5. Sörensen, O., Percy, D. & Dales, S. *Archs Neurol.* **37**, 478–484 (1980).
6. Sörensen, O., Dugre, R., Percy, D. & Dales, S. *Infect. Immunity* **37**, 1248–1260 (1982).
7. Wege, H., Koga, M., Watanabe, R., Nagashima, K. & ter Meulen, V. *Infect. Immunity* **39**, 1316–1324 (1983).
8. Richert, J. R., Driscoll, B. F., Kies, M. W. & Alvord, E. C. *J. Immun.* **122**, 494–496 (1979).
9. Vandevelde, M. *et al. Acta neuropath.* **56**, 285–293 (1982).
10. Lipton, H. L. & Dal Canto, M. C. *Science* **192**, 62–64 (1976).
11. Lipton, H. L. & Dal Canto, M. *J. neurol. Sci.* **42**, 391–405 (1979).
12. Panitch, H., Petursson, G., Georgsson, G., Palsson, P. & Nathanson, N. *Lab. Invest.* **35**, 452–460 (1976).
13. Tschannen, R., Steck, A. J. & Schäfer, R. *Neurosci. Lett.* **15**, 295–300 (1979).
14. Steck, A. J., Tschannen, R. & Schäfer, R. *J. Neuroimmun.* **1**, 117–124 (1981).
15. Panitch, S. H., Swoveland, P. & Johnson, K. P. *Neurology* **29**, 548–549 (1979).
16. Drzeniek, R. & Rott, R. *Int. Archs Allergy* **36**, 146–152 (1969).
17. Massanari, R. M., Paterson, P. Y. & Lipton, H. L. *J. infect. Dis.* **139**, 297–303 (1979).
18. Deibler, G. E., Martenson, R. E. & Kies, M. W. *Prep. Biochem.* **2**, 139–165 (1972).

Defective translation of measles virus matrix protein in a subacute sclerosing panencephalitis cell line

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Subacute sclerosing panencephalitis (SSPE) is a slowly progressing fatal human disease of the central nervous system (CNS) that is associated with measles virus persistence. Virus nucleocapsids are present in the brain[1,2] and the patient is in a state of hyperimmunization towards this agent. However, although all other structural polypeptides are recognized by the immune system, there is a markedly decreased antibody response towards virus matrix or membrane protein[3,4]. Matrix protein has not been detected in brain cells[5] and infectious virus is not present. The absence of this virus structural polypeptide is thought to account for the apparent restriction in virus maturation both *in vivo* and *in vitro*. SSPE viruses can only rarely be rescued from brain tissue by co-cultivation or cell fusion techniques using tissue culture cell lines susceptible to measles virus infection[6]. Often this procedure fails to yield a lytic budding virus but produces instead a carrier cell line in which the agent is cell associated. These lines (known as SSPE cell lines) also do not contain matrix protein[7,8]. However, the reason for this deficiency is unknown. We have therefore now examined an SSPE cell line which does not yield infectious virus in order to define this process further. We found that although messenger RNA for membrane protein was present, it was unable to form normal matrix protein in translation reactions.

In our laboratory we have examined an SSPE cell line (N-1) obtained by co-cultivation of SSPE brain material with Vero cells[9]. This cell line shows slowly spreading areas of cell fusion, but does not release infectious virus, although particles can be obtained by procedures which favour cell fragmentation[10,11]. During ordinary cell culture procedures, or in co-cultivation and cell fusion experiments, we have never detected release of infectious virus. Results of electron microscopic examination of the N-1 cells are consistent with a defect in virus maturation, and the virus nucleocapsids are randomly distributed throughout the cytoplasm[12]. These cells are thus a good model for the situation observed in CNS cells from SSPE patients.

