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Case Report

Peripheral neuropathy associated with anti-myelin basic protein antibodies in a woman vaccinated with rubella virus vaccine

Maria Grazia Cusi*[,1], Silvia Bianchi[1], Laura Santini[1], Donatella Donati[1], Marcello Valassina[1], Pier Egisto Valensin[1], Livia Cioé[2] and Riccardo Mazzocchio[3]

[1]*Dipartimento di Biologia Molecolare - Sezione Microbiologia, Università di Siena, Via Laterina, 8, 53100 Siena, Italia;* [2]*Istituto Superiore di Sanità Laboratorio di Virologia, 00161 Roma, Italia and* [3]*Istituto di Neurochirurgia, Dipartimento di Scienze Oftalmologiche e Neurochirurgiche, Università di Siena, 53100 Siena, Italia*

Active immunisation with rubella vaccine has not been commonly associated with neurological complications. We report the case of a 23-year-old woman who developed a mild, distal demyelinating neuropathy after immunisation with the live attenuated RA 27/3 rubella strain. Post-immunisation immunologic studies carried over 24 months showed the presence of antibodies to the RV proteins, particularly to the capsid antigen, and to the myelin basic protein (MBP). A similarity between a C antigen motif and a sequence of the MBP was found by computer analysis. The cross-reactivity was confirmed by immunising mice with a synthetic peptide derived from the MBP, which developed a strong humoral response to RV and MBP. This finding raises the possibility that a virus-induced immune response could lead to an autoaggressive reaction responsible for demyelination.

Keywords: rubella virus; myelin basic protein; mimicry

Introduction

It is generally believed that autoimmune diseases result from a combination of exogenous and endogenous factors. It has been suggested that viruses may act as exogenous factors in triggering autoimmunity (Baum *et al*, 1993; Dong *et al*, 1994; Fujinami and Oldstone, 1985; Tan, 1989; Tardieu *et al*, 1984). Potential severe neurological complications associated with the rubella vaccination have raised some concern (Fenichel, 1982; Frey, 1994; Mühlebach-Sponer *et al*, 1995; Schaffner *et al*, 1974; Tingle *et al*, 1985a). Moreover, the persistence of anti-C antibodies against the live attenuated rubella strain for long periods raises the question of whether the specific antigenic stimulus persists in the vaccinees (Cusi *et al*, 1989, 1993). Recently, we had the opportunity of studying a patient with a transient neuropathic syndrome after the rubella vaccination with the live attenuated RA27/3 virus strain. Symptoms of peripheral nerve involvement, mainly consisting of painful bilateral lower limb weakness developed 4 weeks after vaccination. Abnormal nerve conduction tests suggested a mild, distal demyelinating motor neuropathy (Mazzocchio *et al*, 1997). Myelin basic protein constitutes 30% of the total myelin basic protein in the central nervous system, but it is a lesser constituent of the peripheral nervous system (Kamholz *et al*, 1986). Several works have demonstrated that various viruses share epitopes with specific host antigens and may, under appropriate conditions, elicit autoantibodies; for example, cytomegalovirus, hepatitis B, HIV-1, influenza virus have been shown capable of inducing autoimmune reactions (Laing *et al*, 1989) and, particularly, a cell-mediated immune response against myelin basic protein (MBP) has been evidenced in post-infectious encephalomyelitis (Johnson *et al*, 1984). In an attempt to address the issue of possible mechanisms of viral induced autoimmunity, an experimental approach was undertaken by taking advantage of the restricted specificities that can be obtained immunising animals with synthetic peptides. A human MBP peptide (SGKDSHHPART) matching with a motif (aa. 51–61) of rubella virus capsid protein (C) was synthesised and injected in mice which raised

*Correspondence: Maria Grazia Cusi
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antibodies capable to react with the predetermined sequence of RV and also with the MBP.

