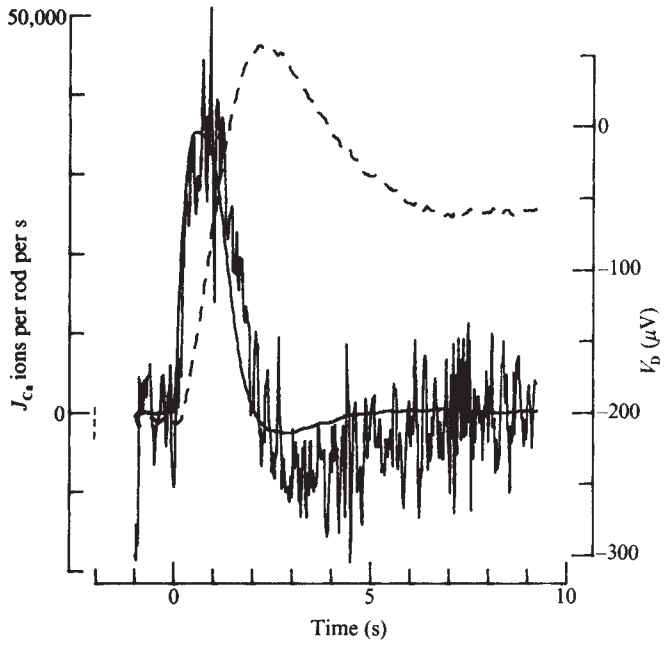

Fig. 4 Time courses of the light-induced Ca^{2+} flux from the rod layer compared with the light-induced reduction in V_d. Stimuli as in Fig. 2. Smooth line: V_d response convolved with an exponential response function of time-constant 130 ms to simulate the time-lag of the Ca electrode. Noisy curve: Ca^{2+} flux from the rods computed from equation 4. Dashes: time integral of Ca^{2+} flux in units of ions per rod on the left-hand scale. Mean of four responses to flashes of energy 110 photons absorbed per rod. The time course of the Ca efflux and the change in V_d are similar. Apparently about half of the Ca^{2+} released within 2 s of the flash is reaccumulated by the rod layer.

side of equation (4) by numerical differentiation of the experimental Ca transient yields the noisy curve in Fig. 4.

The Ca flux can now be directly compared with the waveform of the V_d response if the latter is first convolved with a truncated exponential with a 130-ms time constant to simulate the response lag of the Ca electrode. The result is a smooth line, shown in Fig. 4. The two waveforms are nearly identical, the duration of the Ca efflux being slightly longer and its peak not more than 100 ms later than the transformed V_d response.

The Ca^{2+} flux is large and biphasic. As much as half of the 45,000 ions leaving the outer segments in the first 2 s after the flash are apparently reabsorbed in the next 7 s (Fig. 4, dashed line). Since each rod absorbed 110 photons per flash, about 400 Ca^{2+} ions must have been extruded per photon response. If Ca^{2+} is the internal excitatory transmitter, the total Ca release must have been at least twice as large, because only cytoplasmic Ca^{2+} should reduce the Na^+ permeability of the plasma membrane. The observed release would only increase the cytoplasmic $[Ca^{2+}]$ by about 3 μM, a small fraction of the total Ca^{2+} in rod outer segments[14,25]. But if a steady 25-s light stimulus of intensity 9,500 photons per rod per s is used instead of a flash, the Ca^{2+} outflux is sustained for the entire period of illumination and amounts to about 100 μmol l^{-1} of rod water. The light-sensitive Ca store is therefore large.

The light-induced Ca^{2+} effluxes seen here are fast enough, large enough, and of the spatial distribution to be expected from the Ca hypothesis. Moreover, the observed fast exchange of Ca^{2+} across the plasma membrane of rod outer segments explains the great sensitivity of the dark current to the $[Ca^{2+}]$ in the external medium. It remains to link the Ca^{2+} fluxes to enzymatic events in the disk membranes, such as the hydrolysis of GTP[26–29] and of cyclic GMP[30], and the binding of GDP[29].

Note added in proof: Since this paper was submitted, a preliminary account has appeared in *Fedn Proc.* **39,** 2066 (1980) and similar conclusions are reached by use of surface calcium electrodes by G. H. Gold and J. Korenbrot, *Fedn Proc.* **39,** 1814 (1980).

