Key words: measles virus/mumps virus/oligo-2′,5′-adenylate synthetase/interferon (HuIFN)

Oligo-2′,5′-adenylate Synthetase Activity in K562 Cell Lines Persistently Infected with Measles or Mumps Virus

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(*Accepted 26 April 1988*)

SUMMARY

Fluctuation of oligo-2′,5′-adenylate synthetase (2–5AS) activity was examined in K562 cells infected with vaccine strains of measles virus (strains AIK-C and CAM-70) and mumps virus (strains Torii and Miyahara). Persistent infection was easily established in the mumps virus-infected cells without significant cytolysis or cell killing. In contrast, most of the cells infected with measles virus were killed by extensive cytolysis within 3 to 4 days. The small number of cells that did survive became persistently infected. That these persistently infected cells carried a virus antigen was confirmed by fluorescein isothiocyanate-labelled anti-measles virus rabbit antiserum and anti-mumps virus rabbit antiserum. The cells produced infectious progeny virus as well as interferon (IFN). Little induction of 2–5AS activity by IFN was demonstrated during the early stages of infection by these viruses. Similar results were observed in some of the persistently infected cells but not, however, K-CMP cells (K562 cells persistently infected with CAM-70) or K-MMP cells (K562 cells persistently infected with Miyahara). Failure to induce 2–5AS activity was unchanged in cells cultured for more than 6 months. The decrease of 2–5AS activity observed in K-MTP cells (K562 cells persistently infected with Torii) was the result of suppression of transcription of 2–5AS mRNA. On the other hand, a normal level of mRNA was found in K-AKP cells (K562 cells persistently infected with AIK-C). Therefore, it is suggested that the decrease of 2–5AS activity in K-AKP cells may be due to a failure to translate 2–5AS mRNA.

The establishment of persistent infection in several cultured cell lines by paramyxoviridae, including measles and mumps viruses, has been reported (Andzhaparidze *et al.*, 1983; Barry *et al.*, 1976; Knight *et al.*, 1972; Menna *et al.*, 1975; Truant & Hallum, 1977). Persistent infection of measles virus in the human central nervous system is closely associated with subacute sclerosing panencephalitis (SSPE) (Carter *et al.*, 1983; Hall & Choppin, 1981; Wechsler & Meissner, 1982). It has been suggested that measles virus may also be a casual factor in multiple sclerosis (Haase *et al.*, 1981). In addition to this, mumps virus infection can lead to a chronic infection of the central nervous system. Though the mechanisms and maintenance of these persistent infections are still obscure, various reports have been made and it has been suggested that such persistent infection is the result of the appearance of virus mutants or interferon (IFN) (Andzhaparidze *et al.*, 1982; Carter *et al.*, 1983; Jacobson & McFarland, 1982; Truant & Hallum, 1977). Jacobson & McFarland (1982) reported that anti-IFN serum could shift the virus–cell interaction from a persistent to a productive infection, and infectious progeny virus was also produced in spite of the presence of IFN. The antiviral state may be closely associated with persistent infection due to the inhibitory or suppressive effect of IFN on virus replication.

Therefore, the change in activity of such antiviral systems caused by virus infection may be correlated with persistent infection. We have studied the fluctuation of oligo-2′,5′-adenylate synthetase (2–5AS) activity in K562 cells acutely and persistently infected with mumps or measles virus to investigate this point.

The K562 cells were infected with measles virus vaccine strains CAM-70 (Tanabe Chemical Industries) and AIK-C (Kitasato Institute, Tokyo, Japan) and mumps virus vaccine strains Torii (Takeda Chemical Industries) and Miyahara (Chemo and Serum Therapy Institute, Kumamoto, Japan) at a multiplicity of infection of 0·1. The cells were then generally cultured and passaged every 5 or 7 days, except for the measles virus-infected cells which were not passaged between days 5 and 20 post-infection (p.i.) because of the decreased number of live cells. The virus strains used were passaged and propagated in Vero cells before use in this experiment. Virus titre and IFN activity were assayed as previously described (Fujii *et al.*, 1983; Fujii & Oguma, 1986). Virus-infected cells were smeared onto a glass slide, dried and fixed with cold acetone at 4 °C for 15 min. They were then stained using fluorescein isothiocyanate (FITC)-labelled anti-measles virus rabbit antiserum or anti-mumps virus rabbit antiserum (Denka, Tokyo, Japan) at 37 °C for 60 min. The cells were then washed three times and observed microscopically.