Case history

A 23-year-old woman was immunised with live, attenuated rubella virus vaccine (RA 27/3 strain-Gunevax, Sclavo Spa, Siena, Italy). Two weeks later, she presented malaise, fever, rash, enlarged tender lymph nodes, and arthralgia. At the beginning of the fourth week after vaccination, she complained of mild pain behind both knees. Shortly thereafter, pain also occurred in the calves and at the ankles; while usually bilateral, it was often more intense in the left leg. She reported of feeling her legs heavy and weak. These symptoms fluctuated in intensity, being most pronounced on awakening, diminishing during the day and worsening in the evenings. Over the ensuing week, leg pain became so intense as to influence the patient's posture and gait (she could not straighten her legs and walked with her knees slightly bent). She felt fatigued and hardly capable of deambulating. Occasionally, she also experienced wrist pain and hand paresthesias. Her personal history was unremarkable. On clinical examination, although evaluation of strength in the lower limbs was limited by the presence of muscle pain, no gross proximal or distal muscle weakness was apparent. There were no deep tendon abnormalities, sensory loss, or meningeal signs. Electrophysiological tests were performed 1 week and 2 weeks after the onset of leg pain. They demonstrated a mild but clear-cut nerve dysfunction (see Table 1): the tibial and peroneal nerves had prolonged distal motor latencies; the left tibial nerve also had reduced motor conduction. There were no conduction blocks between proximal and distal stimulation sites. F-wave and H-reflex latencies were normal. Sensory findings were normal in the superficial peroneal nerves, while the conduction velocity of the left sural nerve was just below normal values. Median nerve values were also normal. Needle EMG examination was normal. Laboratory tests, including rheumatoid arthritis parameters, were normal. MHC class analysis performed on the patient cells showed a HLA-DR2 type. The patient refused lumbar puncture. After 1 month, symptoms improved spontaneously. Nerve conduction studies also showed improvement (see Table 1) with complete normalisation 2 months later. A subsequent clinical and electrophysiological control was carried out about 11 months later because of the reappearance of pain and fatigue. Electrophysiology again showed increased distal motor latency in the left peroneal

Table 1 Nerve conduction study

		Patient				
		First time		*After 1 month*		
Nerves		*right*	*left*	*right*	*left*	*Control*
Deep peroneal						
DML	ms	5.7	6.66	5.61	5.88	<5.3
CMAP (A)	mV	13.4	13.8	12.5	10.8	>1.7
CMAP (K)	mV	12.5	13.6	12.0	10.5	>1.7
MCV	m/s	50.7	48.6	49.0	45.0	>41.0
F-wave	ms	44.8	46.6	46.2	45.0	<53.0
Tibial						
DML	ms	7.04	7.05	5.13	5.04	<6.6
CMAP (A)	mV	11.3	7.87	8.15	6.08	>1.7
CMAP (K)	mV	10.5	7.22	8.78	5.54	>1.7
MCV	m/s	44.0	35.3	44.2	43.3	>39.5
F-wave	ms	49.6	49.6	47.0	47.0	<54.3
H-reflex	ms	28.5	29.1	28.8	28.8	<33.7
Median						
DML	ms	3.64				<4.0
CMAP (W)	mV	9.45				>7.7
CMAP (E)	mV	9.19				>7.7
MCV	m/s	63.2				>50.0
F-wave	ms	25.1				<30.0
Superficial peroneal						
SCV	m/s	46.0	43.2	49.0	48.8	>42.0
SAP	μV	18.6	17.9	12.5	19.1	>7.2
Sural						
SCV	m/s		40.5		45.5	>41.5
SAP	μV		29.4		15.2	>10.3

DML: distal motor latency; MCV: motor conduction velocity; CMAPa: compound muscle action potential amplitude, (A): ankle; (K): knee; (W): wrist; (E): elbow; SCV: sensory conduction velocity; SAPa: sensory action potential amplitude

nerve and both tibial nerves (not shown). Needle EMG showed a decreased motor unit recruitment in the left tibialis anterior muscle and in the intrinsic muscles of both feet. This time, cerebro-spinal fluid (CFS) analysis was possible. It showed normal cell count, glucose and protein content. Search for anti-RV antibodies was negative. The patient was treated with B1, B6 and B12 vitamins, fully recovering within a month. No clinical abnormality was reported in the course of the second year. No further electrophysiological tests were performed.

