Immune Responses to Mumps Vaccine in Adults Who Were Vaccinated in Childhood

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Background. In a mumps outbreak in the United States, many infected individuals were adults who had received 2 doses of mumps vaccine. The persistence of cellular immunity to mumps vaccine has not been defined.

Methods. This was an observational, nonrandomized cohort study evaluating cell-mediated and humoral immunity to mumps in 10 vaccinated and 10 naturally immune adults. Mumps-specific T cell activation and interferon (IFN)-γ production were measured using lymphoproliferative and flow cytometry assays, and mumps immunoglobulin (Ig) G was measured using enzyme-linked immunosorbent assay.

Results. T cell immunity to mumps was high in both groups; 70% of vaccinated and 80% of naturally immune individuals had a positive (>3) stimulation index (SI) (P = 1.0). The mean percentages of mumps-specific CD4+ T cells that expressed CD69 and produced IFN-γ were equivalent in the 2 groups: 0.06% and 0.12%, respectively (P = .11). The mean SIs in the groups were also equivalent, although IFN-γ concentrations from cultures stimulated with mumps antigen were higher in naturally immune adults than in vaccinated adults (P = .01). All adults were positive for mumps IgG.

Conclusion. T and B cell immunity to mumps was detected in adults at least 10 years after immunization. Except for IFN-γ release, responses in vaccinated adults paralleled those observed in naturally immune individuals.

Wild-type mumps virus continues to cause outbreaks throughout the world despite widespread immunization programs. An outbreak in the United States in 2006 resulted in 5783 confirmed or probable mumps cases [1]. In this outbreak, high rates of mumps cases were observed among college students 18–24 years old [2]. The mumps vaccination status was not known in all patients; however, in a subset of these patients (n = 1798), 49% had received at least 2 doses of measles-mumps-rubella (MMR) vaccine, and 14% had received 1 dose [1]. It is unclear whether mumps outbreaks in vaccinated individuals result from failure to respond to vaccination (defined as primary vaccine failure) or waning immunity (defined as secondary vaccine failure) [3–6].

Although studies have reported high rates of seroconversion after mumps vaccination (>90%) [3, 7], efficacy studies have shown rates as low as 64% after 1 vaccine dose, rising to 88% after 2 doses [4–6]. Immunogenicity studies measuring only mumps-specific IgG levels probably overestimate vaccine efficacy. One study evaluated neutralizing antibodies to mumps virus in patients who contracted mumps disease and who had received 1 mumps vaccine dose. These data showed low levels of vaccine-specific neutralizing antibody titers despite high mumps IgG levels [8]. Results of these studies parallel findings with measles vaccine and support the idea that subsets of individuals fail to respond to primary immunization but will respond to a second vaccine dose [9].

Primary vaccine failure clearly contributes to disease susceptibility, but it remains unclear whether mumps vaccination is also associated with waning immunity and subsequent secondary vaccine failure. The possibility of secondary failure is supported by findings of studies using mumps antibody avidity assays in small groups of vaccinated individuals who contracted mumps disease. In these studies, some individuals who had been vaccinated showed high-avidity titers associated with a recall response to antigen and not a primary response [10–12]. These studies were conducted in populations...
in which the majority of persons had received 1 mumps vaccine dose; therefore, this has not been evaluated extensively in populations in which 2 doses are routine.

The immunogenicity of the mumps vaccine depends on the generation of mumps-specific antibodies, particularly neutralizing antibodies, and on the development of cell-mediated immunity [13–15]. Speculation has arisen concerning whether mumps outbreaks may result if there are antigenic differences between circulating wild-type virus and vaccine strains. Mumps vaccine strains vary globally [16], and some data suggest that there are differences between mumps virus strains in the neutralizing ability of serum from vaccinated individuals [3], potentially leaving highly vaccinated populations at risk for infection with a strain not fully neutralized by vaccine immunity.

The contribution of cell-mediated immunity to protection against mumps virus infection has not been clearly defined, but evidence supports the importance of T lymphocytes as the main protagonist in the recovery from acute viral infection and in providing long-lasting protection against disease. Although neutralizing antibodies most likely represent the first defense against reinfection with viruses, individuals lacking humoral immunity appear to be protected against infection or severe disease. One study of measles virus showed that, despite the inability to detect antibody responses to measles, positive lymphocyte responses were associated with protection against measles virus infection [17]. Animal studies in macaques demonstrated a limited role of humoral immunity against measles virus replication and control and a more crucial role of cell-mediated immunity [18, 19]. Mumps virus protection would be expected to follow a similar pattern of immunity because it belongs to the same family of viruses as measles virus.

