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Research report

# Development of a simplified and convenient assay for cell-mediated immunity to the mumps virus

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ABSTRACT

Because methods for measuring cell-mediated immunity (CMI) to the mumps virus are expensive, time-consuming, and technically demanding, the role of CMI in mumps virus infection remains unclear. To address this issue, we report here the development of a simplified method for measuring mumps virus-specific CMI that is suitable for use in diverse laboratory and clinical settings. A mumps vaccine was cultured with whole blood, and interferon (IFN)-γ released into the culture supernatant was measured using an enzyme-linked immunosorbent assay. IFN-γ production in blood from vaccinated subjects markedly increased in response to the vaccine and decreased before the antibody titer decreased in some cases, suggesting that this assay may be used as a simple surrogate method for measuring CMI specific for the mumps virus.

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## 1. Introduction

The mumps vaccine was licensed in the United States and Japan in 1967 and 1981, respectively. In 1977, the United States Advisory Committee on Immunization Practices recommended a single dose for routine vaccination, and the number of mumps patients subsequently decreased dramatically (Dayan et al., 2008; Dayan and Rubin, 2008). However, because outbreaks were observed even among vaccinated people, two doses of the mumps vaccine were recommended in 1989 (van Loon et al., 1995; Rubin and Plotkin, 2013). In 2010, the mumps vaccination coverage was approximately 90.5% in the United States (Centers for Disease Control and Prevention (CDC), 2011a,b) but only approximately 30% in Japan because mumps vaccination was not part of the routine immunization schedule.

In response to mumps virus infection, the host activates mechanisms such as humoral and cell-mediated immunity (CMI). The levels of circulating antibodies can be readily measured and are often used to estimate immunity to mumps virus infection. Neutralizing antibodies against the mumps virus protect against infection, but their effective titer is unknown (Plotkin, 2010). Furthermore, although CMI is evaluated using methods such as lymphocyte proliferation assays, cytotoxic assays, or IFN-γ production in the supernatant of peripheral blood mononuclear cell (PBMC) cultures (Chiba et al., 1982; Jokinen et al., 2007; Vandermeulen et al., 2009; Centers for Disease Control and Prevention (CDC), 2011a,b), these methods are time-consuming and require an academic laboratory, which hampers efforts to evaluate the role of CMI in mumps virus infection.

To clarify the characteristics of mumps-specific CMI, we report the development of a simplified CMI measurement system that uses whole blood to determine mumps-specific

*Abbreviations:* CMI, cell-mediated immunity; CTL, cytotoxic T lymphocyte; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ; IR, interferon-γ release; PBMC, peripheral blood mononuclear cell.

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IFN-γ release (IR); this assay is based on our published IR assay (Otani et al., 2009).

## 2. Materials and methods

### 2.1. Study population

Five healthy adults (age, 37–57 years) and 12 young adults (age, 18–24) were enrolled in the study. Enzyme immunoassay (EIA) titers <4 are considered to indicate inadequate immunity, and medical workers in Japan whose titer levels correspond to this range are recommended to receive the mumps vaccine by the Japanese Society for Infection Prevention and Control. In the present study, subjects aged 37–57 years had an EIA titer >4. The remaining 12 subjects with uncertain vaccination histories and EIA titers <4 were vaccinated against mumps.

The Ethics Committee of Hyogo College of Medicine approved this study, and we collected blood after obtaining written informed consent from all subjects. Peripheral blood samples were obtained from the healthy adults to evaluate the conditions of the IR assay.

