Antigenic relationships between six genotypes of the small hydrophobic protein gene of mumps virus

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Six different genotypes of mumps virus, A, C, D, G, H and I, genotyped on the basis of the small hydrophobic protein gene sequence, were subjected to antigenic comparison. Monoclonal antibodies directed against the haemagglutinin–neuraminidase protein of the SBL-1 strain of genotype A were used in immunofluorescence tests with different mumps virus strains. In addition, the six virus genotypes were compared by cross-neutralization tests with human post-vaccination sera after vaccination with the Jeryl Lynn (JL) strain of mumps virus and with rabbit hyperimmune sera directed against the A or D genotypes of mumps virus. Genotypes C, D, G, H and I could not be antigenically separated. In contrast, three different virus strains of genotype A, SBL-1, JL and Kilham, were distinct and were found to represent three different serotypes within the A genotype of mumps virus. Vaccination of Swedish children with the JL strain of mumps virus resulted in clearly lower neutralization titres against the SBL-1 strain, which is endemic in Sweden, compared to the homologous vaccine titres.

Introduction

Mumps virus belongs to the family Paramyxoviridae, genus Rubulavirus (Rima et al., 1995). The single-stranded mumps virus genomic RNA contains seven genes in the following order on the genome map: the nucleocapsid (N), phospho (P), membrane (M), fusion (F), small hydrophobic (SH), haemagglutinin–neuraminidase (HN) and large (L) protein genes (Elango et al., 1988; Elliott et al., 1989). The SH protein gene of mumps virus is the most variable part of the mumps virus genome. Its mRNA is 310 nt long and encodes a virus membrane protein of 57 aa (Elango et al., 1989; Takeuchi et al., 1996). SH gene analysis has been used to genotype mumps virus strains. Comparison of the nucleotide sequences of the SH gene from mumps virus strains collected worldwide has shown the existence of ten genotypes, namely A–J (Wu et al., 1998; Jin et al., 1999; Kim et al., 2000, Tecle et al., 2001), although the authenticity of genotype E has been questioned (Jin et al., 1999). The distribution of mumps virus genotypes varies extensively both temporally and geographically (Afzal et al., 1997a, b; Örvell et al., 1997a; Jin et al., 1999; Takahashi et al., 2000; Tecle et al., 1998, 2001, 2002). Co-circulation of different mumps virus genotypes in a specific geographical location is a well-known phenomenon (Afzal et al., 1997b; Örvell et al., 1997a; Jin et al., 1999; Tecle et al., 1998, 2001, 2002) and circulation of mumps virus genotypes may vary even between different geographical locations within the same country (Takahashi et al., 2000).

Reinfections occur with mumps virus and vaccination with monovalent vaccines may show a lack of efficient protection against all mumps virus genotypes (Künkel et al., 1994; Gut et al., 1995; Afzal et al., 1997b; Kim et al., 2000; Nöjd et al., 2001). This phenomenon may be due to pronounced antigenic differences that exist between the HN proteins of different mumps virus strains (Server et al., 1982; Örvell, 1984; Yates et al., 1996; Örvell et al., 1997b; Nöjd et al., 2001). Genotype A has been shown to be antigenically distinct from genotypes B, C and D, but knowledge about genotypes E–J is lacking (Yates et al., 1996; Örvell et al., 1997b; Nöjd et al., 2001). For surveillance of immunity against mumps virus, it is important to follow the distribution of genotypes in different countries and also to measure genotype-specific immunity in the population (Nöjd et al., 2001). In Sweden, mass vaccination against mumps virus was started with the Jeryl Lynn (JL) strain of genotype A in 1982. This vaccine has been reported to contain two different viruses, JL isolates 2 and 5 (JL2 and JL5) (Afzal et al., 1993). There was a temporal relationship between
the start of vaccination and the disappearance of the neuro-pathogenic genotypes C and D from the community from 1986 onwards. In contrast, the less neuro-pathogenic SBL-1 strain of genotype A is still endemic in Sweden (Tecle et al., 1998; Nöjd et al., 2001).

