

Mumps virus neutralizing antibodies do not protect against reinfection with a heterologous mumps virus genotype

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Abstract

In April 1999, a previously healthy 22-year-old woman was taken ill with fever and bilateral swelling of the parotid glands. A chronic course of disease extending from April to December was found with swelling of the parotid glands, fatigue, low grade fever, episodes of tachycardia and nightswetting. Mumps virus RNA of genotype A character based on the SH (small hydrophobic) protein gene classification was demonstrated in three serum samples collected during the course of clinical disease. Different criteria for reinfection were fulfilled including demonstration of IgG antibodies by ELISA in a preinfection serum sample. The preinfection serum sample of the patient was able to efficiently neutralize the infectivity of a heterologous genotype D strain but was unable to neutralize the homologous genotype A virus. The findings in the present study may offer an explanation of a mechanism behind previously observed vaccine failures and the occurrence of reinfection with heterologous mumps virus strains. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Mumps virus belongs to the family Paramyxoviridae, genus Rubulavirus [1]. The single stranded RNA genome contains seven genes; the nucleocapsid (N), phospho (P), membrane (M), fusion (F), small hydrophobic (SH), haemagglutinin-neuraminidase (HN) and (L) protein genes [2,3]. The SH gene is the most variable gene of mumps virus strains, it encodes a protein of 57 amino acids and it has been used to genotype mumps virus in nine genotypes, named A-I [4,5]. Genotype specific epitopes for induction of neutralizing antibodies have been shown between the A genotype in comparison to the B, C and D genotypes [6,7]. The possibility of reinfection with mumps virus causing clinical symptoms has been debated for a long time. In

a recent comprehensive study, reinfection with mumps virus has been demonstrated by the application of different serological methods [8]. However, the presence of IgG in the absence of IgM-antibodies in sera from patients with mumps virus reinfection makes serological diagnosis problematic. In such cases it is often necessary to demonstrate the presence of virus RNA by the polymerase chain reaction (PCR).

Mumps virus infection is believed to cause a benign disease of short duration. Few cases of chronic mumps virus infection have been described in the literature. Chronic mumps virus infection in the central nervous system (CNS) extending for several years of one human case has been described by Finnish workers [9]. There were signs of chronic neurogenic lesions without active denervation. In the present study the occurrence of a chronic case of mumps is described. The patient was taken ill in spite of the presence of a preexisting humoral immunity directed against a heterologous genotype of mumps virus. The results provide information about the importance of genotype specific immunity for protection against the different genotypes of mumps virus.

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2. Materials and methods

2.1. Materials for investigation

Five consecutive serum samples from a patient with chronic mumps virus infection were analyzed by serological testing and molecular analyses.

Twenty-five serum samples from an age-matched control group were investigated for determination of neutralization titres against the A and D genotypes of mumps virus.

2.2. ELISA testing of serum samples

Titres of IgG and IgM antibodies in the patient's serum samples were determined by ELISA according to accredited test procedures at the Department of Clinical Virology, Huddinge University Hospital. A preparation of purified glycoproteins of the SBL-1 strain was used as the antigen for determination of IgG titers [10]. For IgM determination, a previously described IgM capture procedure was used [10].

2.3. Virus neutralization assay

The procedure for end-point neutralization has been described previously [11]. Serial two-fold dilutions of sera in a volume of 0.15 ml of Eagle's Medium containing 2% fetal calf serum (FCS) were mixed with an equal volume of virus containing 100–200 TCID₅₀/0.1 ml. The mixtures were shaken and incubated at 20°C for 1 h. After that time period 0.1 ml of the antigen-antibody mixtures was inoculated on Green Monkey Kidney (GMK) cells in tissue culture tubes; two tubes were inoculated per antibody dilution. The inoculated tubes were incubated at 37°C. The tubes were inspected for cytopathic effects in a light microscope and final readings were made after 7 days incubation. A neutralizing antibody titre of less than 2 was defined as seronegativity.

2.4. Molecular analyses

Genotyping by PCR was performed on materials which could be amplified in a nested PCR reaction with primers common to all known genotypes of the SH protein gene [12]. Isolation of mumps virus RNA from the serum samples was performed as described previously [12]. For nucleotide sequencing of the SH gene, a 415 bp fragment was amplified by PCR as described previously [12]. For each fragment one of the inner primers (BJSHPR2) was 5'-biotinylated and the outer primer (BJSHPR1) was coupled at the 5' end to a universal M13 primer sequence. Direct DNA sequencing was performed as described previously [7].