Serological studies

Sera samples were drawn before and at 1, 2, 3, 6, 12 and 24 months after vaccination. Serological tests against an extensive range of viral antigens, particularly those associated with the central nervous system (coxsackie, herpes simplex 1, cytomegalo, varicella-zoster, Epstein-Barr, measles and mumps viruses) showed no significant titres. Rubella specific antibodies were assayed by haemagglutination-inhibition (HAI), neutralisation (NT) assays as previously described (Cusi *et al*, 1989) and by ELISA (Behring, L'Aquila, Italy) according to the manufacturer's instructions. Western blot (WB) was carried out using as antigens the RV proteins and the bovine MBP (Sigma, Milan, Italy), which shared 5/10 amino-acids with the C protein in the peptide sequence considered in this study.

Immunisation of mice

Four Balb/c mice (2 months old) were injected intramuscularly four times with 50 μg of synthetic peptide dissolved in 50 μl of saline, previously conjugated with keyol limpet haemocyanin (KLH) (Briand *et al*, 1985). A week after the last immunisation the animals were sacrificed and the sera were tested for immunological analysis.

Western blot

MBP (8 μg/well) or RV proteins (10 μg/well) separated onto a 12% SDS–PAGE were transferred to nitrocellulose paper as reported by Towbin *et al* (1979). Strips were incubated at room temperature with the patient sera diluted 1:200 or immunised mice sera diluted 1:50 in PBS per 2 h. After washing with PBS 2% bovine serum albumin (BSA), 0.5% Nonidet P-40, peroxidase conjugated goat anti-human or rabbit anti-mouse Ig (Biorad, Milan, Italy) were added. After 1 h of incubation, the strips were washed as above and 4 Cl-1-naphtole was added. Strips were then photographed and pictures of the strips were analysed with an instant imager (Adobe Photoshop Program, by Adobe, USA) in order to give the density value of the bands.

ELISA

One hundred μl/well of conjugated peptide (2 μg/ml), or MBP or RV proteins (1 μg/ml) were adsorbed on a 96 well microplate overnight at 4°C. After 1 h saturation with PBS 2% BSA, dilutions of the immunised mice sera (from 1:100 to 1:6200) or the patient IgG (from 20 μg/ml to 0.3 μg/ml) purified by affinity chromatography (Trap GII Kit by Pharmacia, Milan, Italy) were incubated 1 h at 37°C. After a washing with PBS containing 0.5% NP-40 and 1% BSA, the plates were incubated 1 h at 37°C with peroxidase conjugated rabbit anti-mouse or goat anti-human IgG (Biorad, Milan, Italy). After washings, the substrate (3,3′ 5,5′ tetramethylbenzidine from Sigma, Milan, Italy) was added and the plates read at 450 nm (Behring, L'Aquila, Italy). Negative controls were included in each test.

Post-immunisation immunologic studies

Studies performed on preimmunised serum of the patient did not reveal the presence of antibody to the RV by HAI, NT and WB. After immunisation with the RA27/3 rubella virus strain, the patient showed seroconversion by HAI and ELISA tests for antibody to RV, although a decline in HAI antibody titre over the next 3 months was noticed (Table 2). IgM were absent in all the serum samples. Neutralising antibodies were detected only in the serum samples drawn at 3, 6 and 12 months after immunisation. WB performed with the rubella structural proteins, revealed a strong reaction to the C antigen and a weak reaction to the E1 and E2 proteins in all the sera but the last one, which showed only the presence of the anti-C antibodies. Since it has been described that anti-MBP antibodies can be induced after viral infections (Fujinami and Oldstone, 1985; Johnson, 1982), the patient serum samples drawn before and after

Table 2 Immunologic response before and after immunisation

Sample	*ELISA* (IU/ml)	*HAI*	*NT*	*WB* *RV*	*MBP*
Prevaccination	neg	neg	neg	neg	neg
Postvaccination					
One month	45	1:256	neg	pos	pos
Two months	36	1:512	neg	pos	pos
Three months	57	1:128	1:32	pos	pos
Six months	52	1:256	1:64	pos	pos
One year	43	1:128	1:16	pos	pos
Two years	40	1:64	<1:4	pos[a]	pos