Measles virus proteins can be detected in infected cells by immunoprecipitation and comprise: the large protein (L), haemagglutinin (H), phosphoprotein (P), nucleocapsid (N) and its degradation product (NC), the fusion protein, both uncleaved (F_0) and cleaved (F_1), and the matrix protein (M). The P protein is poorly recognized by the serum used in this work, but the small band (X) detected in infected cells and known to be related[13] to P was precipitated from both cell lysates and *in vitro* translation products. We confirmed that this protein is unrelated to matrix protein using the technique of Cleveland mapping[25]. All of these proteins were detected in lysates of

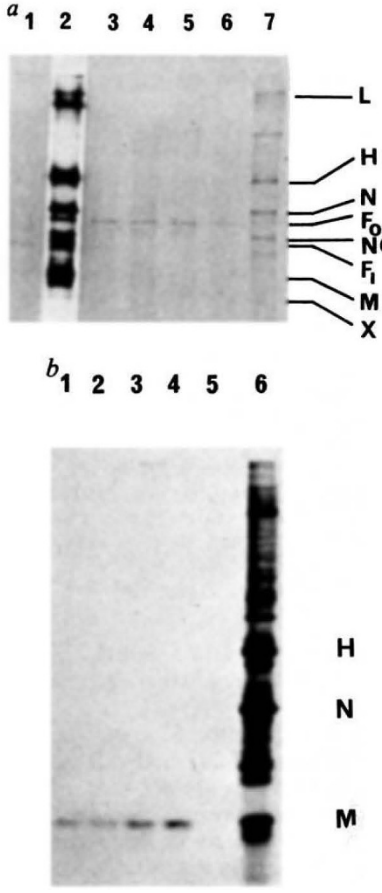

Fig. 1 *a*, Immunoprecipitation of measles-specific proteins from N-1 cells. Cells were labelled with ^{35}S-methionine (1,200–1,400 Ci mMol^{-1}, Amersham Buchler) for 2 h when the cytopathic effect was maximal. Lysates were prepared and analysed by immunoprecipitation and electrophoresis on a 10% SDS-polyacrylamide gel as described elsewhere[3]. Gels were fluorographed and exposed to Fuji X-ray film. Samples were precipitated with: tracks 1, 2, 7, rabbit hyperimmune anti-measles serum; track 3, control ascites fluid; tracks 4–6, ascites fluids containing monoclonal antibodies directed against Edmonston measles virus matrix protein. Track 1, lysate prepared from uninfected Vero cells; track 2, lysate prepared from Edmonston virus-infected Vero cells; tracks 3–7, lysates prepared from N-1 cells. *b*, Specificity of monoclonal antibodies. Lysates were prepared from Edmonston measles virus-infected Vero cells and immunoprecipitated with: tracks 1–4, anti-measles virus matrix protein monoclonal antibodies; track 5, monoclonal antibody raised against coronavirus; track 6, total cell lysate, without immunoprecipitation.

Edmonston measles virus-infected Vero cells. All except matrix protein could be demonstrated in lysates prepared from N-1 cells (Fig. 1*a*). Matrix protein was also not detected by immunofluorescence or radioimmunoassay using four monoclonal M-specific antibodies (Fig. 1*b*) which also cross-react with the non-defective SSPE virus Lec[14]. However, the levels of virus protein expression were low in these cell populations, as only a small percentage of cells (10–20%) were infected. Analysis by radioimmunoprecipitation was complicated by background host-cell proteins (Fig. 1, track 1) and nucleocapsid degradation products which migrated in the matrix protein region (38,000 molecular weight). The long exposures required to search for trace amounts of matrix protein were found unsatisfactory, and it was therefore necessary to analyse protein production *in vitro*. This results in a decreased background in immunoprecipitation[14]. Matrix protein production could be prevented by a defect in either transcription or translation, and it is known that this inhibition is to some extent host-controlled, as cell fusion experiments have occasionally resulted in the rescue of infectious virus[6]. Therefore, *in vitro* translation was

