eram, na maioria dos casos, intranucleares. As alterações histopatológicas associadas a manifestações neurológicas e à detecção do vírus são discutidas em relação às manifestações clínicas agudas e tardias.

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REFERENCES

1. AARLI, J.A. - The immune system and the nervous system. **J. Neurol. (Berl.), 229**: 137-154, 1983.

2. ADAMS, J.M.; BAIRD, C. & FILLOY, L. - Inclusion bodies in measles encephalitis. **J. Amer. med. Ass., 195**: 290-298, 1966.

3. ANDERSON, J.R. - Viral encephalitis and its pathology. **Curr. Top. Path., 76**: 23-60, 1988.

4. BACZKO, K.; LIEBERT, U.G.; CATTANEO, R. et al. - Restriction of measles virus gene expression in measles inclusion body encephalitis. **J. infect. Dis., 158**: 144-150, 1988.

5. BROWN, H.R.; GOLLER, N.L.; THORMAR, H. et al. - Measles virus matrix protein gene expression in a subacute sclerosing panencephalitis patient brain and virus isolated demonstrated by cDNA hybridization and immunocytochemistry. **Acta neuropath. (Berl.), 75**: 123-130, 1987.

6. BUDKA, H.; LASSMAN, H. & POPOW-KRAUPP, Th. - Measles virus antigen in panencephalitis. An immunomorphological study. Stressing dendritic involvement in SSPE. **Acta neuropath. (Berl.), 56**: 52-62, 1982.

7. CERVOS-NAVARRO, J.; KANNUKI, S. & NAKAGAWA, Y. - Blood-Brain-Barrier (BBB). Review from morphological aspect. **Histol. Histopath., 3**: 203-213, 1988.

8. CHOPPIN, P.W. - Measles virus and chronic neurological diseases. **Ann. Neurol., 9**: 17-20, 1980.

9. DACHY, A. & THIRY, L. - Les affections neurologiques en rapport avec la rougeole. Considerations virologiques et immunologiques. **Acta. paediat. belg., 24**: 171-194, 1970.

10. FOURNIER, J.G.; PRIVATE, A. & BOUTEILLE, M. - Structural changes in the cell nucleus during measles virus infection in cerebellar explants. **J. Ultrastruct. Res., 77**: 319-328, 1981.

11. GENDELMAN, H.E.; WOLINSKY, J.S.; JOHNSON, R.J. et al. - Measles encephalitis: lack of evidence of viral invasion of the central nervous system and quantitative study of the nature of demyelination. **Ann. Neurol., 15**: 353-360, 1984.

12. GHADIALLY, F.N. - Ultrastructural pathology of the cell and matrix. London, Butterworths, 1982. p. 108-109.

13. GIBBS, F.A. & ROSENTHAL, I.M. - Eletroencephalographic in natural and attenuated measles. **Amer. J. Dis. Child., 103**: 395-400, 1962.

14. HAASE, A.T.; SWOVELAND, P.; STOWRING, L. et al. - Measles virus genome in infections of the central nervous system. **J. infect. Dis., 144**: 154-160, 1981.

15. HANKINS, R.W. & BLACK, F.L. - Western blot analysis measles virus antibody in normal persons and in patients with multiple sclerosis, subacute sclerosing panencephalitis, or atypical measles. **J. clin. Microbiol., 24**: 324-329, 1986.

16. HANNINEN, P.; ARSTILA, P.; LANG, H.; SALMI, A. & PANELIUS, M. - Involvement of the central nervous system in acute uncomplicated measles virus infection. **J. clin. Microbiol., 11**: 610-613, 1980.

17. JOHNSON, R.T. - Viruses and chronic neurological diseases. **Johns Hopk. med. J., 150**: 132-140, 1982.

18. JOHNSON, R.T. - The pathogenesis of acute viral encephalitis and postinfectious encephalomyelitis. **J. infect. Dis., 155**: 359-365, 1987.

19. JOHNSON, R.T.; HIRSCH, R.L.; GRIFFIN, D.E. & WOLINSKY, J.S. - Clinical and immunological studies of measles encephalitis. **Trans. Amer. Neurol. Ass., 106**: 42-45, 1981.

20. JOHNSON, R.T.; GRIFFIN, D.E.; HIRSCH, R. & VAIBERG, A. - Measles encephalitis. **Clin. exp. Neurol., 19**: 13-16, 1983.

21. JOHNSON, R.T.; GRIFFIN, D.E.; HIRSCH, R.L. et al. - Measles encephalomyelitis - clinical and immunologic studies. **New Engl. J. Med., 310**: 137-141, 1984.

22. JOHNSON, R.T.; GRIFFIN, D.E. & MOENCH, T.R. - Pathogenesis of measles immunodeficiency and encephalomyelitis: parallels to AIDS. **Microb. Pathogenesis**, 4: 169-174, 1988.