A persistent infection without any detectable c.p.e. was readily established after infection with the Torii strain but the Miyahara strain showed slight cytolysis. In contrast, cells infected with the AIK-C strain had a significant c.p.e. due to fusion, and sometimes became as much as 10-fold larger than uninfected cells. Roughly 40 to 50% of infected cells died within 5 to 10 days p.i. Thirty days p.i. a persistent infection was established. The CAM-70 strain did not induce cell fusion in this experiment. Approximately 70 to 80% of the infected cells died within 5 to 10 days p.i. and cell growth was not observed for 10 to 20 days p.i. After 20 days cultivation, cell growth was noticed and almost all the growing cells were shown to be FITC-positive. A persistent infection was established by 40 days p.i.

The cells were treated with 500 IU/ml of human leukocyte IFN-α (3×10^6 IU/vial, Lot 02-31, Japan Red Cross Society, $> 10^7$ IU/mg protein), human fibroblast IFN-β (3×10^6 IU/vial, Lot L-0523, Toray, $> 10^7$ IU/mg protein) or human recombinant IFN-γ (9×10^6 IU/vial, Lot GTK-001, Toray, $> 10^7$ IU/mg protein) for 24 h. Oligo-2′,5′-adenylate synthetase activity in cells treated with IFN was measured by the methods of Fujii *et al.* (1984, 1987) at 5, 10, 20 and 40 days p.i. As shown in Table 1, very little 2–5AS activity was induced by IFN in cells infected with the Torii strain (K-MT cells) or the AIK-C strain (K-AK cells) at any of the times indicated in the table. On the other hand, although induction of 2–5AS activity by IFN was poor in cells infected with the CAM-70 strain (K-CM cells) or the Miyahara strain (K-MM cells), at 5 and 10 days p.i., the enzyme activity was restored and inducible after 20 days cultivation.

Enzyme induction, virus yield and spontaneous production of IFN in four persistently infected cell lines are summarized in Table 2. The 2–5AS activity was induced by IFN-α and IFN-β in K562, K-CMP (K562 cells persistently infected with the CAM-70 strain) and K-MMP (K562 cells persistently infected with the Miyahara strain), but not in K-AKP (K562 cells persistently infected with the AIK-C strain) and K-MTP (K562 cells persistently infected with the Torii strain) cells. IFN-γ did not affect the induction of 2–5AS in any of the five cell lines. These persistently infected cells yielded about 10^4 to 2×10^4 p.f.u./ml of infectious virus and 8 IU/ml of IFN was spontaneously produced by K-CMP and K-MTP cells. All of these persistently infected cells were found to be FITC-positive.

Poor induction of 2–5AS by IFN was also confirmed by analysis of reaction products using HPLC (Fig. 1). HPLC was carried out with a Pharmacia FPLC system in a Mono Q HR 5/5 column at a flow rate of 1·0 ml/min as previously described (Fujii *et al.*, 1988). Solvent A was 50 mM-Tris–HCl pH 8·0, 7 M-urea. Solvent B was 50 mM-Tris–HCl pH 8·0, 1 M-NaCl, 7 M-urea. Elution was with a linear gradient from 9% to 34% of solvent B in 26 min. Authentic oligo-2′,5′-adenylate (2–5A) (trimer and tetramer) (Pharmacia), was used as a marker and the position of elution of these markers is indicated on the chromatogram. Investigation of the molecular sizes of the 2–5A products obtained from 2–5AS derived from K562 and K-CMP cells (Fig. 1 *a* and *b*) showed the synthesis of dimer, trimer, tetramer and pentamer forms. The amount of 2–5AS

Table 1. *Activity of 2–5AS in virus-infected cells*

Cell line	Time p.i. (days)	IFN (IU/ml) 0	IFN (IU/ml) 500	FITC-positive (%)
K562	5	7·8*	162·3	–
K-AK		20·6	13·9	80–90
K-CM		27·5	46·1	60–70
K-MT		15·5	10·1	80–90
K-MM		10·4	16·3	80–90
K562	10	5·4	169·9	–
K-AK		3·3	4·6	100
K-CM		ND†	ND	100
K-MT		10·4	8·9	100
K-MM		7·6	10·8	100
K562	20	6·7	188·6	–
K-AK		11·2	13·7	100
K-CM		8·5	384·5	100
K-MT		9·1	17·7	100
K-MM		10·6	204·4	100
K562	40	6·2	175·8	–
K-AK		7·9	19·5	100
K-CM		5·6	177·4	100
K-MT		6·1	5·8	100
K-MM		9·4	211·6	100

* 2–5AS activity (nmol/mg/h).
† ND, Not done.