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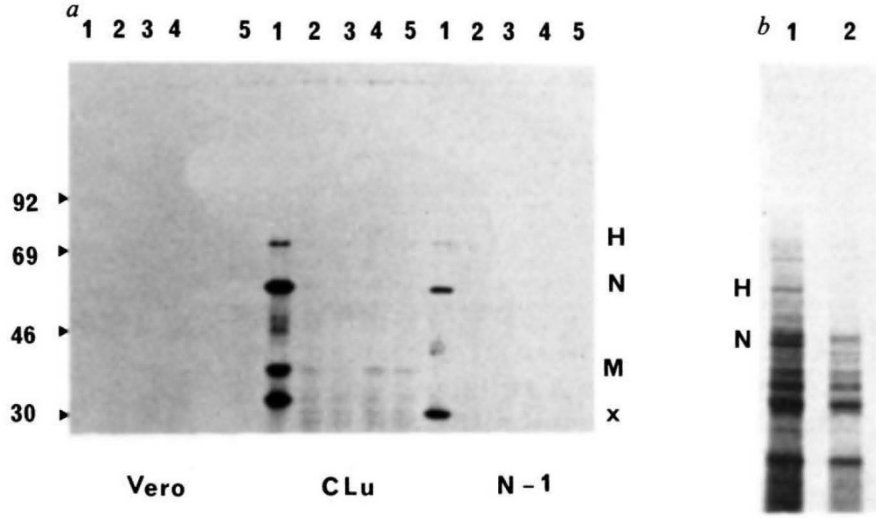

Fig. 2 *In vitro* translation of mRNA from persistently infected cells. *a*, Track 1, sample immunoprecipitated with rabbit anti-measles antiserum, preadsorbed with uninfected Vero cell antigens; tracks 2–5, samples immunoprecipitated with different monoclonal antibodies specific for matrix protein. *b*, Protein products from the *in vitro* translation of mRNA isolated from N-1 SSPE cells (track 1) and uninfected (track 2) without immunoprecipitation. mRNA was extracted from cytoplasmic preparations of uninfected Vero, N-1 and carrier Lu106 cells[22] and translated in a rabbit reticulocyte lysate system[23] (provided by Dr S. Siddell). Products were diluted in RIPA buffer containing protease inhibitors[3] and immunoprecipitated using rabbit anti-measles virus serum preadsorbed with uninfected Vero cell antigens. The same products were also immunoprecipitated with four monoclonal antibodies directed against Edmonston virus matrix protein. Samples were then analysed on a 10% SDS-polyacrylamide gel and visualized by fluorography. Positions of ^{14}C-labelled molecular weight markers (Amersham) are indicated.

used to investigate such control in SSPE virus persistent infections[15]; putative host-cell regulatory factors are eliminated in these experiments.

When mRNA was extracted from N-1 cells and translated in an *in vitro* system containing both ^{35}S-methionine and ^{35}S-cysteine, matrix protein was not detected among the products either with or without immune precipitation (Fig. 2). In contrast, matrix protein was produced in large amounts by mRNA extracted from carrier Lu106 (CLu) cells, a persistent infection initiated with measles virus Edmonston[16] which does release infectious particles. Matrix protein produced *in vitro* gave an unsatisfactory reaction with monoclonal antibodies, presumably because the protein lacked certain modifications. Polyclonal rabbit antiserum was not so sensitive to these differences. Thus, the conclusion that matrix protein was not formed in the *in vitro* translation reaction rests primarily on immunoprecipitation performed using this rabbit serum. As N-1 SSPE-cells did not form matrix protein *in vitro*, and as we were never able to demonstrate the protein by short pulse-labelling of tissue cultures, we were able to exclude completely the possibility that the protein is very unstable within the cell and rapidly degraded[17].