23. LANCELLOTTI, C.L.P. & ROSEMBERG, S. - As complicações neurológicas pelo vírus do sarampo. **Pediatria (S. Paulo), 6**: 10-12, 1984.

24. LEBON, P. - Virus de la rougeole et système nerveux central. **Ann. Pédiat., 34**: 555-559, 1987.

25. LYON, G. - Viral encephalitis in children. **Acta paediat. belg., 34**: 143-150, 1981.

26. MALAMUD, N. - Encephalomyelitis complicating measles. **Arch. Neurol. Psychiat. (Chic.), 38**: 1025-1038, 1937.

27. MALAMUD, N. - Sequelae of postmeasles encephalomyelitis: clinicopathologic study. **Arch. Neurol. Psychiat. (Chic.), 41**: 943-954, 1939.

28. MEHTA, P.D.; PATRICK, B.A.; SOBCZYK, W. et al. - Immunoglobulin G subclass antibodies to measles virus in patients with subacute sclerosing panencephalitis or multiple sclerosis. **J. clin. Microbiol., 27**: 62-65, 1989.

29. MEULEN, V. ter; KATZ, M. & MULLER, D. - Subacute sclerosing panencephalitis: a review. **Curr. Top. Microbiol. Immunol., 57**: 1-38, 1972.

30. MEULEN, V. ter; MULLER, D.; KACKELLI, Y.; KATZ, M. & MYERMANN, R. - Isolation of infectious measles virus in measles encephalitis. **Lancet, 2**: 1172-1175, 1972a.

31. MEULEN, V. ter & HALL, W.W. - Slow virus infections of the nervous system: virological, immunological and pathogenetic considerations. **J. gen. Virol., 41**: 1-25, 1978.

32. MEULEN, V. ter & CARTER, M.J. - Measles virus persistency and disease. **Progr. med. Virol., 30**: 44-61, 1984.

33. MICCO, C. de & TOGA, M. - Le statut immunitaire du systéme nerveux central. **Rev. neurol., 144**: 776-788, 1988.

34. MICHENER, R.C. & HENLEY, W.L. - Focal convulsions associated with subclinical measles infection. **Clin. Pediat. (Phila.), 22**: 643-649, 1983.

35. MILLER, H.G.; STANTON, J.D. & GIBBONS, J.L. - Parainfectious encephalomyelitis and related syndromes. **Quart. J. Med., 25**: 427-505, 1956.

36. MOENCH, T.R.; GRIFFIN, D.E.; OBRIECHT, C.R.; VAISBERG, A.J. & JOHNSON, R.T.- Acute measles in patients with and without neurological involvement: distribution of measles virus antigen and RNA. **J. infect. Dis., 58**: 433-442, 1988.

37. NAKAI, M. & IMAGAWA, D.T. - Electron microscopy of measles virus replication. **J. Virol., 3**: 187-197, 1969.

38. OHUCHI, M.; CHUCHI, R.; MIFUNE, K.; ISHIHARA, T. & OGAWA, T. - Characterization of the measles virus isolated from the brain of a patient with immunosuppressive measles encephalitis. **J. infect. Dis., 156**: 436-441, 1987.

39. OJALA, A. - On changes in the cerebrospinal fluid during measles. **Ann. Med. intern. Fenn., 36**: 321-331, 1947.

40. OYANAGI, S.; MEULEN, V ter; KATZ, M. & KOPROWSKI, H. - Comparison of subacute sclerosing panencephalitis and measles virus: an electron microscope study. **J. Virol., 7**: 176-187, 1971.

41. REINICKE, V.; MORDHORST, C.H. & INGERSLEV, N. - Central nervous system affection in connection with "ordinary" measles. **Scand. J. infect. Dis., 6**: 131-135, 1974.

42. RUSSELL, W.C. - Paramyxovirus and Morbillivirus infections and their relationship to neurological disease. **Progr. Brain Res., 59**: 113-132, 1983.

43. SCHADLICH, H.J.; KARENBERG, A.; NEKIC, M. & FELGENHAUER, K. - Acute encephalitis with intrathecal synthesis of measles antibodies. **J. Neuroimmunol. (Amst.), 20**: 171-172, 1988.

44. SCHADLICH, H.J.; KARENBERG, A.; NEKIC, M. & FELGENHAUER, K. - Acute encephalitis with intrathecal synthesis of measles antibodies. **J. Neurol. (Berl.), 235**: 248-251, 1988a.

45. WECHSLER, S.L. & MEISSNER, H.C. - Measles and SSPE viruses: similarities and differences. **Progr. med. Virol., 28**: 65-95, 1982.

46. WILD, T.F. - Measles virus and chronic infections. **Path. et Biol., 29**: 429-433, 1981.

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