The aim of the present study was to investigate the antigenic relationships between mumps virus strains by including a larger number of virus strains of different genotypes into the comparison. An attempt was made to define both different serotypes of mumps virus and explain the seeming paradox of the persistence of the SBL-1 strain of mumps virus in Sweden, in spite of the continued mass vaccination with the JL virus strain of the same genotype (Tecle et al., 1998).

Methods

**Viruses.** Ten virus strains belonging to genotypes A, C, D, G, H and I were studied. Three virus strains belonged to genotype A, SBL-1 (Orvell, 1978, 1984), Kilham (Kilham, 1951; Wolinsky et al., 1974) and JL (Merck). The virus strains of genotypes C (V27) and D (V0e and RW) have been described previously (Orvell et al., 1997a, b). The Kilham and RW virus strains were a generous gift from J.S. Wolinsky, Department of Virology, University of Texas Health Science Center, Houston, TX, USA. Virus strains Gloucester 1/UK 96 (genotype G) and Manchester 1/UK 95 (genotype H) were a generous gift from L. Jin, Enteric, Respiratory and Neurological Virus Laboratory, Central Public Health Laboratory, London, UK (Jin et al., 1999, 2000). The Lo 18 strain of genotype G was a generous gift from M. L. Bentley and M. A. Afzal, Division of Virology, National Institute for Biological Standards and Control (NIBSC), Hertfordshire, UK. The ODATE-1 (30041) virus strain of genotype I has been described previously (Saito et al., 1996, 1998). Before use in experiments, viruses were grown in Green monkey kidney (GMK) cells maintained in Eagle’s minimal essential medium containing 2% foetal calf serum. A total of 12 Danish virus isolates, genotypes C, D, H and I, was studied by nucleotide sequencing part of the HN gene but it was not possible to grow these isolates in GMK cells (Tecle et al., 2001).

**Antibodies.** Monoclonal antibodies (mAbs) directed against the HN protein of the SBL-1 strain of mumps virus were used. The 11 mAbs used have been characterized by serological analysis and competitive ELISA in a previous study (Orvell, 1984). One group of three antibodies (group II), mAbs 2041, 5500 and 2072, could inhibit haemagglutinating (HA) activity and exhibited the highest neutralizing titres compared to other antibodies. Their binding locations on the HN protein have been defined previously (Orvell et al., 1997b). Other antibodies (group IV), mAbs 2034, 2082 and 5342, inhibited NA activity but did not block HA activity. Five rabbits were hyperimmunized with purified virions of the mAbs 2034, 2082 and 5342, inhibited NA activity but did not block HA activity and exhibited the highest neutralizing titres compared to other antibodies. Their binding locations on the HN protein has been described previously (Orvell et al., 1997a, b). The Kilham and RW virus strains were a generous gift from J.S. Wolinsky, Department of Virology, University of Texas Health Science Center, Houston, TX, USA. Virus strains Gloucester 1/UK 96 (genotype G) and Manchester 1/UK 95 (genotype H) were a generous gift from L. Jin, Enteric, Respiratory and Neurological Virus Laboratory, Central Public Health Laboratory, London, UK (Jin et al., 1999, 2000). The Lo 18 strain of genotype G was a generous gift from M. L. Bentley and M. A. Afzal, Division of Virology, National Institute for Biological Standards and Control (NIBSC), Hertfordshire, UK. The ODATE-1 (30041) virus strain of genotype I has been described previously (Saito et al., 1996, 1998). Before use in experiments, viruses were grown in Green monkey kidney (GMK) cells maintained in Eagle’s minimal essential medium containing 2% foetal calf serum. A total of 12 Danish virus isolates, genotypes C, D, H and I, was studied by nucleotide sequencing part of the HN gene but it was not possible to grow these isolates in GMK cells (Tecle et al., 2001).