[a]positive only to the rubella virus C protein

vaccination were tested against the MBP by WB, in order to reveal the reactivity. All samples, except for the serum drawn before vaccination, resulted positive (Figure 1). As controls, sera from healthy subjects obtained 1 month after RA27/3 vaccination always resulted totally negative (data not shown). The anti-MBP response did not show a decline during the 2 years after vaccination, in fact, the intensity of the bands revealed in WB and analysed at the computer, resulted similar in all the strips tested with the serum samples drawn at different times after immunisation (Figure 1). The cross-reaction of the patient sera to the MBP and the synthetic peptide was also evidenced in ELISA. The results are shown in Figure 2.

Attempts to isolate infectious virus from peripheral mononuclear cells or to detect virus genome by RT–PCR (using the sense primer 5′TGCTTTG-CCCCATGGGACCTCGAG 3′ nt 8359-8382 and the antisense primer 5′ GGCGAACACGCTCATCA-CGGT 3′ nt 8679-8659 from Genset, Paris, France) were unsuccessful.

Production of cross-reactive antibodies in mice immunised with an MBP peptide

Using computer-assisted analysis (Chou and Fasman, 1974; Hopp and Woods, 1981), the sequence of the RV C protein was compared with the human MBP sequence and immunological studies were carried out to look for the cross-reactivity. A peptide derived from the MBP sequence (SGKDSHHPART aa. 55-65) and sharing a homology (7/10 amino-acids) with the RV C protein (aa. 51-61) was synthesised (Eurosequence, The Netherlands) and used as antigen to immunise mice.

Sera of the immunised mice were screened by ELISA and WB against the RV and the MBP. Negative controls represented by samples obtained from mice immunised with the KLH were included in each test. The results are shown in Figure 3. We used as antigen both the conjugated and unconjugated peptide, but the results were similar. The sera immunised with the peptide reacted strongly both with RV and MBP; the cross-reaction was also evidenced by WB which showed a dark band corresponding to the MBP and a weak band corresponding to the C protein of the RV (data not shown).

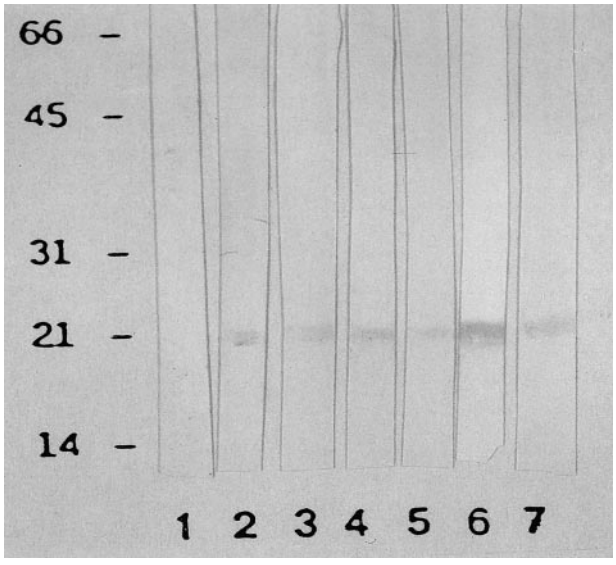

Figure 1 Western blot (WB) analysis of myelin basic protein (MBP) specific antibodies with serum samples drawn from the patient with neurological symptoms before vaccination (lane 1) and, one (lane 2), two (lane 3), three (lane 4), six (lane 5), 12 (lane 6) and 24 months (lane 7) after immunisation. The density of the band of each strip evaluated as described in 'Case history', was respectively 3,78, 2,3, 4,6, 4,8, 7,8 and 5,5 from lane 2–7. Molecular weight standards are shown on the left (Biorad, Milan, Italy).