Table 2. *Induction of 2–5AS activity by IFN in persistently infected cells*

Cell line	2–5AS induction by: No IFN	IFN-α	IFN-β	IFN-γ	Virus release (p.f.u./ml)	IFN production (IU/ml)
K562	5·8*	207·3	242·4	6·7	–	<2
K-AKP	6·3	12·5	9·6	9·0	$8·4 \times 10^3$	<2
K-CMP	8·1	299·5	306·4	5·6	$1·1 \times 10^4$	8
K-MTP	7·4	8·3	6·6	6·8	$2·4 \times 10^4$	8
K-MMP	10·2	306·6	288·7	10·3	$1·8 \times 10^4$	2

* 2–5AS activity (nmol/mg/h).

dimer, trimer and tetramer forms was reduced when 2–5AS from K-AKP and K-MTP cells was studied (Fig. 1 *c* and *d*). There was a 20- to 40-fold increase in the amount of 2–5A produced by 2–5AS from K562 cells as compared with that from K-AKP and K-MTP cells.

Cloning of cells by the limiting dilution method was performed and induction of 2–5AS by IFN was examined. All of the seven clones obtained from K-MTP cells produced infectious virus and were FITC-positive. In these clones 2–5AS induction was also completely suppressed. A similar result was recognized in K-AKP clones including those susceptible to cell fusion (clones 1, 2, 3, 4, 5 and 6) or not (clones 7, 8, 9 and 10). In four (11, 12, 13 and 14) out of a total of 14 clones enzyme activity was inducible by IFN to about 50% of the activity in the control K562 cells (Table 3).

Northern blot analysis was carried out on mRNA preparations from K562 cells and from three persistently infected cell lines to determine the size and the amount of transcript identified by human 2–5AS DNA. Total cytoplasmic RNA (60 μg) was electrophoresed on formaldehyde–agarose gels, blotted and hybridized to the ^{32}P-labelled probe as previously described (Fujii *et al.*, 1988; Rave *et al.*, 1979; Sawada & Fujinaga, 1980). For detection of human 2–5AS RNA, we

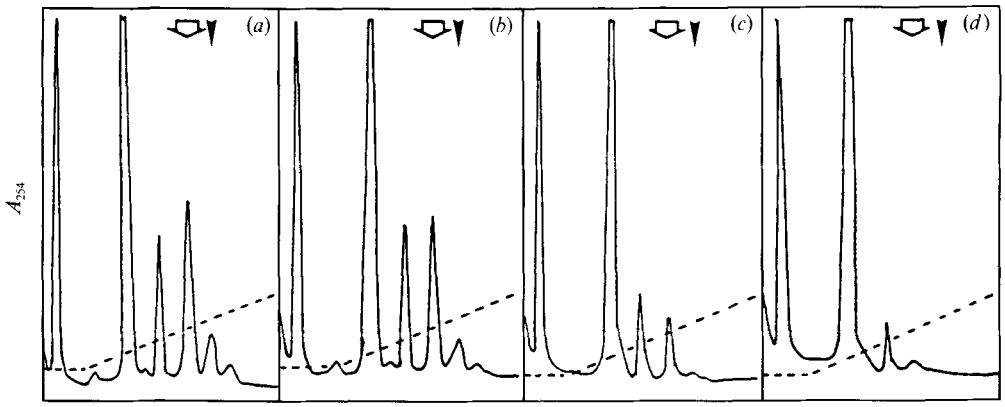

Fig. 1. Analysis of reaction products by FPLC. Oligonucleotides produced by 2–5AS from K562 (*a*), K-CMP (*b*), K-AKP (*c*) and K-MTP (*d*) cells treated with 500 IU/ml of IFN were separated by using a Mono Q HR 5/5 column. Arrows indicate the position of the trimer (⇦) and tetramer (⥣) of 2–5A.