Received 12 December 1979; accepted 12 May 1980.

1. Hagins, W. A., Penn, R. D. & Yoshikami, S. *Biophys. J.* **10,** 380–412 (1970).
2. Penn, R. D. & Hagins, W. A. *Biophys. J.* **12,** 1073–1094 (1972).
3. Cohen, A. I. *Vision Res.* **10,** 445–453 (1970).
4. Rüppel, H. & Hagins, W. A. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 257 (1973).
5. Yoshikami, S., Robinson, W. E. & Hagins, W. A. *Science* **185,** 1176–1179 (1974).
6. Baylor, D. A. & Fuortes, M. G. F. *J. Physiol., Lond.* **207,** 77–92 (1970).
7. Yoshikami, S. & Hagins, W. A. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 245 (1973).
8. Hagins, W. A. & Yoshikami, S. *Expl Eye Res.* **18,** 229–305 (1974).
9. Pinto, L. H., Brown, J. E. & Coles, J. A. in *Vertebrate Photoreception* (eds Fatt, P. & Barlow, H.) 159 (1977).
10. Hagins, W. A. & Yoshikami, S. in *Vertebrate Photoreceptor* (eds Fatt, P. & Barlow, H.) 97 (1977).
11. Liebman, P. A. *Invest. Ophthal.* **13,** 700–702 (1974).
12. Hendricks, T., Daeman, F. J. M. & Bonting, S. L. *Biochim. biophys. Acta* **345,** 468–473 (1974).
13. Szuts, E. & Cone, R. A. *Fedn Proc.* **33,** 1471 (1974).
14. Szuts, E. & Cone, R. A. *Biochim. biophys. Acta* **468,** 194–208 (1977).
15. Hendricks, T., van Haard, P. M. M., Daeman, F. J. M. & Bonting, S. L. *Biochim. biophys. Acta* **467,** 175–184 (1977).
16. Kaupp, U. B. & Junge, W. *FEBS Lett.* **81,** 229–232 (1977).
17. Baylor, D. A., Lamb, T. D. & Yau, K.-W. *J. Physiol., Lond.* **288,** 589–611 (1979).
18. Heinemann, U., Lux, H. D. & Gutnick, M. J. *Expl Brain Res.* **27,** 237–243 (1977).
19. Simon, W., Ammann, D., Oehme, M. & Morf, W. E. *Ann. N. Y. Acad. Sci.* **307,** 52–70 (1978).
20. Reuter, H. & Seitz, N. *J. Physiol., Lond.* **195,** 451–470 (1968).
21. Mullins, L. J. *J. gen. Physiol.* **70,** 681–695 (1977).
22. Blaustein, M. P., Ratzlaff, R. W. & Kendrick, N. K. *Ann. N. Y. Acad. Sci.* **307,** 195–212 (1978).
23. Baker, P. F. *Ann. N. Y. Acad. Sci.* **307,** 250–268 (1978).
24. Carslaw, H. S. & Jaeger, J. C. *Conduction of Heat in Solids* 2nd edn (Oxford University Press, London, 1959).
25. Hagins, W. A. & Yoshikami, S. *Ann. N.Y. Acad. Sci.* **264,** 314–325 (1975).
26. Robinson, W. E. & Hagins, W. A. *Biophys. J.* **16a** (1977).
27. Robinson, W. E. & Hagins, W. A. *Photochem. Photobiol.* **29,** 693 (1979).
28. Wheeler, G., Matsuo, Y. & Bitensky, M. W. *Nature* **269,** 822–824 (1977).
29. Robinson, W. E. & Hagins, W. A. *Nature* **280,** 398–400 (1979).
30. Yee, R. & Liebman, P. A. *J. biol. Chem.* **253,** 7902–7909 (1978).

