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Mumps-specific cross-neutralization by MMR vaccine-induced antibodies predicts protection against mumps virus infection

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ABSTRACT

Background: Similar to other recent mumps genotype G outbreaks worldwide, most mumps patients during the recent mumps genotype G outbreaks in the Netherlands had received 2 doses of measles, mumps and rubella (MMR) vaccine during childhood. Here, we investigate the capacity of vaccine-induced antibodies to neutralize wild type mumps virus strains, including mumps virus genotype G.
Methods: In this study, we tested 105 pre-outbreak serum samples from students who had received 2 MMR vaccine doses and who had no mumps virus infection ($n = 76$), symptomatic mumps virus infection ($n = 10$) or asymptomatic mumps virus infection ($n = 19$) during the mumps outbreaks. In all samples, mumps-specific IgG concentrations were measured by multiplex immunoassay and neutralization titers were measured against the Jeryl Lynn vaccine strain and against wild type genotype G and genotype D mumps virus strains.
Results: The correlation between mumps-specific IgG concentrations and neutralization titers against Jeryl Lynn was poor, which suggests that IgG concentrations do not adequately represent immunological protection against mumps virus infection by antibody neutralization. Pre-outbreak neutralization titers in infected persons were significantly lower against genotype G than against the vaccine strain. Furthermore, antibody neutralization of wild type mumps virus genotype G and genotype D was significantly reduced in pre-outbreak samples from infected persons as compared with non-infected persons. No statistically significant difference was found for the vaccine strain. The sensitivity/specificity ratio was largest for neutralization of the genotype G strain as compared with the genotype D strain and the vaccine strain.
Conclusions: The reduced neutralization of wild type mumps virus strains in MMR vaccinated persons prior to infection indicates that pre-outbreak mumps virus neutralization is partly strain-specific and that neutralization differs between infected and non-infected persons. Therefore, we recommend the use of wild type mumps virus neutralization assays as preferred tool for surveillance of protection against mumps virus infection.

1. Introduction

At the end of 2009, large mumps outbreaks started in the Netherlands and continued for 3 years, followed by some years with smaller and more local mumps outbreaks [1,2]. Most of the patients were young adults who had received 2 measles, mumps, and rubella (MMR) vaccinations during childhood at 14 months and 9 years of age, according to the Dutch national immunization program [3,4]. Similar to other recent mumps outbreaks among MMR vaccinated persons worldwide, the mumps outbreaks in the Netherlands were dominated by mumps genotype G virus strains, whereas the Jeryl Lynn mumps strain of the MMR vaccine belongs to genotype A. It has been shown previously that vaccinated persons develop sufficient neutralizing antibodies against wild type mumps genotype G virus, although the neutralization capacity is lower against the

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wild type strain as compared with the vaccine strain [5,6]. However, no correlate of protection has been defined so far that distinguishes MMR vaccinated persons with sufficient immunological protection from those who are not protected against mumps virus infection.

Here, we aimed to investigate the capacity of vaccine-induced antibodies to neutralize various mumps virus strains and to establish a correlate of protection based on pre-outbreak neutralizing antibody titers. Pre-outbreak serum samples were selected from MMR vaccinated students who were infected with mumps virus during the outbreak and these samples were compared with samples from non-infected students. The correlation between mumps-specific IgG concentrations and functional antibodies against the Jeryl Lynn vaccine strain was determined. Furthermore, we studied strain-specific neutralization between the Jeryl Lynn vaccine strain, mumps virus genotype G, which circulated in the Netherlands during the recent mumps outbreaks among vaccinated persons, and mumps virus genotype D, which caused a mumps outbreak among unvaccinated children in 2007–2009 in the Netherlands [7]. We investigated pre-outbreak samples from both persons with symptomatic and asymptomatic infections to determine if reduced neutralization of wild type strains affects the occurrence of clinical mumps in mumps virus infected persons.