**Immunofluorescence (IF) analysis.** The procedure for IF analysis was similar to that described previously (Rydbeck et al., 1986). GMK cells infected with the different strains of mumps virus were transferred to glass slides. The cells were then air dried and fixed in cold (−20 °C) acetone. mAbs were used at a 1:50 dilution of the original ascites fluid. After incubation with the antibodies at 37 °C for 30 min., the slides were washed with PBS, after which goat anti-mouse fluorescein-labelled antibodies were added and the incubation was repeated. After washing, Evans blue at a final concentration of 0.03% was added and the preparations were examined under a fluorescence microscope.

**Neutralization assays.** The procedure for end-point neutralization has been described previously (Orvell, 1976). Serial twofold dilutions of rabbit hyperimmune sera or human sera in a volume of 0.15 ml were mixed with an equal volume of virus (100–200 TCID₅₀/0.1 ml). The mixtures were shaken and incubated at room temperature for 1 h. After that time-period, 0.1 ml of the antigen–antibody mixtures were inoculated onto GMK cells in tissue culture tubes; two tubes were inoculated per antibody dilution. The inoculated tubes were incubated at 37 °C and inspected for cytopathic effect and final readings after 7 days of incubation.

**Nucleotide sequencing.** A selected coding area of the HN gene covering aa 341–380 of the HN protein was sequenced for 16 virus strains belonging to genotypes C, D, G, H, I and J. The SH gene of ten clonal isolates of the JL virus strain were also sequenced. The procedures for PCR, nucleotide sequencing and construction of phylogenetic trees have been described previously (Orvell et al., 1997a, b; Tecle et al., 2000).

Results

Genotypic characterization of the ODATE-1 virus strain

The ODATE-1 strain was isolated in 1993 during a mumps virus epidemic in the Akita prefecture in Japan. (Saito et al., 1996, 1998). The ODATE strain was associated with a high incidence of aseptic meningitis. Phylogenetic analysis of the SH gene of the ODATE strain shows that it belongs to genotype I of mumps virus and it groups together with Korean virus strains isolated in 1998, described by Kim et al., 2000 (Fig. 1).

Antigenic comparison of the HN protein of different genotypes

Eight neutralizing and three non-neutralizing (group I) mAbs directed against the HN protein of the SBL-1 strain of mumps virus (Orvell, 1984) were used for comparison of B cell epitopes between genotypes A, C, D, G, H and I (Table 1). The antibodies had been characterized previously for their ability to block different biological activities of the virus. Group II antibodies blocked haemolytic activity (HL) but not HA or NA activity; group III antibodies blocked HA and HL activity and group IV antibodies could inhibit NA and HL but not HA activity. The binding locations of the group III antibodies have been defined in a previous study on neutralization escape mutants formed by growing the virus in the presence of these antibodies. Antibodies 2041, 5500 and 2072 were found to create mutants with an exchange of an amino acid at position 354, 352 and 269, respectively, of the 582 aa long HN protein of the SBL-1 virus (Orvell et al., 1997b).

The eight mAbs belonging to group I, II and IV reacted with all ten virus strains (Table 1). The antibodies of group III, mAbs 2041, 5500 and 2072, did not react with virus strains belonging to genotypes C, D, G, H or I. A different reactivity
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was obtained when the three antibodies were tested with the three viruses of genotype A, the SBL-1, Kilham and JL virus strains. Antibody 5500 reacted with all three virus strains, antibody 2041 reacted with the Kilham strain but not with the JL virus and antibody 2072 reacted neither with the Kilham nor with the JL virus strain.