Co-infection by lactic dehydrogenase virus and C-type retrovirus elicits neurological disease

Larry R. Pease & William H. Murphy

Department of Microbiology and Immunology, The University of Michigan Medical School, Ann Arbor, Michigan 48109

Intraperitoneal injection of neuropathogenic strains of lactic dehydrogenase virus (LDV)[1–3] causes a histologically distinctive[4] fatal paralytic disease characterized by an inflammatory destruction of motor neurones in the brain stem and cord in C58 mice aged over 9 months. To elicit the disease in the naturally susceptible C58 strain requires an age-associated[5] or X-ray induced[6] loss of immunological competence[5], LDV infection[1–3] and genetic susceptibility[1,6]. Genetic studies[1,7] of the common inbred mouse strains showed that susceptibility to the disease was not linked to the major histocompatibility complex but correlated with the FV-1^n allele, susceptibility to spontaneous leukaemia, and infection by neuropathogenic strains of LDV[1–3]. These observations suggested that neuropathogenic strains of LDV elicit the disease only in those strains of mice that carry multiple copies of N-tropic C-type retroviruses in their genomes[8,9] and that are permissive for retrovirus replication. Presumably the expression of these viral genomes (high titres of virus in tissues correlating with age[9]) is the important factor. Here we present genetic evidence to support this hypothesis and briefly discuss the possible implications.

In Table 1 the mice in group 1 were selected to test whether there was a correlation between susceptibility to the disease, the number of copies of N-tropic C-type retroviruses in their genomes[8,9], their haplotype and their FV-1 genotype. The FV-1^b allele[10] dominantly restricts N-tropic C-type retrovirus replication. All of the strains (*a–e*) that were FV-1^n and are known to contain multiple copies of N-tropic C-type retroviruses in their genomes[8,9] were susceptible. The data for the congeneic AKR strains are of interest because they show that

0028-0836/80/300398—03$01.00

Table 1 Susceptibility of inbred mice to paralytic disease

Group			H-2 type	FV-1 type	Genomic copies of C-type retroviruses	Incidence of paralysis† age (months)		
						6	9	12
Inbred strains*								
	a	C58/J	k	n	Many		10/10	15/15
	b	AKR/Boy	k	n	Many		13/20	
	c	AKR-H-2^b/Boy	b	n	Many	0/17		5/6
	d	C3H/Fg Boy	k	n	Many			16/16
	e	PL/J	u	n	Many			6/11
	f	AKR-FV-1^b/Boy	k	b	Many		0/24	
1	*g*	C3H/HeJ	k	n	Few	0/8		0/12
	h	DBA/2J	d	n	Few	0/13		0/14
	i	B6/Boy	b	b	Few		0/13	
	j	B10	b	b	Few	0/19		0/10
	k	B6-FV-1^n/Boy	b	n	Few		0/12	
	l	B10.BR	k	b	Few	0/18		
F_1 hybrids								
	a	C58/wm × C3H/HeJ	k/k	n/n		2/8	7/12	
	b	C58/wm × DBA/2J	k/d	n/n		2/8	5/8	
2	*c*	C58/wm × B10	k/b	n/b		0/26		0/10
	d	C58/wm × B10.BR	k/k	n/b		0/20		0/14
	e	C58/wm × BALB/cWm	k/d	n/b		0/11		0/19
Backcross progeny								
	a	(C58 × B10) × B10	k/b or b/b	n/b or b/b		0/43		
3	*b*	C58 × (C58 × B10)	k/k or k/b	n/n or n/b		18/53		6/12
	c	(C58 × B10) × C58	k/k or k/b	n/n or n/b		1/93		1/20

* In group 1, strains *a–c* are considered to have a 'high' incidence of leukaemia ranging from 40 to 90% depending on the colony. Strains *d* and *e* have a 'moderate' incidence of leukaemia ranging from 20 to 40%. The remaining mice listed have a 'low' incidence of spontaneous leukaemia (usually < 5%)[16]. AKR-H-2^b/Boy is congeneic to AKR/Boy and is H-2^b rather than H-2^k. AKR-FV-1^b/Boy is congeneic to AKR/Boy but is FV-1^b rather than FV-1^n. B6/Boy is a subline of C57BL/6 and is FV-1^b. The congeneic line B6-FV-1^n/Boy is FV-1^n. The C58/wm and BALB/cWm mice are our stock inbred lines. The following abbreviations were used: C58 for C58/wm, B10 for C57BL/10Sn, and B10.BR for B10.BR/SgSn. In Groups 2 and 3 the female is listed first for each breeding set; mice were 2–5 months old when mated.