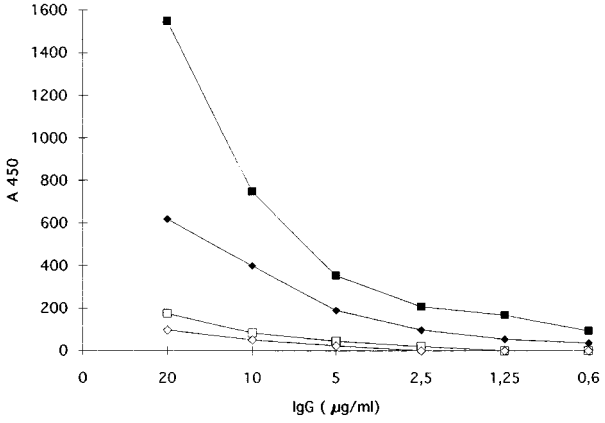

Figure 2 Human purified IgG were assayed for binding to MBP (◆) and peptide (■) by ELISA. Full symbols represent the last serum sample of the patient, open symbols represent the serum of a healthy subject vaccinated with the RA27/3 strain.

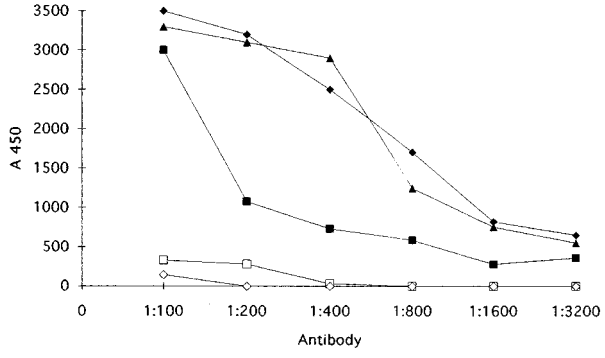

Figure 3 Mice antibodies were assayed for binding to RV (■), MBP (▲), and peptide (◆) by ELISA. Full symbols represent the positive serum, open symbols represent the negative serum.

Discussion

Neuropathy may appear after natural rubella, while the exanthem is still evident, coinciding closely with the development of acute arthropathy (Ford *et al*, 1992; Howson *et al*, 1991; Tingle *et al*, 1985b, 1997). However, in the present case, the onset of pain, the most prominent and perhaps unique neuropathy symptom, occurred after the rash period in the absence of clinical and laboratory evidence of joint inflammation (Mazzocchio *et al*, 1997). The more consistent type of electrophysiological alteration was local slowing of motor conduction in the more distal segments of leg nerves, suggesting the presence of a focal demyelinating motor neuropathy, such as observed in mild cases of Guillain-Barré syndrome (Brown, 1993). Pain syndromes with abnormal nerve conduction responses suggesting a neuropathy have been described in children following immunisation with rubella vaccines no longer in use (Schaffner *et al*, 1974; Holt *et al*, 1976). In these studies, however, no electrodiagnostic abnormalities were found in lower limb nerves nor could the pathogenesis of neuropathy be elucidated.

Screening tests for RV specific antibodies showed low to moderate levels of antibodies in all the sera tested. Antibody responses assessed by immunoblotting and directed to each of the three major structural proteins of rubella virus, E1, E2 and C, revealed the presence of IgG against the C antigen in all the patient samples and the lack of antibodies against the E1 protein, which contains the neutralising epitopes (Cusi *et al*, 1993), in the last serum drawn 2 years after vaccination. When patient sera obtained after vaccination were tested in WB against the structural rubella virus proteins, anti-C antibodies were detected in all samples, while anti-E1 and anti-E2 antibodies were absent in the serum, 2 years post-immunisation. Serological findings were indicative of a depressed response to rubella virus compared with the responses from controls who had no complications following vaccination with the same rubella strain. Moreover, they could be further differentiated for the appearance of specific anti-MBP antibodies soon after the vaccination. Reactivity to MBP has predominantly been observed in subjects carrying HLA-DR2 (Wucherpfennig and Strominger, 1995), as in our case. Demyelination mechanisms include a direct cytolytic effect on Schwann cells with subsequent myelin breakdown or immune pathological phenomena induced by the virus or immune responses against infected myelin cells (Chantler *et al*, 1995; Kristensson and Norrby, 1986; Theofilopoulos, 1995; Wucherpfennig and Strominger, 1995). Demyelination is not a common feature in rubella infection of the central nervous system but has been described in acute infectious encephalomyelitis (Johnson *et al*, 1984) and in progressive rubella panencephalitis (Wolinsky *et al*, 1982). Although the presence of such antibodies does not imply their pathogenic role (Burns *et al*, 1983), the finding of molecular mimicry between the capsid protein of rubella virus and a sequence of the MBP opens up the possibility that a cross-reactive immune response might trigger autoimmune demyelination. We cannot explain why this woman developed antibodies to the MBP which is constitutively expressed mainly in the central nervous system and at low level in the peripheral nervous system, but we can speculate that some events like a viral infection may also act indirectly by eliciting autoimmune responses. T cells reactive to MBP epitopes (Burns *et al*, 1983) presumably escape thymic clonal deletion and may remain in the periphery in a resting state. Under certain conditions (e.g. viral infection) these cells may become activated and cause disease. One mechanism could account for the activation and clonal expansion of autoreactive T cells by a viral peptide having similarity with an immunodominant self peptide (Baum *et al*, 1993; Gianani and Sarvetnick, 1996; Oldstone, 1987). We would thus propose that this case findings further support the arguments in favour of a causal relation between rubella immunisation and neuropathy. Evidence for biologic plausibility consists of the observation that neuropathies and other related neurologic conditions can occur, though uncommonly, after natural infection with the wild-type rubella virus (Howson *et al*, 1991). Thus, the elimination of proven mimicry epitopes of viral vaccines by genetic modifications could make viral vaccines safer and reduce the frequency of postvaccinal neuropathies.