Comparison of a partial protein sequence of the HN protein from 21 virus strains

The results described showed that viruses of genotypes C, D, G, H and I were antigenically different from the SBL-1 strain of genotype A. It was also found that there existed antigenic differences between the three virus strains of genotype A. It was considered of interest to compare the amino acid sequence in the region where antibodies 2041 and 5500 bind. Of 16 virus strains collected at different time-points, a portion of the HN gene of each strain was sequenced and the amino acid sequence deduced (Fig. 2). In comparison to the SBL-1 strain, all virus strains of genotypes C–I investigated contained glutamine instead of proline at position 354 and aspartic acid instead of glutamic acid at position 356. Genotype H differed by containing the amino acids serine, tyrosine and proline at positions 357–359. These results could explain why the two antibodies could not bind to viruses of genotypes C, D, G, H or I. The lack of reactivity of mAb 2041 with the JL virus could not be explained, as the virus material should contain both the JL2 and the JL5 virus clones (Afzal et al., 1993); the sequence of the JL2 virus is identical to the SBL-1 strain in this region (Yates et al., 1996) and thus a reaction with mAb 2041 would have been expected if the virus material had contained the JL2 virus.

Table 1. Immunofluorescence reactivity of different mAbs in tests with ten strains of mumps virus

mAbs were grouped according to their ability to block different biological activities of mumps virus (Örvell, 1984). The group assignment of each mAb is shown in parentheses.

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Genotype</th>
<th>Immunofluorescence with mAb number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1933 (I)</td>
</tr>
<tr>
<td>SBL–I</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>Kilham</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>JL</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>V27</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>V6</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>RW</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>Gloucester 1/UK 96</td>
<td>G</td>
<td>+</td>
</tr>
<tr>
<td>Lo 18</td>
<td>G</td>
<td>+</td>
</tr>
<tr>
<td>Manchester 1/UK 95</td>
<td>H</td>
<td>+</td>
</tr>
<tr>
<td>30041</td>
<td>I</td>
<td>+</td>
</tr>
</tbody>
</table>
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Fig. 2. Deduced amino acid sequences obtained from nucleotide sequencing of part of the HN gene of 21 virus strains. The 16 virus strains representing members of genotypes C, D, G, H, I and J were sequenced in the present study. The JL2 and JL5 sequences were obtained from the work of Yates et al. (1996). The two mutant virus strains 310 and 320 were obtained by growing the SBL-1 virus in the presence of mAbs 2041 and 5500, respectively. These mutant viruses were sequenced in a previous study (Orvell et al., 1997b).

Nucleotide sequencing of ten clonal isolates of the JL virus strain

The JL virus material that had been used for antigenic comparison was subjected to plaque purification as described previously (Orvell et al., 1997b). Ten different clonal isolates plaque-purified three times were used for sequencing the SH protein gene (Fig. 3). Not one belonged to the JL2 strain but all ten clonal isolates were found to belong to the JL5 strain described previously (Afzal et al., 1993) by both phylogenetic analysis and comparison of protein sequences. It can be seen from Fig. 3 that phylogenetic analysis of sequences obtained previously of the SH gene of JL fall into one of two different groups, the JL2 group (D90232, CAA45239, JL-A, X63707, P22110 and JQ2367) or the JL5 group (JQ2368, NC003044, NP150070, AF201473, AAK83229, AAF70394 and the present clonal isolates 1, 4, 6, 10, 17, 15, 9, 5, 2 and 18).

Analysis of neutralizing antibodies in rabbit hyperimmune sera and human sera

The findings described above indicated that genotype A was antigenically distinct from virus strains of genotypes C, D, G, H and I. An antigenic difference was also found between the SBL-1, Kilham and JL5 virus strains of genotype A. It was considered of interest to see if antigenic differences could also be found between genotypes and within genotype A in tests with polyclonal antibodies from rabbits and humans. The results from testing with rabbit hyperimmune sera can be seen in Table 2. The pattern of neutralization titres did not allow a clear-cut antigenic distinction between genotypes C, D, G, H and I as these genotypes were all neutralized to high titres by antibodies directed against the Kilham (rabbit 203) and the RW (rabbits 204 and 205) strains but to lower titres by antibodies directed against the SBL-1 strain (rabbits 201 and 202). In contrast, a clear-cut serological distinction could be made...
Table 2. End-point neutralization titres in rabbit hyperimmune sera