The observed failure in matrix protein production could result from production of a defective mRNA, or from failure to produce that message at all. Accordingly, mRNAs isolated from CLu and N-1 cell lines, whose translation products are shown in Fig. 1, were analysed by electrophoretic separation on an agarose gel, transfer to nitrocellulose and hybridization to nick-translated, cloned cDNA copies of sequences contained in the measles virus Edmonston mRNAs for nucleocapsid and matrix proteins[18,19]. The results of this analysis are shown in Fig. 3. In both cell lines examined, three classes of RNA were detected. The largest consists of the virus genome (g), and migrates with a true molecular weight of 4.5×10^6 in a fully denaturing gel[20]. The intermediate-sized RNA molecules (is) are of an unknown nature although they were observed to remain in the A(−) fraction after all mRNA had been removed. Such molecules might represent defective interfering RNAs, polymerase mistakes or RNA processing intermediates and may be similar to the polycistronic messages observed in vesicular stomatitis virus infections[21]. The smaller molecular weight species was the most plentiful molecule in the A(+) fraction and represented mRNA[18,19]. A mRNA for nucleocapsid protein was readily demonstrated in both cell lines (Fig. 3*a*), providing an internal, positive control for these experiments.

When cloned copies of the matrix protein mRNA were used as a probe, mRNA was still demonstrable in both cell lines (Fig. 3*b*). The quantitative reduction in the level of matrix

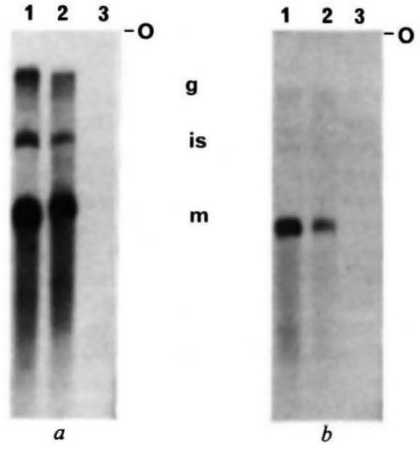

Fig. 3 Analysis of mRNA by filter hybridization. The mRNA preparations translated in Fig. 2 (1 μg per track) were denatured in formamide and separated at 100 mA on a 1.5% agarose gel buffered with MOPS and containing 5% formaldehyde (37%, Merck). RNAs were then transferred in 1 × SSC to nitrocellulose filters (NEN Gene-Screen)[24]. Filters were baked at 80 °C for 2 h and hybridized to ^{32}P-labelled plasmids (pBr322, $6–9 \times 10^8$ c.p.m. per μg) containing inserted sequences derived from Edmonston measles virus nucleocapsid (panel *a*) and matrix (panel *b*) mRNAs. In each panel: track 1, mRNA from carrier Lu106 cells; track 2, mRNA from SSPE-N-1 cells; track 3, mRNA extracted from uninfected Vero cells. O, Origin; g, genome; is, intermediate sized RNA; m, mRNA.

protein mRNA in N-1 cells observed in Fig. 3*b* cannot, however, explain the lack of matrix protein. The amount of this mRNA in N-1 cell preparations often exceeds that in samples prepared from other infections in which matrix protein production is readily demonstrable (see Fig. 4).

Messenger RNA obtained from two normal Edmonston virus infections was analysed by the blotting technique. The amount of matrix protein mRNA in 1 μg of the N-1 cell sample was equivalent to that contained in 1 μg of the second Edmonston-infected cell sample (Fig. 4*a*, tracks 3, 6). When these mRNAs were translated *in vitro*, M protein was readily detected in the immunoprecipitated products of both Edmonston-infected cell mRNAs, but not in those specified by the N-1 cell message (Fig. 4*b*, track 6). Thus, we conclude that the N-1 SSPE cell line clearly contained a mRNA specific for matrix protein which could not be translated to produce the normal protein *in vitro* or in infected cells. The basis of this defect is unknown but it could arise through premature termination or failure to initiate translation. A small peptide or a protein which might be completely different antigenically from normal matrix protein (for