3. Results

3.1. Description of disease history

In April 1999, a previously healthy 22-year-old female university student engaged in long distance running was taken ill with bilateral swelling of the parotid glands, a feeling of ill-health, fatigue and shortness of breath at exercise. She was consulting a medical doctor at an out-patient clinic in the beginning of May. She reported that she had been taken ill in mumps at the age of 4 years and that she had received vaccination with measles, mumps and rubella (MMR) virus at the age of 12 years. The patient was given medicine to alleviate breathing. After medical treatment her breathing while exercising was easier, but during the summer she continued to feel decreased strength at exercise, fatigue and bilateral fluctuating swelling of the parotid glands. Late in the summer she also felt uncomfortable from episodes of tachycardia and nightswetting. Due to her ill health she attended the Infectious Disease Clinic in the beginning of October. At medical examination bilateral swelling of the parotid glands and a low-grade fever were recorded. Erythrocyte sedimentation rate and C-reactive protein (CRP) were not elevated and a number of laboratory tests on blood, liver and thyroid function were all within normal values. Some laboratory tests to assess immunological function were performed. Quantities of IgA, IgG and IgM immunoglobulin class, subclass determination and CD4/CD8 counts showed no abnormalities. In the middle of December the patient was beginning to recuperate with less fatigue and reduced swelling of the parotid glands. At her last visit to the Infectious Disease Clinic, on 8 February 2000, the patient felt perfectly healthy and there was no longer any enlargement of the parotid glands.

3.2. Results of virus laboratory tests

The results of virus laboratory tests performed on the patient's serum samples are shown in Table 1. PCR reactivity was found in the three samples collected during her clinical illness (serum samples B–D), but not in the serum samples taken before and after the disease period. The PCR products of the three samples were sequenced. The three nucleotide sequences were identical and were found to correspond to the so called, 'SBL strain' of genotype A (data not shown, [10]). In all the five samples the presence of high titres of IgG antibodies in the absence of IgM antibodies was found.

The serum sample A from 1996 contained neutralizing antibodies against genotype D, but neutralizing antibodies against genotype A were not found.

All other serum samples contained neutralizing antibodies against genotype D, but neutralizing antibodies

against genotype A were not found except for the convalescent (E) serum sample. A significant increase of the titre of neutralizing antibodies against genotype A was found in the convalescent serum sample.

In comparison to the sera of the patient, most of the 25 sera from persons in the same age group contained lower titres of neutralizing antibodies against both genotypes (Table 1, Fig. 1). The percent seronegativity in the group against genotype A and D was 68% (17/25) and 64% (16/25), respectively. The titres against genotype D were generally higher, but in some serum samples the titre against genotype A was on the same level as the titre against genotype D (serum no. 16, 18, 19, 20 and 23).

The quotas of D/A neutralization titres varied from four or more (serum A,B,C,D,E,21,22,24 and 25) to one or less (serum no. 16, 18, 19, 20 and 23).

4. Discussion

In this investigation a chronic case of mumps was identified. There were no signs of any immunological dysfunction judged from the patient's history or in laboratory testings. The patient informed that she had suffered from mumps at the age of 4 years. It was confirmed that the patient had undergone a previous mumps virus infection by the demonstration of IgG antibodies and neutralizing antibodies against genotype D in a serum sample from 1996, before the disease period. Circulating viral RNA was identified in the serum of the patient three times for a period of 6 months and the time period coincided with the patient's

symptoms of mumps. The virus that was identified was similar to the so called 'SBL-1' strain of genotype A. This mumps virus strain is the current predominant mumps virus strain in Sweden and it is associated with parotitis but seldom causes meningitis [10]. It has been shown that the D genotype of mumps virus was cocirculating with genotype A in Sweden from 1970 to 1985, and after that time period the D genotype has disappeared [10]. The C genotype of mumps has been identified in Sweden in the year 1983 and 1984 [10]. This C genotype is immunologically similar to genotype D [7]. The patient's serum sample from 1996 exhibited neutralizing antibodies against genotype D. It is, therefore, probable that she had undergone an earlier infection with a genotype D like virus. The vaccination with genotype A (Jeryl Lynn strain) that the patient received in 1989 had not resulted in any demonstrable neutralizing antibodies against the A genotype in the same serum sample from 1996. In spite of the fact that the patient had recourse to neutralizing antibodies against genotype D she was not protected against infection with genotype A. After the disease period the patient had built up neutralizing antibodies against genotype A.

It has been shown previously that immunological differences exist between the A and the D genotype of mumps virus [6,7]. The major population of neutralizing antibodies formed in rabbits after hyperimmunization with the A and D genotypes of mumps virions have been found to be type specific [7]. Neutralization titres against the homologous strain was always higher than against the heterologous strain. It was, therefore, of interest to investigate if a similar situation existed in

Table 1
The results of serological and PCR tests performed on consecutive serum samples from a patient with chronic mumps virus infection (serum A–E), neutralization antibody titers were determined against the A and D genotype of mumps virus in the preinfection serum sample (A), samples collected during clinical illness (B–D) and the convalescent serum sample (E) and in sera from 25 persons in the age group 20–25 years old^a