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References

Baum H, Butler P, Davies H, Sternberg MJE, Burroughs AK (1993). Autoimmune disease and molecular mimicry: an hypothesis. *TIBS* **18:** 141–144.

Briand JP, Muller S, Van Regenmortel MHS (1985). Synthetic peptide as antigens: pitfalls of conjugation methods. *J Immunol Meth* **78:** 58–69.

Brown WF (1993). Acute and chronic inflammatory demyelinating neuropathies. In: *Clinical Electromyography*. Brown WF and Bolton CF (eds). Butterworth-Heinemann, Boston, pp 535–559.

Burns JB, Rosenzweig A, Zweiman B, Lisak RP (1983). Isolation of myelin basic protein-reactive T-cell lines from normal human blood. *Cell Immunol* **81:** 435–440.

Chantler JK, Smyrnis L, Tai G (1995). Selective infection of astrocytes in human glial cell cultures by rubella virus. *Lab Invest* **72:** 334–340.

Chou PY, Fasman CD (1974). Prediction of protein conformation. *Biochemistry* **13:** 222–245.

Cusi MG, Cellesi C, Valensin PE (1993). Possibility of reinfection after immunisation with RA27/3 live attenuated virus vaccines. *Arch Virol* **129:** 337–340.

Cusi MG, Metelli R, Valensin PE (1989). Immune responses to wild and vaccine rubella viruses after vaccination. *Arch Virol* **106:** 63–72.

Dong X, Hamilton KJ, Satoh M, Wang J, Reeves WH (1994). Initiation of autoimmunity of the p53 tumor suppressor protein by complexes of p53 and SV40 large antigen. *J Exp Med* **179:** 1243–1252.

Fenichel GM (1982). Neurological complication of immunization. *Ann Neurol* **12:** 119–128.

Ford DK, Tingle AJ, Schulzer M (1992). Sequential follow up observations of a patient with rubella associated persistent arthritis. *Ann Rheum Dis* **51:** 407–410.

Frey T (1994). Report of an International Meeting of Rubella Vaccines and Vaccination, 9 August 1993, Glasgow, United Kingdom. *J Infect Dis* **170:** 507–509.

Fujinami RS, Oldstone MBA (1985). Amino Acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* **230:** 1043–1045.

Gianani R, Sarvetnick N (1996). Viruses, cytokines, antigens, and autoimmunity. *Proc Natl Acad Sci USA* **93:** 2257–2259.

Holt S, Hudgins D, Krishnan KR, Critchley EMR (1976). Diffuse myelitis associated with rubella vaccination. *Brit Med J* **2:** 1037–1038.

Hopp TP, Woods KR (1981). Prediction of protein antigen determinants from amino acid sequences. *Proc Natl Acad Sci USA* **78:** 3824–3838.