The present study investigated mumps-specific cellular and humoral immunity in adults who had received 2 doses of mumps-containing vaccines in childhood in order to evaluate whether immunity persists years after vaccination. In addition, we evaluated mumps-specific immunity in adults who reported a history of mumps virus infection in childhood as a control group. We focused on cell-mediated immunity as an important component of the immune response to mumps. Previous studies had evaluated mumps-specific cell-mediated immunity using skin tests, in vitro lymphocyte proliferation, and cytokine measurement in peripheral blood mononuclear cell (PBMC) cultures in response to stimulation with mumps antigen. We were interested in evaluating the memory CD4⁺ T cell response on exposure to mumps antigen in vitro. Few published studies have evaluated cell-mediated responses to mumps antigen by cytokine enzyme-linked immunospot assay and/or flow cytometry [20–22]. Using the very sensitive technique of flow cytometry, we were able to evaluate mumps-specific CD4⁺ T cells and single-cell expression of interferon (IFN)–γ in memory CD4⁺ T cells in response to mumps antigen [22, 23].

**METHODS**

**Study population.** Twenty healthy adults were included in the study. Participants had no chronic illness and no known immunosuppressive conditions. All participants were recruited through the Stanford University Medical Center, and inclusion criteria included receipt of 2 doses of mumps-containing vaccine or mumps disease without vaccination, in adults born after 1970. Ten adults (20–35 years old) were born in the United States after 1970, after the reported incidence of mumps had decreased following licensure of mumps vaccine in 1967 [24]. The 10 US-born adults had no history of mumps virus infection, had no known exposure to mumps, and had received 2 documented doses of MMR vaccine during childhood, with an interval of at least 10 years from their last MMR vaccination. Ten adults born outside the United States (20–48 years old) were also included in the study. They reported a history of mumps virus infection during childhood, with no history of mumps vaccination before their infection. Three of these adults were born in France, 2 each in The Netherlands and the United Kingdom, and 1 each in Russia, Switzerland, and China. No mumps cases were identified in our area during the study period, and there have been <30 mumps cases annually since the practice of giving 2 doses of MMR vaccine was initiated in 1989 [25], making exposure to wild-type virus in the United States unlikely although possible. In the 10 foreign-born participants, exposure to circulating mumps virus outside the United States was probable. The study was approved by the Stanford University Committee for the Protection of Human Subjects; written informed consent was obtained from each adult. Blood samples were collected from each participant.

**Mumps antigen.** Mumps antigen was prepared from lysates of Vero cells inoculated with live mumps virus vaccine (Mumps-vax; Merck) containing >20,000 TCID₅₀. Vero cell lysates were made in parallel from flasks that had been seeded with the same concentration of cells as the antigen flasks served as an uninfected cell control. Mumps virus–infected cells were harvested at peak cytopathic effect (~90%), sonicated, centrifuged, frozen and thawed 3 times, and stored at −80°C until used. Live mumps virus preparations before harvesting yielded 1.0 × 10⁴–1.0 × 10⁵ pfu/mL. After harvesting, 0.1 mL of undiluted antigen was seeded onto uninfected Vero cells; no plaques were detected. The antigen preparation, which contained 320 μg/mL cell and viral protein, was equivalent to total protein in uninfected cell lysate.

**T cell proliferation assay.** The T cell proliferation assay was performed as described elsewhere [26, 27]. In brief, PBMCs were added to microtiter plates at 3.0 × 10⁵ cells/well in RPMI 1640 medium (Mediatech) with 10% heat-inactivated normal human serum (Sigma); all PBMC cultures were prepared within 24 h after blood was sampled. T cell proliferation was measured by ³H-thymidine uptake after incubation of PBMCs with dilutions of 1:8, 1:16, and 1:32 of mumps antigen and Vero control in
duplicate wells for 5 days. Preliminary studies were performed with multiple dilutions of mumps antigen (range, 1:8–1:512); dilutions of 1:8, 1:16, and 1:32 stimulated T cells from all positive donors. The stimulation index (SI) was determined as the ratio of the mean counts per minute in antigen wells to that in control wells; an SI of ≥3.0 was considered to be positive [26], and the highest SI from either concentration was used for statistical analysis. Phytohemagglutinin (PHA; 0.1 mg/mL; Difco Laboratories) was used as a positive control, and PBS was used as a negative control. The SI for PHA stimulation was calculated as the mean counts per minute in PHA-stimulated wells divided by that in PBS wells. The mean counts per minute were compared between groups for Vero cell and PBS controls, and no differences were found.