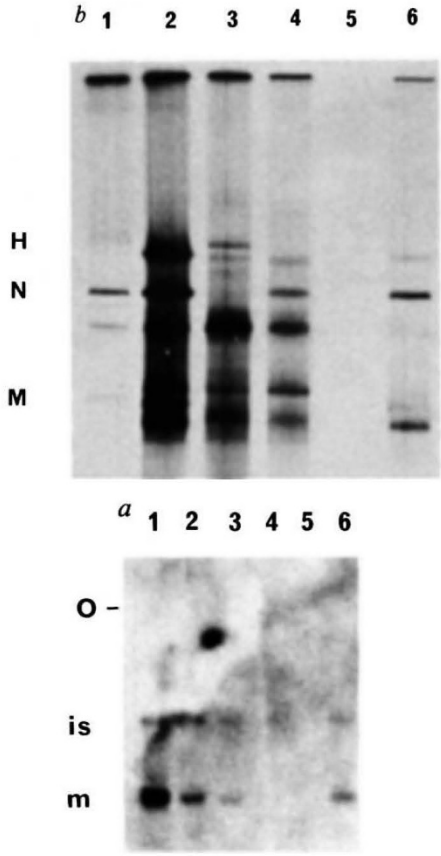

Fig. 4 *a*, Relative quantitation of mRNA samples. Tracks 1, 2, mRNA sample obtained from Edmonston virus-infected Vero cells (preparation 1); tracks 3, 4, mRNA sample from Edmonston-infected Vero cells (preparation 2); track 5, uninfected Vero cell mRNA; track 6, N-1 cell mRNA. 1 μg of mRNA was loaded onto tracks 1, 3, 5 and 6; 0.2 μg of mRNA were loaded onto tracks 2 and 4. *b*, *In vitro* translation. Track 1, Edmonston virus proteins immunoprecipitated from virus-infected Vero cell lysates proteins immunoprecipitated from translation products specified by; track 2, Edmonston virus-infected Vero cell mRNA (preparation 1); track 3, uninfected Vero cell mRNA; track 4, N-1 cell mRNA; track 5, No mRNA addition; track 6, Edmonston virus-infected Vero cell mRNA (preparation 2).
Methods: In *a*, two samples of mRNA isolated from measles virus (Edmonston)-infected Vero cells were electrophoresed with mRNA isolated from N-1 cells on a 1.5% agarose gel as described in Fig. 3 legend. The RNA was then transferred to a nitrocellulose filter and hybridized to ^{32}P-labelled DNA containing sequences derived from the matrix protein mRNA. In *b*, mRNA (1 μg) derived from uninfected cells, SSPE N-1 cells or Edmonston virus-infected Vero cell mRNA (preparations 1 and 2) were translated in the rabbit reticulocyte lysate system. The products from these reactions were immunoprecipitated using rabbit anti-Edmonston virus serum, analysed on a 10% SDS-polyacrylamide gel and visualized by fluorography. Antiserum used in this experiment was not preadsorbed with uninfected cell antigens and therefore recognized several host proteins contaminating the original virus antigen preparation (track 3).

example, due to a frameshift mutation) would not be detected in these experiments.

Whatever the molecular mechanism, the end result must be a lack of functional matrix protein in the infected cell. Matrix protein is thought to act as a trigger in the budding process, bringing together internal nucleocapsid structures and virus glycoproteins inserted in the cell membrane. Consequently, any defect in matrix protein production should lead to a cell-associated phenotype. The mRNA defect described here is only one process which could produce this effect. A Sendai virus persistent infection *in vitro* is known in which a drastic reduction in matrix protein stability within the cytoplasm leads to a similar deficiency in the levels of functional protein[17]. A transcriptional defect could also accomplish the same end. It is therefore possible that measles viruses might adopt different strategies in order to achieve persistence. No single mechanism has yet been identified *in vivo*. However, this report constitutes the first such identification in an SSPE cell line *in vitro* and we are now searching for matrix protein-specific information in post-mortem samples of SSPE patient brain.