2. Methods

2.1. Study subjects and pre-outbreak serum samples

All pre-outbreak sera used in this study were samples from a medically ethically approved serological study previously described (NL38042.041.11) [8]. This retrospective study was performed among students from the city of Utrecht in 2012. Besides a self-sampled dried blot spot sample and a filled out questionnaire concerning MMR vaccination history, risk factors and mumps symptoms, a serum sample was retrospectively obtained from each student. In addition, MMR vaccination history was verified for 80.5% of the students from the data recorded in the nationwide vaccination registration system (Praeventis) [8]. In total, 619 pre-outbreak serum samples collected between 2007 and 2010 were obtained and included for the initial analysis to identify mumps virus infections based on mumps-specific antibody concentrations [8]. For this study, a selection was made consisting of 105 pre-outbreak serum samples from persons who had received 2 MMR doses and who had symptomatic mumps virus infection, asymptomatic mumps virus infection or no mumps virus infection during the mumps outbreaks that followed. The serological criteria for mumps virus infection were a fourfold increase in IgG concentration or a single-point cutoff at 1500 RU/ml [8]. Persons with symptomatic mumps virus infection fulfilled at least one of these serological criteria and indicated in the questionnaire that they developed mumps symptoms during the period 2009–2012 ($n = 10$). Persons with asymptomatic mumps virus infection fulfilled also at least one of the serological criteria, but they did not report any mumps symptoms in the questionnaire ($n = 19$). Non-infected persons did not fulfill any of the serological criteria for mumps virus infection and did not report mumps symptoms ($n = 76$). The 105 pre-outbreak serum samples selected for this study included all available samples from infected persons that fulfilled the criteria in this study and a selection of the samples from non-infected persons. The geometric mean IgG concentrations in the selected pre-outbreak samples from persons without a mumps virus infection did not differ from the total cohort from the initial study (192 RU/ml versus 171 RU/ml; $p = 0.618$) [8].

2.2. Cell culturing and mumps virus isolation

Vero cells (Monkey African Green Kidney, ECACC) were cultured at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal calf serum (FCS), penicillin, streptomycin, and L-glutamine. For inoculation of the Jeryl Lynn mumps virus strain, DMEM supplemented with 2% FCS, penicillin, streptomycin, and L-glutamine was used. The same medium composition was used for isolation of wild type mumps virus strains, with the addition of nystatin. Virus culturing was performed at 36 °C in 5% CO_2. Mumps virus Jeryl Lynn strain was seeded on Vero cells and passaged 2 times before harvesting. Mumps virus strains belonging to genotype G (MuVi/Utrecht.NLD/40.10) and genotype D (MuVi/Sint Philipsland.NLD/02.08) were isolated from laboratory-confirmed mumps virus-positive throat swabs and were passaged 2 times and 4 times respectively before harvesting. Virus stocks were stored at −80 °C until use. Aliquots were made from all virus stocks to avoid multiple freeze–thaw cycles.

2.3. Multiplex IgG immunoassay

Mumps-specific IgG concentrations were measured with a fluorescent bead-based multiplex immunoassay (MIA) using Luminex technology as described previously [9]. Purified Jeryl Lynn antigen was coupled to carboxylated beads for detection of mumps-specific IgG. Samples were 1:200 diluted in assay buffer (phosphate buffered saline (PBS) containing 0.1% Tween-20 and 3% bovine serum albumin). On each plate, the WHO International Standard Anti Rubella Immunoglobulin RUBI-1-94 (The National Institute for Biological Standards and Control), controls and blanks were included. Antibody concentrations were expressed in local (RIVM) units per milliliter (RU/ml) and were based on the fluorescent intensity of the reference serum curve RUBI-1-94, which has a mumps-specific IgG concentration of 4384.512 RU/ml and was selected as alternative serological standard for mumps to enable comparison and bridging of our results to other studies [8]. When the 1:200 sample dilution fell outside the range of the reference serum curve, further dilutions up to 1:50,000 were used for analysis.

2.4. Focus-reduction neutralization test (FRNT)

Neutralization by vaccine-induced antibodies was tested by FRNT, partly based on the protocol described by Vaidya et al. [10]. Neutralization tests were performed in 96-wells plates. Four-fold dilutions were made in DMEM supplemented with 2% FCS, penicillin, streptomycin, and L-glutamine. Viruses and samples were mixed and incubated for 2 h at 37 °C. Medium was removed from pre-cultured Vero cells and 50 µl of virus mixture was added to each well. Plates were incubated for 4 h at 36 °C, before the mixture was removed from the Vero cells and 200 µl of 0.8% carboxymethylcellulose in DMEM was added to each well. Plates were incubated for 40 h at 36 °C with 5% CO_2 before they were washed with PBS and thereafter fixed with a mixture of acetone and methanol (2:3). After 10 min, plates were washed with ice cold PBS, and then incubated with block buffer (PBS containing 1% bovine serum albumin) for 30 min at 36 °C. Anti-mumps nucleoprotein antibody (7B10, Abcam) was diluted in block buffer (1:3000) and 100 µl was added to each well. After incubation for one hour at 36 °C, plates were washed 3 times with PBS containing 0.1% Tween-20 (PBST) before 100 µl of goat-anti-mouse IgG-HRP (DAKO) diluted in block buffer (1:2000) was added to each well and plates were incubated for one hour at 36 °C. Plates were washed 3 times with PBST and wells were stained with 50 µl of True Blue peroxidase substrate (Kirkegaard & Perry Laboratories). The numbers of plaques were counted and the 50% neutralizing dose (ND_{50}) of each sample was calculated with the Kärber for-

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