Howson CP, Howe CJ and Fineberg HV (1991). Evidence concerning rubella vaccines and arthritis, radiculoneuritis and thrombocytopenic purpura. In: *Adverse effects of pertussis and rubella vaccines: a report of the Committee to Review the Adverse Consequences of Pertussis and Rubella Vaccines*. Division of health promotion and disease prevention, Institute of Medicine, National Academy Press, Washington, DC, pp 187–205.

Johnson RT (1982). *Viral infections of the nervous system*. Raven Press: New York, pp 180–187.

Johnson RT, Griffin DE, Hirsch RL, Wolinsky JS, Roedenbeck S, Imelda De Soriano L, Vaisberg A (1984). Measles encephalomyelitis-clinical and immunologic studies. *New Engl J Med* **310:** 137–141.

Kamholz J, De Ferra F, Puckett C, Lazzarini R (1986). Identification of the three forms of human myelin basic protein by cDNA cloning. *Proc Natl Acad Sci USA* **83:** 4962–4966.

Kristensson K, Norrby E (1986). Persistence of RNA viruses in the central nervous system. *Ann Rev Microbiol* **40:** 159–184.

Laing P, Knight JG, Harris AG, Oxford JS, Webster RG, Markwell M, Paul SM, Pert CB (1989). Influenza viruses induce autoantibodies to a brain-specific 37-kDa protein in rabbit. *Proc Natl Acad Sci USA* **86:** 1998–2002.

Mühlebach-Sponer M, Zbinden R, da Silva VA, Gnehm HE (1995). Intrathecal rubella antibodies in an adolescent with Guillain-Barré syndrome after mumps-measles-rubella vaccination. *Eur J Pediatr* **154:** 166.

Mazzocchio R, Donati D, Valensin PE, Mariottini A, Cusi MG (1997). Transient peripheral neuropathy following rubella vaccination. *J Peripheral Nervous System* **2:** 96–97.

Oldstone MBA (1987). Molecular mimicry and autoimmune disease. *Cell* **50:** 819–820.

Schaffner W, Fleet WF, Kilroy AW, Lefkowitz LB, Herrmanr KL, Thompson J, Karzon DT (1974). Polyneuropathy following rubella immunization. *Am J Dis Child* **127:** 684–688.

Tan EM (1989). Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* **44:** 93–151.

Tardieu M, Powers ML, Hafler DA, Hauser SL, Weiner HL (1984). Autoimmunity following viral infection: demonstration of monoclonal antibodies against normal tissue following infection of mice with retrovirus and demonstration of shared antigenicity between virus and lymphocytes. *Eur J Immunol* **14:** 561–565.

Theofilopoulos AN (1995). The basis of autoimmunity: part I – mechanisms of aberrant self-recognition. *Immunol Today* **16:** 90–98.

Tingle AJ, Chantler JK, Kettyls GD, Larke RPB, Schulzer M (1985a). Failed rubella immunization in adults: association with immunologic and virological abnormalities. *J Infect Dis* **151:** 330–336.

Tingle AJ, Chantler JK, Pot HK, Paty DW, Ford DK (1985b). Postpartun rubella immunization: association with development of prolonged arthritis, neurological sequelae, and chronic rubella viremia. *J Infec Dis* **152:** 606–612.

Tingle AJ, Mitchell LA, Grace M, Middleton P, Mathias R, MacWilliam L (1997). Randomised double-blind placebo-controlled study on adverse effects of rubella immunisation in seronegative women. *Lancet* **349:** 1277–1281.

Towbin H, Staehelin T, Gordon J (1979). Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some application. *Proc Natl Acad Sci USA* **76:** 4350–4356.

Wolinsky JS, Waxham MN, Hess J, Townsend J, Baringer J (1982). Immunochemical features of a case of progressive rubella panencephalitis. *Clin Exp Immunol* **48:** 359–366.

Wucherpfennig KW and Strominger JL (1995). Molecular mimicry in T-cell mediated autoimmunity: viral peptides activate human T-cell clones specific for myelin basic protein. *Cell* **80:** 695–705.