### 2.2. IR assay

A live mumps vaccine (Hoshino strain, Kitasato Institute, Tokyo, Lot LC005, $>10^4$ tissue culture infective dose of 50/ml) was employed for the IR assay. The live vaccine was prepared by reconstituting the virus stock with sterile distilled water according to the manufacturer's protocol. Heparinized whole blood (100 μl) was added to flat-bottomed microtiter plates and incubated with varying amounts of the mumps vaccine diluted with RPMI 1640 medium in a final volume of 200 μl/well. The cocultivations were conducted within 1 h of drawing the blood samples. The culture supernatants (100 μl) were collected 96 h after cultivation, and the IFN-γ concentration was quantified using an enzyme-linked immunosorbent assay (IFN-γ Assay Kit; BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. Either phytohemagglutinin (final concentration, 2.5 μg/ml) or medium was added to the blood rather than the mumps vaccine to serve as the positive or negative control, respectively. The amount of IFN-γ released in the negative and positive control wells in all experiments was <4 pg/ml and >100 pg/ml, respectively,

### 2.3. Antibody titration

The levels of mumps virus-specific antibodies in the sera were determined by a commercial laboratory (SRL, Inc. Tokyo) using an EIA.

## 3. Results

### 3.1. Sensitivity of the IR assay for detecting the mumps vaccine

To determine whether the IR assay described above was suitable, IFN-γ levels were determined as a function of the amount of vaccine added to the blood samples. IFN-γ production was high in five healthy adults at vaccine doses of 50 or 75 μl and decreased with a dose of 100 μl in all subjects except for one (Fig. 1). This decrease at the highest dose may have been the result of a buffering effect of the vaccine; however, the cause is unclear because vaccine matrix is not known. Therefore, 100 μl of whole blood was cultured with a mixture of 75 μl of vaccine and 25 μl of RPMI 1640 medium in each well for subsequent IR assays. We next preformed the IR assay under the same conditions described above except that the mumps vaccine inactivated using ultraviolet light was used as the antigen. We observed similar levels of IFN γ production and therefore used the live mumps virus vaccine in subsequent experiments.

### 3.2. Determination of the culture duration of the IR assay

We found that IFN-γ production increased in all samples in response to the addition of the mumps antigen (Fig. 2). Although IFN-γ was produced after 48 h, additional IFN-γ production was obtained after 96 h. After culture for at least 6 days, hemolysis was observed in some of the samples. Therefore, culture for 96 h was deemed appropriate for determining whether IFN-γ was produced when the mumps vaccine was cultured with whole blood.

### 3.3. Evaluation of the stability of whole blood

To determine whether the IR assay required the analysis of whole blood immediately after collection and to evaluate the functional stability of whole blood, whole blood samples were cultured with antigen 0, 4, or 8 h after collection, and IFN-γ levels were measured after 96 h (Fig. 3). The samples were maintained at room temperature until the assay was performed. The IFN-γ levels differed only negligibly between the samples cultured with the antigen immediately after collection and those cultured after 4 h. However, the IFN-γ levels decreased in two of the samples cultured for 8 h. These results suggest that it is appropriate to culture whole blood with the antigen within 4 h after sample collection. In the following study, all of the samples were assayed within 1 h after collection.

### 3.4. Comparison of IR assay results and mumps virus-specific antibody titers before and after vaccination

Samples acquired from twelve subjects who were vaccinated because of their low or undetectable antibody titers were assayed for CMI and antibody titers before and after vaccination. At 4 weeks after vaccination, the antibody titer of subjects 3, 6, 8, 11, and 12 increased by over 2 fold relative to the values before vaccination, and the other seven subjects with undetectable antibody titers had an EIA titer >2 (Table 1). The IFN-γ production of subjects 2, 3, 5, 7, 8, 10, and 12 increased by more than 2 fold but that of the five other subjects showed less than a 2-fold increase at 4 weeks after vaccination. However, subjects 9 and 11, who were among the four subjects who provided samples 2 weeks after vaccination, exhibited a >2-fold increase in IFN-γ production at 2 weeks and a decrease at 4 weeks after vaccination.

## 4. Discussion

CMI to the mumps virus was measured before and after vaccination using our new, simplified IR method. We detected changes in CMI after vaccination and found that CMI decreased in some cases; therefore, a larger study population needs to be analyzed. Several methods are used to evaluate CMI to the

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