ENRICHMENT OF MEASLES VIRUS-LIKE RNA IN THE NUCLEOCAPSID FRACTION ISOLATED FROM SUBACUTE SCLEROSING PANENCEPHALITIS BRAINS

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A procedure has been developed which facilitates the detection of measles virus RNA sequences in human brains. The procedure involves isolating subviral components (nucleocapsids) from brain tissues prior to RNA purification, followed by hybridization of these RNAs to cDNA synthesized from measles virus 50 S RNA template. Using these techniques we were able to obtain an RNA fraction which was manyfold enriched in measles virus-specific RNA, relative to unfractionated subacute sclerosing panencephalitis (SSPE) brain RNAs. 70–100% of the measles virus-specific RNA present in these SSPE brain samples were recovered in this enriched fraction.

brain tissue   measles virus   nucleocapsid isolation   subacute sclerosing panencephalitis (SSPE)
RNA enrichment   virus RNA

INTRODUCTION

Measles virus causes an acute infection in humans and has been implicated as the etiologic agent in a number of progressive diseases. Viruses which appear to be variants of classical measles virus have been isolated from the brains of patients with subacute sclerosing panencephalitis (SSPE) (Payne and Baublis, 1971). Measles virus has also been implicated as a possible agent in other diseases including multiple sclerosis and systemic lupus erythematosus (for a review, see Morgan and Rapp, 1977).

The cultivation of viruses from diseased tissue is difficult, time-consuming and often impossible. One cannot always be certain that autopsy specimens have been collected in such a way as to minimize the breakdown of intracellular components, including viruses. In the time between death and tissue collection considerable nucleolytic and proteolytic damage may occur.

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* Deceased.

The use of high-specific radioactivity nucleic acids as viral probes provides the capability of assaying a tissue for virus, even though virus cannot be grown because it is defective or has been damaged. However, unlike the situation which occurs with virus-infected cells in culture, only a small portion of the brain cells may contain virus particles. This can make the virus nucleic acid more difficult to detect, since the virus-like nucleic acid will be very dilute. Here we describe a technique for the preferential enrichment of measles virus-like RNA from SSPE brains.

RESULTS AND DISCUSSION

It is generally thought that measles virus RNA is very stable when in its encapsidated intracellular state. Fig. 1 shows the sedimentation profiles of [³H]RNA purified from measles virus nucleocapsids which were incubated in a clarified SSPE brain lysate as described in the legend. The incubation of the [³H]uridine-labeled nucleocapsids in the clarified lysate
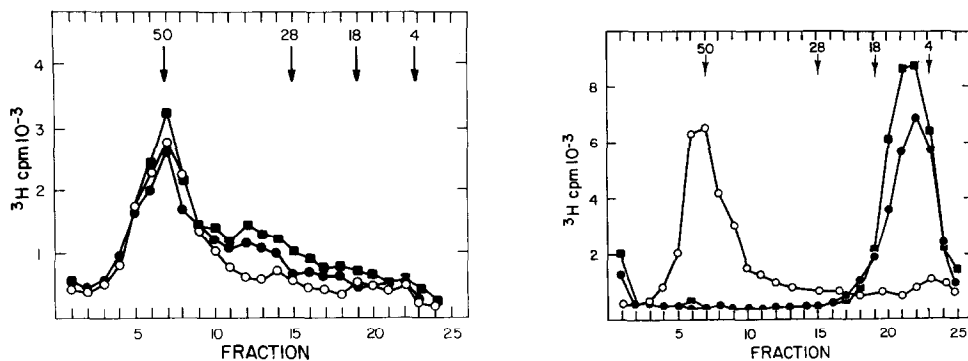