**IFN-γ production.** Supernatants from PBMCs stimulated with mumps antigen (at dilutions 1:8, 1:16, and 1:32) were collected on days 5–7, stored at −80°C, and tested for IFN-γ by ELISA (BioSource). No spontaneous release of IFN-γ was detected in the supernatants of PBMCs stimulated with Vero cell control. The minimum detectable value of IFN-γ was determined to be <4 pg/mL. The peak IFN-γ concentration was used for data analysis.

**Intracellular cytokine flow cytometry assay.** The intracellular cytokine flow cytometry assay was performed on heparinized whole blood, as reported elsewhere [23, 28–30]. First, 200-µL aliquots of heparinized whole blood were placed into 1.5-mL microcentrifuge tubes, and costimulation factors anti-CD28 and anti-CD49d (both from BD Biosciences) were added to each tube. Samples were stimulated with mumps antigen or Vero cell lysates (prepared as described above) at different volumes (8, 16, 24, and 32 µL). Preliminary studies were performed with multiple volumes of mumps antigen (range, 8–64 µL); the volumes yielding the best results (8, 16, 24, and 32 µL) were subsequently used. Staphylococcal enterotoxin B (0.5 mg/mL; Sigma) was used as a positive control in all experiments. Samples were incubated for 16 h at 37°C in 5% CO2, according to preliminary kinetic studies that determined the optimal incubation period (data not shown). Brefeldin A (Sigma), at a final concentration of 10 µg/mL, was added to each tube for the final 4 h of the incubation period. After incubation, 20 µL of 20 mmol/L EDTA (Sigma) was added to each tube to remove adherent cells, followed by FACS Lysing Solution 1× (BD Biosciences) to lyse red blood cells, and tubes were incubated at room temperature. The tubes were then centrifuged, the supernatant was discarded, and the cell pellet was resuspended in freezing solution (10% dimethyl sulfoxide [Sigma] in fetal calf serum). Samples were frozen at −80°C and then processed and stained within 2 weeks.

**Cell surface markers and intracellular cytokine staining.** Frozen samples were thawed, washed with a wash buffer (Dulbecco’s PBS 1×, 0.5% bovine serum albumin [Sigma], and 0.1% sodium azide [0.5 g; Sigma]), and permeabilized with FACS Permeabilizing Solution (BD Biosciences). A mixture of fluorescent mouse anti-human monoclonal antibodies was added to each sample: CD4–peridinin chlorophyll protein–cyanin 5.5, IFN-γ–fluorescein isothiocyanate, CD45RO–phycoerythrin, and CD69–allophycocyanin (all from BD Biosciences). Staining reactions were incubated for 30 min at room temperature. The stained cells were washed with FACS wash buffer and then fixed with 1% paraformaldehyde (Electron Microscopy Science).

**Flow cytometry analysis.** Samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences). Approximately 50,000 events (CD4+ T cells) were collected for each sample. Acquisition and analysis were performed with CellQuest Pro software (version 4.0.1; BD Biosciences). Lymphocytes were gated using forward versus side scatter, and then gates were set to analyze CD4+ T cells (using side scatter vs. CD4). Memory CD4+ T cells were determined by gating on CD4+ T cells that were CD45RO+. Frequencies of responding cells were reported as percentages of CD69+ and IFN-γ+ events.
Antibody assay. Serum samples were tested with a qualitative ELISA for IgG to mumps virus (Wampole Laboratories). The mumps antigen used in this test was from the Enders strain. Tests were conducted according to the manufacturer’s instructions.

Statistics. T cell proliferation was reported as mean ± SE SI. The mean IFN-γ concentration was used for data analysis. The mean percentages of CD4+ T cells and memory CD4+ T cells that expressed CD69 and produced IFN-γ were reported as percentages with their respective SE. The results of ELISAs for mumps IgG were reported as positive or negative. Student’s unpaired t test or Fisher’s exact test was used to compare data between vaccinated and naturally infected adults. Differences for which P ≤ .05 were considered statistically significant. Pearson’s correlation analyses were performed using GraphPad Prism software (version 4.0; GraphPad).

RESULTS

T cell proliferation assay. The mean ± SE SI for mumps antigen was 9.21 ± 2.90 in vaccinated adults, equivalent to the mean ± SE SI of 20.01 ± 6.18 for adults with natural immunity to mumps (P = .13). The percentages of adults with a positive SI (≥3.0) were comparable between the groups—70% in vaccinated adults and 80% in adults with naturally acquired mumps immunity (P = 1.0) (figure 1), and the mean ± SE SIs for PHA were also equivalent for the 2 groups: 73.17 ± 22.42 and 110.23 ± 42.64, respectively.