Table 3. *Activity of 2–5AS in cloned cells*

Cell line	IFN (IU/ml) 0	IFN (IU/ml) 500	FITC-positive (%)	Fusion of cells*
K562	5·6†	249·3	–	–
K-MTP-1	6·0	6·6	100	–
K-MTP-2	5·3	5·0	100	–
K-MTP-3	6·3	10·1	100	–
K-MTP-4	6·5	7·6	100	–
K-MTP-5	7·7	10·5	100	–
K-MTP-6	8·4	7·1	100	–
K-MTP-7	5·2	7·9	100	–
K-AKP-1	10·4	16·1	90–100	+
K-AKP-2	8·8	21·1	90–100	+
K-AKP-3	7·6	15·4	90–100	+
K-AKP-4	12·5	16·0	90–100	+
K-AKP-5	6·6	20·6	90–100	+
K-AKP-6	10·4	24·4	90–100	+
K-AKP-7	8·6	20·4	90–100	–
K-AKP-8	6·4	18·0	90–100	–
K-AKP-9	10·2	12·3	90–100	–
K-AKP-10	10·2	6·9	90–100	–
K-AKP-11	8·6	80·7	90–100	–
K-AKP-12	12·2	97·0	90–100	–
K-AKP-13	10·8	110·1	70–80	–
K-AKP-14	8·8	120·7	70–80	–

* Fusion (+) indicates more than 30% of cells fused.
† 2–5AS activity (nmol/mg/h).

used a ^{32}P-labelled 1·4 kb fragment from pSP25R (Shiojiri *et al.*, 1986). As shown in Fig. 2, there was a drastic decrease in the amount of 2–5AS mRNA in K-MTP cells as compared with that in K562 cells. In contrast K-AKP cells had a normal level (in non-fusing clone K-AK1) or slightly reduced level (fusing clone K-AK2) of 2–5AS mRNA in spite of the failure of IFN to induce 2–5AS activity. The abundance of the larger mRNA (about 3·2 kb) increased in K-AK2 cells relative to that of the low molecular size mRNA (about 1·8 kb). The pattern of mRNA obtained from K-AK1 cells was similar to that from control K562 cells. Furthermore, in the case of K-MTP cells treated with IFN for 3, 6 or 12 h, the amount of transcript did not increase above that found in K-MTP and K562 cells not treated with IFN.