Fig. 1. Effect of nucleocapsid pelleting procedure on RNA degradation. Approximately 2 g of a frozen brain section containing approximately equal amounts of gray and white matter, was diluted 1 : 5 (w/v) in phosphate-buffered saline (pH 7.5) and homogenized at medium speed in a Vertis homogenizer. The cellular debris was pelleted for 10 min at 1000 X g, and the supernatant decanted. [³H]Uridine-labeled measles virus nucleocapsids purified as described previously (Kiley et al., 1974) were then added to either 1 ml of NTE (100 mM NaCl, 25 mM Tris pH 7.8, 1 mM EDTA) buffer (o), or to brain extract which was then allowed to incubate for zero (●) or 21 h (■) at 22°C. The nucleocapsid fraction was then isolated from each sample by centrifugation through NTE buffer containing 15% (w/v) sucrose at 100 000 X g for 4 h. The resulting pellet was resuspended in 1 ml NTE buffer, incubated (30 min at 65°C, pH 8.3) with proteinase K (100 µg/ml) and 1% SDS, and then extracted with phenol—chloroform to purify the RNA. The RNAs were layered on to a 15—30% (w/v) sucrose gradient and centrifuged for 11 h at 100 000 X g in order to determine the sedimentation profiles of the different RNAs shown above. Sedimentation is from right to left with ³H-labeled Vero cell RNA and measles virus RNA serving as markers.

Fig. 2. Effect of phenol extraction purification procedure on RNA degradation. Purified ³H-labeled measles virus nucleocapsid material was added to brain lysate and RNA purified as described in Fig. 1, except that the nucleocapsid-pelleting step was omitted. Shown are the RNA profiles for nucleocapsids incubated in buffer (o) or in cell lysate for 1 (●) or 24 (■) h.

did not result in extensive breakdown of the added [^{3}H]uridine-labeled nucleocapsid RNA. It was observed that the measles virus genomic RNA was degraded substantially if the nucleocapsid purification step was omitted (Fig. 2). Still, the degraded material in Fig. 2. was >85% acid precipitable and large enough to hybridize (Table 1). It is interesting that the size of the measles virus RNA recovered is the same whether nucleocapsids are incubated in brain lysate for 1 or 24 h. This indicates that nucleolytic digestion occurs during the extraction procedure itself, and that the measles virus RNA is quite stable as long as it is within its protein coat. When measles virus mRNA was added to the SSPE-1 brain lysate, it was rapidly degraded. Less than 5% of the material was acid precipitable after 15 min of incubation at 20°C.

Considerable RNA degradation was observed in all other lysates prepared from either

TABLE 1

Hybridization of measles [^{3}H]cDNA with various nucleic acids

	RNA or DNA cDNA	Total $C_0 t$	% [^{3}H]cDNA adsorbed to HA	Hybridization $C_0 t_{1/2}$ (ms · l^{-1})
Measles 50 S RNA	300	0.75	56	0.05
Measles-infected Vero cell RNA	9×10^5	513	58	13.5
SSPE-1 brain cell debris RNA (fraction A)	1.5×10^7	195	54	160
SSPE-1 brain nucleocapsid RNA (fraction B)	6.4×10^3	6.4	60	0.42
Unfractionated SSPE-1 brain RNA	2.9×10^6	780	54	39
Uninfected Cell RNA Vero	6×10^4	120	1.0	—
Fetal human liver DNA	8×10^6	2312	1.5	—

Hybridization assays were performed by the method of Kohne and Britten (1971) as follows. RNAs extracted from a section of the brain of an SSPE patient were extracted as described in the text and incubated at 3–100 μg/ml (nucleocapsid-containing fractions) or at 2–4 mg/ml (total RNA-containing) for 1–50 h at 65°C in 0.48 M phosphate buffer (PB) in the presence of 6–12 pg (300–600 c.p.m.) cDNA probe. At various $C_0 t$ values an aliquot of the reaction mixture was passed over a hydroxyapatite (HA) column equilibrated to 62°C in 0.14 M PB. Hybridized material which adhered to the column under these conditions was eluted with 0.48 M PB and the percentage of counts sticking to the column determined. Numerous points were taken for each sample. Shown above are the $C_0 t_{1/2}$ values for the kinetic reactions, the ultimate hybridization plateau level, the ratio of probe to test nucleic acid for each reaction mixture, and the total $C_0 t$ for indicated reactions. Less than 2% self-reaction of the [^{3}H]cDNA occurred.