End-point neutralization titres in rabbit hyperimmune sera directed against the A or D genotype of mumps virus in tests with six different mumps virus genotypes. Genotypes of each strain are shown in parentheses.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Neutralization titre with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBL (A)</td>
</tr>
<tr>
<td>Rabbit Immunized with strain Genotype</td>
<td></td>
</tr>
<tr>
<td>201</td>
<td>SBL</td>
</tr>
<tr>
<td>202</td>
<td>SBL</td>
</tr>
<tr>
<td>203</td>
<td>Kilham</td>
</tr>
<tr>
<td>204</td>
<td>RW</td>
</tr>
<tr>
<td>205</td>
<td>RW</td>
</tr>
</tbody>
</table>

Table 3. Neutralization titres in ten sera samples from children aged between 2 and 4 years old in tests with four different strains of mumps virus

<table>
<thead>
<tr>
<th>Human serum number</th>
<th>Vaccinated with strain Genotype</th>
<th>Neutralization titre with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>JL (A)</td>
</tr>
<tr>
<td>1</td>
<td>JL A</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>JL A</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>JL A</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>JL A</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>JL A</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>JL A</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>JL A</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>JL A</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>JL A</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>JL A</td>
<td>4</td>
</tr>
</tbody>
</table>

nt, Not tested.

between the three virus strains belonging to genotype A. Kilham virus was distinguished by being neutralized at high titre by the homologous rabbit serum but with distinctly lower titres with antibodies against SBL-1 or RW viruses. The JL5 virus strain was separated from the other two by being neutralized to high titres by rabbit hyperimmune sera directed against the RW virus strain (rabbits 204 and 205), a finding that was absent in tests with the SBL-1 virus strain. In its neutralization profile with the five rabbit hyperimmune sera tested, the JL5 virus strain was similar to the non-A genotypes.

Ten samples of human serum from children aged between 2 and 4 years were tested (Table 3). The children had been vaccinated with the JL virus strain. The neutralizing titres varied from 4 to 64 in tests with the JL strain. Neutralization titres were significantly lower, with four- to eightfold lower titres recorded in tests with the SBL-1 strain. In tests with the Kilham and V6 virus strains, lower neutralization titres were found compared to the JL strain but the difference was not as pronounced as with the SBL-1 strain.

Discussion

An antigenic comparison between different mumps virus strains was performed with the present mAbs about two decades ago (Örvell, 1984). All five different mAbs described belonging to group III with high neutralizing titres, mAbs 2041, 5500, 2072, 2073 and 2075, did not react with the RW virus strain of genotype D in biological tests (Örvell, 1984). In a subsequent study, the antibodies 2041, 5500 and 2072 were found not to react with a number of virus strains of genotypes C and D collected in Stockholm from 1971 to 1985 (Örvell et al., 1997b). The lack of reaction of antibodies 2041, 5500 and
2072 with the C and D genotypes from Stockholm could be explained by the presence of different amino acids at the positions where the antibodies were found to bind on the protein (Örvell et al., 1997b). For antibodies 2041 and 5500, the lack of binding was due to the presence of the amino acids glutamine instead of proline at position 354 and aspartic acid instead of glutamic acid at position 356, which were found in the C and D genotypes. When a number of virus strains belonging to genotypes C, D, G, H, I and J were investigated in the present study, they were all found to contain the same amino acids, glutamine and aspartic acid, at these positions. Also, the Urabe strain of genotype B contains the same amino acids at these positions (Yates et al., 1996). These findings indicate that there exist a pronounced conservation of this biologically important epitope/epitopes in the non-A genotypes.