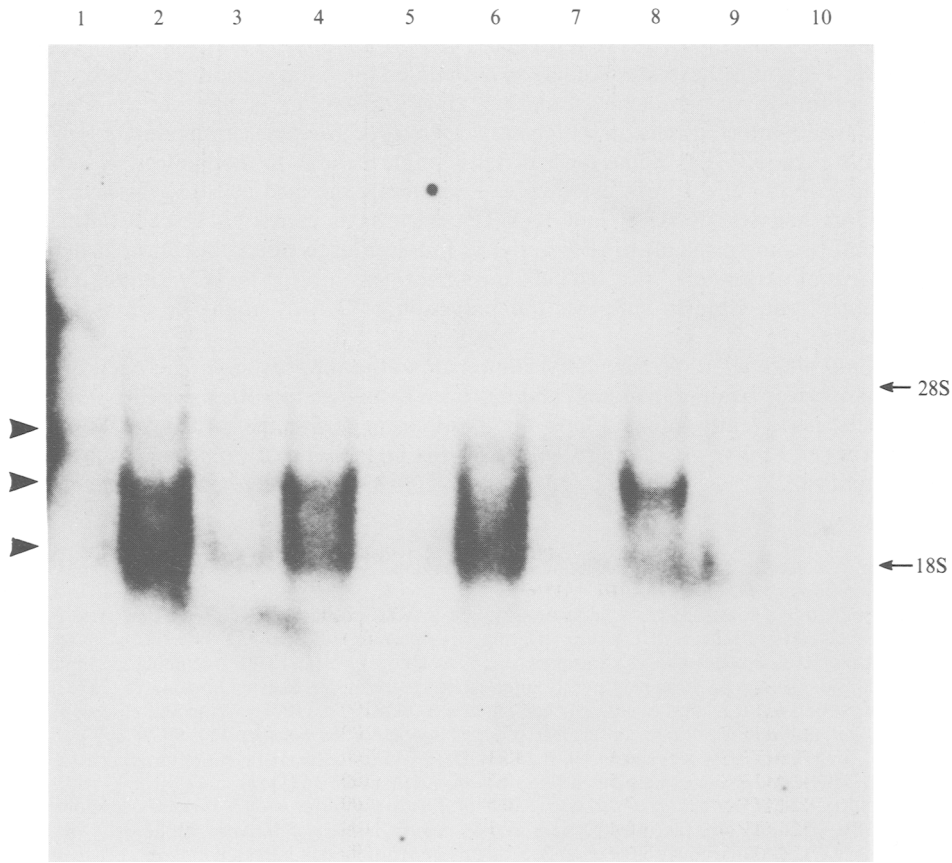

Fig. 2. Analysis of 2–5AS mRNA transcripts in persistently infected cells. Total cytoplasmic RNA (60 μg) from cells treated with 500 IU/ml of IFN (even numbered lanes) for 24 h was electrophoresed on formaldehyde–agarose gels and Northern blots were hybridized to a ^{32}P-labelled probe. Odd numbered lanes represent cells treated in the same way but with no IFN added. Lanes 1 and 2, strain K562; lanes 3 and 4, K-CMP; lanes 5 and 6, K-AKP non-fusion type clone; lanes 7 and 8, K-AKP fusion type clone; lanes 9 and 10, K-MTP.

The possible presence of an inhibitor of 2–5AS was also investigated. The supernatant obtained from the persistently infected cell lines K-AKP and K-MTP had no capacity to inhibit 2–5AS activity from K562 cells. This result was consistent with the results of a FPLC analysis in which the enzyme bound to poly(I : C)–agarose beads, and other cellular proteins were washed out from the 2–5AS reaction mixture. Therefore, suppression of this enzyme is not the result of an inhibitor.

During this study the correlation between the establishment and/or maintenance of persistent infection and the fluctuation of 2–5AS activity was investigated in four persistently infected cell lines. In two cell lines (K-AKP and K-MTP) IFN induced a low-level of 2–5AS activity. The viral suppression of enzyme induction was not associated with the yield of progeny virus (Table 2) or the fusion capacity of the virus (Table 3). On the other hand, there was no suppression of 2–5AS induction in the other two persistently infected cell lines (K-CMP and K-MMP cells). Failure to induce 2–5AS activity did not contribute to the establishment and maintenance of the persistent infection in K562 cells.

Recently, it has been reported that the activity of 2–5AS is scarcely induced by IFN treatment in several cell lines, including Vero cells persistently infected with an SSPE isolate of measles virus, lymphocytes infected with human immunodeficiency virus type 1, P3HR-1 cells

persistently infected with Epstein–Barr virus and BALB3T3 cells persistently infected with Moloney murine leukaemia virus (Crespi *et al.*, 1986; Fujii *et al.*, 1988; Fujii & Oguma, 1986; Preble *et al.*, 1985). Furthermore, Anderson & Fennie (1987) have also reported that the IFN-mediated inhibition of vesicular stomatitis virus replication is not observed in human adenovirus type 5-infected cells, in which early region 1A products can modulate IFN activity (Anderson & Fennie, 1987). Some types of virus might be able to modulate IFN activity and decrease its antiviral effect in infected cells. Persistently infected cells in which IFN fails to induce 2–5AS activity (K-AKP and K-MTP) may have increased susceptibility to virus infection and become resistant to IFN activity. In addition to this, replication of the progeny (carried) virus of persistently infected cells might not be inhibited by IFN activity. Therefore, the capacity of the virus to suppress the induction of 2–5AS might be of importance in pathogenesis.

It is still unknown what mechanism(s) contribute to the suppression of 2–5AS induction. As the suppression was strain-dependent, and has been recognized in other cell lines (SK-AS cells and FL cells) persistently infected with the Torii strain of mumps virus or AIK-C strain of measles virus (N. Fujii *et al.*, unpublished data), it is suggested that suppression may be caused by the virus itself.

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(*Received 20 November 1987*)