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1. Conolly, J. H., Allen, I. V., Hurwitz, L. J. & Miller, J. H. D. *Lancet* **i**, 542–544 (1967).
2. Freeman, J. M., Magoffin, R. L., Lennette, E. H. & Herndon, R. M. *Lancet* **ii**, 129–131 (1967).
3. Hall, W. W., Lamb, R. A. & Choppin, P. W. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2047–2051 (1979).
4. Wechsler, S. L., Weiner, H. L. & Fields, B. N. *J. Immun.* **123**, 884–889 (1979).
5. Hall, W. W. & Choppin, P. W. *Virology* **99**, 443–447 (1979).
6. Katz, M. & Koprowski, H. *Arch. ges. Virusforsch.* **41**, 390–393 (1973).
7. Lin, F. H. & Thormar, H. *Nature* **285**, 490–492 (1980).
8. Machamer, C. E., Hayes, E. C. & Zweeringk, H. J. *Virology* **108**, 515–520 (1981).
9. Doi, Y. *et al. Jap. J. med. Sci. Biol.* **25**, 321–333 (1972).
10. Ohuchi, M., Ohuchi, R. & Homma, M. *Microbiol. Immun.* **23**, 877–888 (1979).
11. Ohuchi, M., Ohuchi, R. & Homma, M. *Microbiol. Immun.* **24**, 1023–1033 (1980).
12. Dubois-Dalcq, M., Horta-Barbosa, L., Hamilton, R. & Sever, J. L. *Lab. Invest.* **30**, 214–250 (1974).
13. Rima, B. K., Lappin, S. A., Roberts, M. W. & Martin, S. J. *J. gen. Virol.* **56**, 447–450 (1981).
14. Carter, M. J. & ter Meulen, V. *Brain Res.* **59**, (in the press).
15. Stephenson, J. R., Siddell, S. G. & ter Meulen, V. *J. gen. Virol.* **57**, 191–197 (1981).
16. Norrby, E. *Arch. ges. Virusforsch.* **20**, 215–224 (1967).
17. Roux, L. & Waldvogel, F. A. *Cell* **28**, 293–302 (1982).
18. Gorezki, M. & Rozenblatt, S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3686–3690 (1980).
19. Rozenblatt, S., Gesang, C., Lavie, V. & Neumann, F. S. *J. Virol.* **42**, 790–797 (1982).
20. Baczko, K., Billeter, M. & ter Meulen, V. *J. gen. Virol.* (in the press).
21. Herman, C. H., Schubert, M., Keene, J. D. & Lazzarini, R. A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4662–4665 (1980).
22. Barrett, T., Wolstenholme, A. J. & Mahy, B. W. J. *Virology* **98**, 211–225 (1979).
23. Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).
24. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
25. Cleveland, D. W., Fischer, S. G., Kirschner, W. & Laemmli, U. K. *J. biol. Chem.* **252**, 1102–1106 (1977).

Reversible induction of natural killer cell activity in cloned murine cytotoxic T lymphocytes

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Natural killer (NK) activity is a poorly understood component of the immune system, generally identified as the ability to kill certain tumour cells[1,2]. Perhaps the most controversial issue has been the lineage to which cells displaying this activity belong. Extensive studies of surface antigens on cells with NK activity in both mouse and man have led to enigmatic results, such cells apparently bearing markers of both T-cell (Thy-1 and E receptor) and myeloid (Mac-1 and OKM1) lineages[3–6]. A fresh approach to this problem would be to take cells of known lineage and test whether they express, or could be induced to express, NK cell function. Using this approach we show here that monoclonal cytotoxic T lymphocyte (CTL) lines can be induced, by culture in high concentrations of spleen cell supernatant, to express a new lytic activity apparently identical with that of splenic cells NK activity. Preliminary evidence implicates both interleukin-2(IL-2) and interferon (IFN) as mediators of this phenomenon. These findings clearly demonstrate that cells of T cell lineage have the capacity to express NK activity.

Antigen-specific CTL clones were generated from single precursor cells using a standard protocol[7]. They were characterized in the following manner. (1) They had the surface marker profile of the CTL lineage, that is, Ig^-, Thy-1^+, Ly-2^+. (2) Proliferation was greatly augmented by stimulation with spleen cells bearing

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