The results from this and previous studies show that there exists a significant antigenic difference between the SBL-1 strain of genotype A and genotypes B, C, D, G, H and I (Örvell, 1984; Yates et al., 1996; Örvell et al., 1997b). By using mAbs directed against the Urabe strain of genotype B, Yates et al. (1996) could show that their two mAbs, mAbs 1970 and 1711, with the highest HA-inhibiting and neutralizing titres did not react with the Enders, Rubin, SBL-1 or JL2 strains of genotype A but reacted with the group C virus strains. Antibody 1970 bound to JL5 but not to JL2, whereas antibody 1711 bound neither JL5 nor JL2. Yates et al. (1996) also showed an antigenic difference between the SBL-1 and the JL2 and JL5 virus strains. A similar antigenic difference was demonstrated in the present study between the JL5 and the SBL-1 strain. The JL virus preparation in the present study was concluded to contain the JL5 virus but not the JL2 virus. The results from cross-neutralization experiments showed that the SBL-1, JL5 and Kilham virus strains form three distinct serotypes within genotype A. An unexpected finding was that the JL5 isolate was efficiently neutralized by antibodies directed against the RW strain of genotype D. The difference between JL5 and RW viruses in the antigenic area of antibody 2041 is the presence of aspartic acid at position 356 in RW and glutamic acid in JL5. These two amino acids show structural similarities. It is therefore possible that the pronounced neutralization of JL5 by the rabbit hyperimmune sera directed against strain RW is due to the presence of glutamine at position 354 in both virus strains. A new neutralizing epitope at positions 329–340 has been described recently (Cusi et al., 2001). A unique structural similarity between RW and JL5 with the amino acid leucine instead of serine at position 336 (Yates et al., 1996) may also be of importance for the pronounced reactivity between JL5 and RW.

The JL virus has been used from 1982 for vaccination of Swedish children. The significantly lower titres against the SBL-1 virus strain in the sera from vaccinated children may indicate that the JL5 virus is suboptimal for protection against the SBL-1 strain in Sweden. The low neutralization titres against the SBL-1 virus may be one of more possible explanations for the continued occurrence of the SBL-1 strain in Sweden (Tecle et al., 1998; Nöjd et al., 2001). The JL virus strain has been reported to contain the JL2 and JL5 strains in the same vaccine preparation in proportions of 1:5 (Afzal et al., 1993); however, a large amount of phylogenetic data on the dominating sequence after growing the virus in tissue culture has shown that this proportion between JL2 and JL5 is not a static phenomenon. For example, the JL variant of mumps vaccine grown on Vero cells and investigated by Yeo et al. (1993) was found to contain the JL2 virus (X63707 in Fig. 3). The JL2 virus (D90232 in Fig. 3) described by Takeuchi et al. (1991) was also found in the total virus material of infected cells but, in the present study, the vaccine preparation was found to contain JL5 after growing the virus in tissue culture. The JL2 virus has an amino acid sequence that is similar to the SBL-1, Enders and Rubini strains of genotype A in the epitope(s) of mAbs 2041 and 5500, whereas the JL5 virus shows an amino acid sequence that is partly similar to both genotypes A and non-A (glutamic acid at position 356 like genotype A and glutamine in position 354 like non-A genotypes). It is possible that the JL2 virus will give better protection than JL5 against the SBL-1 strain. However, the antigenic match between JL2 and SBL-1 is not ideal. The amino acid sequence of JL2 does not conform with the SBL-1 strain in the neutralizing epitope of antibody 2072 around position 269 (Yates et al., 1996; Örvell et al., 1997b). The epitope of antibody 2072 is unique for SBL-1, as all other known mumps virus strains have two different amino acids, positions 265 and 266, compared to SBL-1 in this region (Yates et al., 1996; Örvell et al., 1997b; Tecle et al., 1998). Also, Yates et al. (1996) have found an antigenic difference between SBL-1 and JL2 by using mAbs directed against the Urabe strain. Three of their neutralizing mAbs reacted with the SBL-1 virus but did not react with the JL2 strain.

In recent years, the neuropathogenicity of different mumps virus strains has been investigated. A number of genotypes (genotypes C, D, G, H, I and J) has been found to exhibit a pronounced neuropathogenic capacity (Saito et al., 1996, 1998; Rubin et al., 1998, 2000; Tecle et al., 1998, 2001, 2002; Saika et al., 2002). The present study could not find any clear-cut antigenic difference between the non-A genotype neuro-pathogenic virus strains. In the case that future research will confirm these findings, it may be important to direct vaccine protection against this group of neuropathogenic viruses.

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References

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