Serum design	Time for collection	NT titer against genotype		IgG	IgM	PCR	Virus genotype
		A(SBL)	D(V6)				
A	Febr., 1996	<2	8	1000	Negative	Negative	–
B	May 5, 1999	<2	8	1000	Negative	Positive	A
C	Oct. 4, 1999	<2	16	1000	Negative	Positive	A
D	Nov. 30, 1999	<2	16	1000	Negative	Positive	A
E	8 February 2000	4	16	1000	Negative	Negative	–
No. 1–15	1998	<2	<2	ND	ND	ND	ND
No. 16	1998	2	<2	ND	ND	ND	ND
No. 17	1998	<2	2	ND	ND	ND	ND
No. 18, 19	1998	2	2	ND	ND	ND	ND
No. 20	1998	4	4	ND	ND	ND	ND
No. 21	1998	<2	8	ND	ND	ND	ND
No. 22	1998	2	8	ND	ND	ND	ND
No. 23	1998	8	8	ND	ND	ND	ND
No. 24	1998	4	16	ND	ND	ND	ND
No. 25	1998	8	32	ND	ND	ND	ND

^a ND, not determined

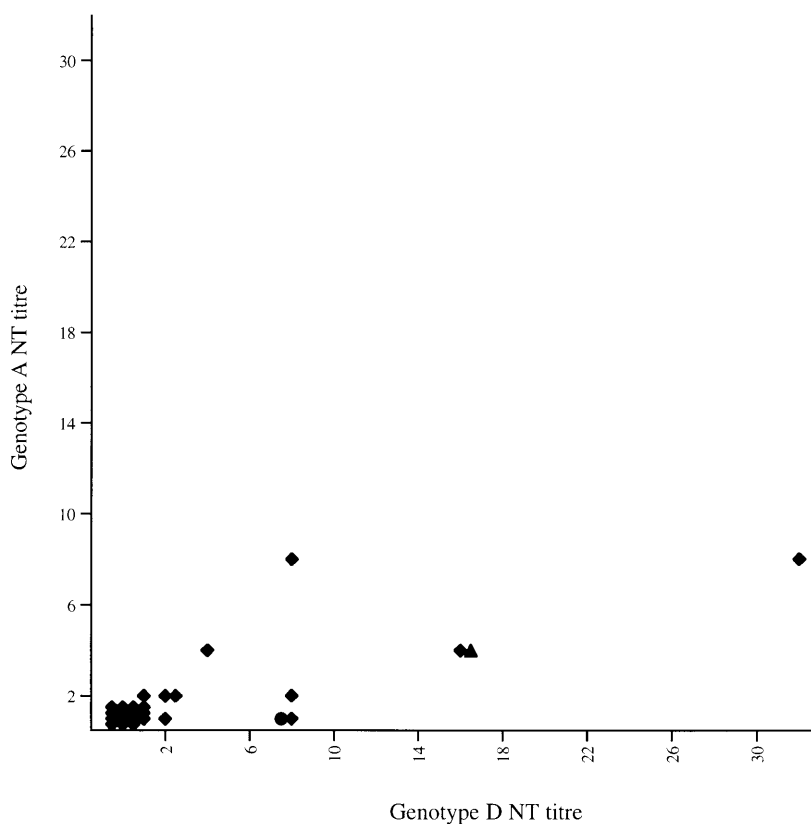


Fig. 1. Relationships between neutralization titres against genotype D and A in 25 sera from persons 20 to 25 years old (◆) and in the two sera of the patient taken before (●) and after (▲) the disease period.

humans by determination of genotype specific titres of neutralizing antibodies. The persons in the group were similar in age to the patient. It was not known if the persons in the group had received vaccination against mumps virus. The percentage of seronegativity was found to be in the order of 60–70% against both the genotypes. This figure for seronegativity was higher than the results from a large study on young persons born in 1980 where an unselected age group showed a seronegative value of 38% [13]. In the latter age group, 60% had a documented vaccination against mumps virus [13].

Mass vaccination against mumps with the Jeryl Lynn strain of genotype A was introduced in Sweden in 1982. Vaccination was carried out at the age of 18 months and in some cases around 12 years of age. As the persons in the present study were somewhat older and probably had escaped vaccination at 18 months of age, it is possible that the 25 individuals in the group born during 1973–1978 had a lower rate of vaccination against mumps virus compared with persons born in 1980 which may explain the higher seronegative values in the former age group. In the present study neutralization titres against genotype D were somewhat higher than the titres against genotype A.

However, when the quotas of D/A neutralization titres were compared with individual sera a pronounced variation was found which is likely to reflect both immunological differences between the genotypes and a different exposure to the two genotypes in the past.

The occurrence of sporadic mumps epidemics is known to have taken place in populations with high vaccine coverage [14,15]. In the epidemics in Switzerland in 1992–1993 and in 1995 and in Portugal in 1996, the populations were vaccinated with a heterologous genotype. Taken together, these reports indicate that the vaccine may not be effective against heterologous genotypes. Determination of genotype specific neutralization titres in the sera of patients before and after mumps virus infection may shed light on the level of cross-protection that can be reached by a prior mumps virus infection with a heterologous genotype. A clear neutralization titre against genotype D did not protect the patient from infection with the A genotype. A similar situation to the one described in the present study may exist also after vaccination with monospecific vaccines containing only one genotype of mumps virus.

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