SSPE or control brains. Since our probe does not react with rRNA (Table 1), and since our extraction procedure disrupts all polysomes which might endure (Kiley et al., 1974), it must be assumed that all material reacting with our measles virus-specific probe is nucleocapsid RNA and is most likely in nucleocapsid protein since free RNA is degraded so rapidly. These experiments indicate that any nucleocapsid particles naturally present in SSPE brain might also be intact and possible to isolate. Therefore, brain tissue was processed and the nucleocapsid fraction obtained from five SSPE brains and a number of controls as described below.

The nucleocapsid fraction was isolated from Vertis-homogenized brain tissue as described in Fig. 1, except that the supernatant fractions of cell lysates were layered on 15% sucrose (rather than a 15—30% sucrose gradient) and the nucleocapsid-containing extracts centrifuged for 4 h (rather than 3 h) to pellet nucleocapsid material. Three different fractions resulted: A) the cellular debris pellet obtained in the initial clarification centrifugation of the Vertis-homogenized brain (see Fig. 1); B) the pellet obtained from ultracentrifugation of the initial, clarified supernatant (if present the nucleocapsids will be in this fraction); and C) the supernatant from the ultracentrifugation step. The RNA was isolated for each fraction.

Unfractionated RNA was also isolated from a separate sample of brain by thawing an identical piece of tissue in 3.2 M sodium trichloroacetic acid (pH 7.0) and purifying the nucleic acids by phenol—chloroform extraction and ethanol precipitation. This nucleic acid-containing pellet was then incubated in NTE buffer (pH 8.3) containing 100 μg/ml proteinase K and 1 % SDS for 30 min at 65°C, re-extracted with phenol—chloroform and ethanol precipitated again. The concentration of measles virus-like RNA in each RNA preparation was then determined by nucleic acid hybridization kinetics (Britten and Kohne, 1968), using high specific radioactivity (2×10^8 dpm/μg) [^{3}H]cDNA synthesized by using measles virus 50 S RNA as a template (Taylor et al., 1976) (Table 1).

The measles virus 50 S RNA used as template for the production of the cDNA was about 60—70% double-stranded after self-hybridization. Spruance et al. (1980) and Bedows and Payne (1981) have also observed that a large fraction of 50 S measles virus nucleocapsid RNA is composed of plus and minus strands. The 50 S RNA was denatured before being used as a template for cDNA. Table 1 summarizes the hybridization characteristics of the [^{3}H]cDNA. The maximum extent of hybridization of the cDNA is about 60%. This incomplete reaction is probably due to the presence of plus and minus measles virus RNA in the 50 S and measles virus-infected cell RNA.

Table 1 compares the hybridization of our measles virus cDNA to various RNA fractions of one of the SSPE brains (SSPE-1). The bulk of the RNA from the SSPE-1 brain tissue sample was present in fraction A (675 μg RNA/g tissue). Fraction B, the nucleocapsid fraction, contained about 100 times less RNA (6.2 μg/g tissue). Less than 1 μg of RNA/g tissue was found in fraction C.

The concentration of measles virus RNA in the A, B, and unfractionated RNA preparations was determined by nucleic acid hybridization kinetic studies. The kinetics of hybridization of measles virus [^{3}H]cDNA plus the various RNA preparations yielded

$C_0t_{1/2}$ values of hybridization of 0.42 ms·l^{-1} for RNA B, 160 ms·l^{-1} for RNA A, and 39 ms·l^{-1} for unfractionated RNA. Thus, the nucleocapsid RNA fraction had a measles virus RNA concentration 93 times higher than the unfractionated SSPE brain RNA. This indicates that the nucleocapsid isolation procedure resulted in a great preferential enrichment of measles virus-specific RNA.