† Mice received 550R of whole body X-ray irradiation 24 h before the intraperitoneal injection of LDV (10^7–10^8 infectious doses) derived from either line *1b* cells or from a stock prepared from the plasma of infected BALB/cWm mice[2]. The plasma stocks of LDV cause the same clinical and histopathological effects as line *1b* derived virus[2]. After infection, mice were held for 30 days and scored for the incidence of paralysis. Paralysed and disease-free mice (3–6 per group) were killed and the diagnosis of paralysis or its absence confirmed histologically as described previously[4]. The sex of mice did not affect their susceptibility in any of the test groups.

when H-2^b was substituted for H-2^k, mice were still susceptible (compare *c* with *b*). When FV-1^b was substituted for FV-1^n, mice were resistant (compare *f* with *b*). The mice in groups *g* and *h* were representative of strains that carry few copies of retroviruses in their genomes but are permissive (FV-1^n) for retrovirus replication. None contracted the disease. The strains in groups *i* and *j* contain few copies of retroviruses in their genomes, are not permissive (FV-$1^{b/b}$) for N-tropic C-type retrovirus replication, and were not susceptible. Introduction of the FV-1^n allele in place of FV-1^b (compare *k* with *i*) or H-2^k in place of H-2^b (compare *l* with *j*), did not confer susceptibility. These results confirmed our findings[1,7] that susceptibility or resistance was not H-2 linked and indicated that mice had to carry multiple copies of N-tropic C-type retroviruses in their genomes and be permissive for retrovirus replication to be susceptible.

The mice in group 2 were used to test whether mice presumed to be genetically susceptible, but containing too few copies of N-tropic C-type retroviruses in their genomes (mice in *g* and *h*, Group 1), would manifest paralytic LDV infection if they became infected by the appropriate C-type retrovirus. Thus, 'genetically susceptible' male mice 'deficient' in virus (C3H/HeJ and DBA/2J) were bred to C58 females. The assumption was that the C58 mothers would transmit virus to the newborn F_1 progeny either chromosomally or by the milk. Table 1 shows that the F_1 hybrids derived from the 'potentially susceptible strains' (groups *a* and *b*) were susceptible. The corresponding genetically resistant (FV-$1^{n/b}$) hybrids were not (groups *c–e*) even when the H-2^k haplotype (group *d*) was substituted for H-2^b (group *c*).

In group 3, test-cross progeny were tested to determine whether resistance segregated in accordance with the hypothesis that the FV-1^b allele determined resistance. The data show that all of the backcross progeny in control group *a*, which in principle inherited an FV-1^b allele, were resistant. In theory, 50% of the test-cross progeny in group *b* should have been FV-$1^{n/n}$ and susceptible if they were infected by an N-tropic C-type retrovirus by their mothers. The results confirmed this expectation with a somewhat lower incidence of disease (34%) in the incompletely susceptible 6-month-old indicator C58 mice[1,6,7]. Moreover, when backcross progeny were nursed on resistant (C58 × B10) mothers (group *c*) there was a strong maternal effect. Such an effect would be consistent with the failure to transmit virus to the progeny by the milk[11], or the transmission of antibody to the progeny by the milk, thereby protecting them from C-type retrovirus infection. Other mechanisms of conferring maternal resistance are not ruled out by the data[11].

If one accepts the genetic data at their face value they support the hypothesis that dual infection by LDV and N-tropic C-type retrovirus(es) was required to elicit a specific paralytic motor neurone disease in genetically susceptible mice. Expression of N-tropic C-type retrovirus genomes in FV-$1^{n/n}$ mice seemed to be an important factor in the susceptible strains. The paradox is that neither LDV[12] or the common C-type retroviruses are frankly cytopathic to cells either *in vitro* or *in vivo*. Although Gardner *et al.*[13] and McCarter *et al.*[14] have reported the occurrence of neuropathogenic C-type viruses in mice, LDV has not been evaluated as an essential contributory factor in such virus stocks. However, these investigators also have not claimed that neurone destruction is a direct cytopathic response to viral infection. The striking feature of the model we describe is that two viruses that are ubiquitous in mice, and that ordinarily do not cause overt disease, seem to act together to elicit a specific disease syndrome in a genetically susceptible host. These findings are of interest because they direct attention to how unrelated viruses may interact together at the cellular level, provide an additional way to characterize phenotypically LDV

and mouse leukaemia virus strains, and may provide an additional insight to the complex interactions between viruses and their hosts in the slow viral infections of the nervous system[15].