Comparison of measles virus RNA recovered from the unfractionated RNA preparation and the nucleocapsid-like pellet indicated that at least 75% of the measles virus sequences in the SSPE-1 brain were recovered in fraction B RNA. Furthermore, in most other SSPE brains examined, an even greater recovery was observed (Table 2). The kinetics of reaction of [³H]cDNA with unfractionated SSPE-1 RNA also provided information as to the frequency of occurrence of the measles virus-specific RNA molecules in SSPE brain cells. On the average, each SSPE-1 brain cell from this patient contained about 400–600 measles virus RNA molecules (the RNA/DNA ratio of brain cells is about 1:1). Measles virus-infected Vero cells contained around 1000–2000 measles virus RNA copies per cell on an average (the RNA/DNA ratio of these cells is 2–3:1).

The T_m values of measles virus cDNA : SSPE-1 RNA hybrids and measles virus cDNA : measles virus-infected cell RNA hybrids were also measured in 0.14 M phosphate buffer (PB) and found to be 79°C and 83°C respectively. These data are consistent with the high extent of reaction seen for measles virus [³H]cDNA and this SSPE RNA (close to 100% when normalized, see Table 1) and indicate that about 4–6% nucleotide sequence difference exists between SSPE virus and the Edmundston measles virus strain. These data are consistent with, and expand somewhat, the data of Hall and Ter Meulen (1976).

Table 2 compares the hybridization reactions between five SSPE brains. (Three identically prepared control brains were also assayed but failed to hybridize (<1%) to the probe.) Three of five SSPE brain RNAs tested hybridized an equivalent amount of cDNA probe as measles virus-infected Vero cell RNA. It is possible that the failure of these other RNAs to hybridize reflects the presence of a high level of competing positive-stranded material. Alternatively, these brains may exclusively contain measles virus nucleocapsid particles of a highly fragmented nature representing only a small portion of the measles virus genome.

Comparison of the reaction rates of nucleocapsid RNA vs. total RNA indicates that in all cases except SSPE-3, there is a 100–3000-fold concentration in measles virus-specific sequences from those SSPE brains. The large disparity between these values may reflect a difference in nuclease levels between brains which could rapidly diminish total RNA concentrations.

Although only 1–3% as much RNA is extracted by the nucleocapsid pelleting technique as by total RNA extraction, the vast majority of measles virus sequences were recovered during the nucleocapsid pelleting technique (Table 2). Thus the ability to concentrate a specific virus RNA by isolating its nucleocapsid fraction has great potential usefulness. The higher concentration of viral nucleic acid or nucleocapsid protein greatly increases the sensitivity of detection of these sequences when assayed by nucleic acid

TABLE 2

Comparative hybridization characteristics of five SSPE brains

	% [³H]cDNA adsorbed to HA	Nucleocapsid RNA hybridization $C_0 \cdot t_{1/2}$ (fraction B)	Total RNA hybridization $C_0 \cdot t_{1/2}$	Fold enhancement in reaction rate	No. of measles genomes per cell	% Measles virus RNA recovered in nucleocapsid fraction (B)	Fraction B RNA/total RNA
SSPE-1	60	0.42	39	93	400– 600	75%	0.011
SSPE-2	39	3.3	≥1000	≥303	100– 200	>95%	0.038
SSPE-3	10	400	≥910	≥2.3	1– 2	70%	0.031
SSPE-4	62	0.44	≥910	≥2068	300– 400	>95%	0.013
SSPE-5	56	0.16	475	2969	800–1600	>95%	0.014
Measles-infected Vero cell RNA	58	0.68	13.5	20	1000–2000	>95%	0.058
All control brains	<1	–	–	–	–	–	–

RNAs from the above SSPE and control brains and measles virus-infected cells were prepared as described in the text and assayed as described in Table 1.

hybridization or by immunologic methods, thus increasing the chance of detecting the virus if it is present at low levels. While it is clear that this approach will not work for every virus species, it is hoped that it will prove useful in determining whether diseases such as multiple sclerosis have a measles virus etiology.

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