This work was supported by funds from the Institute of Gerontology of the University of Michigan and by USPHS grant S07-RR-05383. All the 'Boy' mouse strains were from Dr Edward Boyse. The mice with a J designation and B10 and B10.BR lines were from Jackson Memorial Laboratory.

Received 18 February; accepted 27 May 1980.

1. Murphy, W. H., Nawrocki, J. F. & Pease, L. R. in *Animal Models of Neurologic Disease* (eds Behan, P. O. & Rose, C. F.) (Pitman, London, in the press).
2. Nawrocki, J. F., Pease, L. R. & Murphy, W. H. *Virology* **103**, 259–264 (1980).
3. Martinez, D., Brinton, M. A., Tachovsky, T. G. & Phelps, A. H. *Infect. Immun.* **27**, 979–987 (1980).
4. Lawton, J. W. M. & Murphy, W. H. *Archs Neurol.* **28**, 367–370 (1973).
5. Murphy, W. H., Tam, M. R., Lanzi, R. L., Abell, M. R. & Kauffman, C. *Cancer Res.* **30**, 1612–1622 (1970).
6. Duffey, P. S., Martinez, D., Abrams, G. D. & Murphy, W. H. *J. Immun.* **116**, 475–481 (1976).
7. Pease, L. R., Abrams, G. D. & Murphy, W. H. *J. Immun.* (submitted).
8. Lowy, D. R., Chattopadhyay, S. K., Teich, N. M., Rowe, W. P. & Levine, A. S. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3555–3559 (1974).
9. Chattopadhyay, S. K., Lowy, D. R., Teich, N. M., Levine, A. S. & Rowe, W. P. *Cold. Spring Harb. Symp. quant. Biol.* **39**, 1085–1101 (1974).
10. Lilly, F., Duran-Reynals, M. L. & Rowe, W. P. *J. exp. Med.* **141**, 882–889 (1975).
11. Melief, C. J. M., Louie, S. & Schwartz, R. S. *J. natn. Cancer Inst.* **55**, 691–698 (1975).
12. Rowson, K. E. K. & Mahy, B. W. J. *Virol. Monogr.* **13**, 57–87 (1975).
13. Gardner, M. R. *et al.* in *Amotrophic Lateral Sclerosis* (eds Adams, J. M., Johnson, R. T. & Brazier, M. A. B.) 217–234 (1976).
14. McCarter, J. A., Ball, J. K. & Frei, J. V. *J. natn. Cancer Inst.* **59**, 179–183 (1977).
15. TerMeulen, V. & Hall, W. W. *J. gen. Virol.* **41**, 1–25 (1978).
16. Staats, J. *Cancer Res.* **36**, 4333–4377 (1976).

S100 protein is present in cultured human malignant melanomas

Richard Gaynor, Reiko Irie, Donald Morton & Harvey R. Herschman

Division of Hematology-Oncology, Department of Medicine; Division of Oncology, Department of Surgery; Department of Biological Chemistry and Laboratory of Nuclear Medicine and Radiation Biology, Center for the Health Sciences; University of California, Los Angeles 90024

S100 protein, so called because of its solubility in 100% saturated ammonium sulphate[1], is an acidic cytoplasmic protein specific for the nervous system[2,3]. It is localized primarily to glial elements of the brain, Schwann cells in the peripheral nervous system and satellite cells in sympathetic ganglia[4,5]. This brain-specific protein shows a close immunological relationship among a wide variety of vertebrates, as measured by a quantitative complement fixation technique[6]. Although strict serological conservation and tissue localization have been maintained among different species, no function for S100 protein has been determined. However, the appearance of S100 protein in brain is correlated with maturation of the nervous system in both rat and man[7,8]. Previous *in vitro* studies on the production and regulation of S100 protein in the C6 cell line derived from a rat astrocytoma indicated that cell contact and cessation of division induced S100 protein[9]. Neuroblastoma cell lines lack this protein[10,11] although it is found in other astrocyte cell lines. We are unaware of any reports of cell lines, other than those of glial origin, which produce S100 protein. Melanocytes are derived from neuroectodermal elements, and this common origin of glial cells and melanocytes prompted us to study whether S100 protein might be present in cell lines from human malignant melanomas. We now report the presence of S100 protein in five of seven continuous cell lines of human malignant melanoma.

All lines used in our studies were derived from metastatic melanomas, and were maintained in culture for 12–20 passages. Most lines have been characterized previously[12,13]. After the melanoma cells in tissue culture flasks were confluent, the cells were collected and homogenized. The soluble protein was assayed by quantitative complement fixation for S100 protein, using rabbit antisera to bovine S100. Complement fixation curves for extracts of human brain and one of the melanoma cell lines are shown in Fig. 1. Five of seven cell lines from human melanomas were found to have S100 protein (Table 1). In two of the lines (lines 19 and 12) the amount of S100 protein exceeded that present in human brain medulla. Lymphocyte cell lines of the two patients from which M14 and M29 were derived were negative for S100, as were HeLa cells and a human sarcoma cell line. Cells from line M14 were injected into a nude mouse; the tumour resulting after 30 days had 2.2 μg of S100 per g of tumour. Of the lines studied, only M12 and M19 were melano-

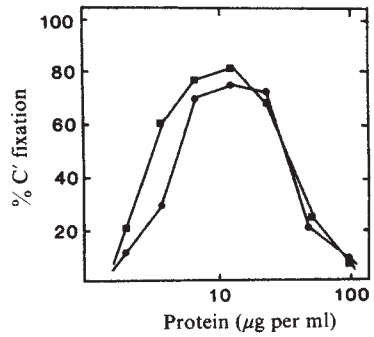

Fig. 1 Complement (C′) fixation curves of S100 for human brain medulla (●) and M29 cell line (■). M29 cell line extract was prepared as described in Table 1 legend. The brain sample was homogenized with Tris buffer, 2 ml per g tissue, and spun at 40,000*g* for 10 min before S100 determination. Quantitative complement fixation using an 8,000-fold dilution of antiserum was carried out on extracts of M29 and human brain medulla.

Table 1 S100 protein values for melanoma cell lines and other tissues

Specimen	μg S100 per mg soluble protein
Melanoma cell lines	
M12	6.2
M14	1.0
M15	1.9
M19	30.0
M21	ND
M24	ND
M29	3.5
Other cells and tissues	
C6 rat glial cells	2.6
Brain cerebrum (human)	2.3
Brain medulla (human)	5.6
HeLa cells	ND
Sarcoma cell line (human)	ND
Lymphocyte cell lines	
L14	ND
L29	ND

Cell lines were cultured in 75-cm^2 culture flasks using RPMI 1640 medium with 20% fetal calf serum and maintained in 5% CO_2. Confluent cells were washed twice with phosphate-buffered saline (NaCl, 8 g l^{-1}; KCl, 0.2 g l^{-1}; $MgCl_2 \cdot 6H_2O$, 0.1 g l^{-1}; $Na_2HPO_4 \cdot 7H_2O$, 2.16 g l^{-1}) and once with complement fixation buffer (0.0 M trisma base, 0.14 M NaCl, 0.5 mM $MgSO_4$, 0.15 mM $CaCl_2$, 0.1% bovine serum albumin). Cells were collected by scraping into complement fixation buffer, homogenized and spun at 40,000*g* for 10 min. Quantitative complement fixation was carried out using a 6,000-fold dilution of antiserum as described by Levine[17]. S100 was quantified as described by Zuckerman *et al.*[18]. The serological specificity of our antiserum has previously been demonstrated by double diffusion in agar and by quantitative complement fixation analysis using soluble protein extracts of a variety of tissues as antigens[17,18]. Purified bovine S100 was used as a standard to quantify the amount of S100 present in the soluble portion of each specimen. ND, Not detectable.

0028–0836/80/300400—02$01.00