IFN-γ production. The mean ± SE IFN-γ concentration was 515 ± 92 pg/mL in the supernatants of PBMC cultures stimulated with mumps antigen for the 10 vaccinated adults. These responses were similar to the results of our previous study [26], in which the mean ± SE IFN-γ concentration in adults was 692 ± 120 pg/mL. The mean ± SE IFN-γ concentration in the adults with naturally acquired mumps immunity was 938 ± 63 pg/mL, which was significantly higher than concentrations in vaccinated adults (P = .0001) (figure 1). There was no correlation between SI and IFN-γ values in either the vaccinated or the naturally immune group.

Flow cytometry analysis. Representative dot plots are depicted in figure 2. The mean ± SE percentages of CD4+ T cells that expressed CD69 and produced IFN-γ after stimulation with Vero cell control (A) or mumps antigen (B and C) in a vaccinated adult. The percentages of CD4+ T cells (gated events) that expressed CD69 (Y-axis) and produced IFN-γ (X-axis) are shown in the right upper quadrants.

Antibody assay. All serum samples from subjects in both groups were positive for mumps IgG antibodies by ELISA.
DISCUSSION

In this study, we showed that mumps vaccination induced strong memory T cell immunity, as demonstrated by T cell proliferation and IFN-γ production in adults who were vaccinated in childhood. These responses persisted for at least 10 years after immunization with MMR vaccine and paralleled the immune responses found in adults who had natural infection during childhood or adolescence. Importantly, this study evaluated memory T cell responses by flow cytometry, demonstrating that a proportion of circulating T cells in both immunized and naturally infected subjects recognized mumps antigen upon in vitro exposure.

The majority of previous studies examining mumps vaccine effectiveness and clinical protection have focused on the acquisition of humoral immunity [33–36]. Weibel et al. [37] demonstrated the persistence of neutralizing antibodies to mumps antigen without significant decline for at least 9 years after the administration of 1 dose of mumps vaccine.

The cellular immune response to mumps vaccine has been less well studied. Initial evaluations during the 1960s used cutaneous delayed hypersensitivity (demonstrated by the mumps skin test), which was shown to correlate with humoral immunity, but these findings were not quantitative [38]. Since then, other studies have shown that the cell-mediated immunity evaluated by lymphocyte blast transformation was significant but transient [13, 15], and in our previous studies [26, 39] we showed that mumps-specific T cell proliferation and IFN-γ production were elicited in infants vaccinated at 6, 9, and 12 months. Two other studies have evaluated the persistence of lymphoproliferative responses to mumps. One documented that lymphoproliferative responses to mumps viruses persisted in two-thirds of the study population for at least 6 years after vaccination [40]. In a recent study from Finland, Jokinen et al. [41] used lymphoproliferative assays and cytokine measurements to demonstrate the persistence of mumps cell-mediated immunity in adults 21 years after MMR vaccination. In that study, no differences were found between naturally immune and vaccinated individuals, but concentrations of IFN-γ were not measured. To date, no studies have evaluated memory CD4+ T cell responses by use of flow cytometry in adults vaccinated against mumps as children.

In our study, vaccinated adults had evidence of T cell immunity to mumps even 10 years after the last vaccination, with a mean SI much higher than the threshold of 3. In addition, the same percentages of vaccinated and naturally immune adults had positive T cell responses. In this study, unlike the study by Jokinen et al. [41], the mean SI in vaccinated adults was lower than that in the adults with previous natural mumps infection, but not significantly lower. However, IFN-γ concentrations in PBMC cultures stimulated with mumps antigen were significantly higher for naturally infected adults than for vaccinated adults. We found no significant correlations between SI and IFN-γ levels in either vaccinated or naturally immune adults; thus, higher IFN-γ concentrations did not predict higher SIs in individuals. This suggests that a threshold level of IFN-γ may be needed to produce a positive T cell response, which was achieved in the vaccinated group. It is important to note that the quantitative level of T cell immunity and IFN-γ concentration needed for protection against disease is unknown. However, the presence of memory T cells should provide efficient expansion of mumps-specific T cells if the individual is reexposed to mumps.

An important finding in our study was that mumps-specific CD4+ T cells were present in both groups of adults. The mean percentages of total CD4+ T cells that expressed CD69 and produced IFN-γ in response to mumps antigen were the same in vaccinated adults and in adults with natural immunity caused by
mumps virus infection. Furthermore, the percentages of mumps-specific memory (CD45RO⁺) CD4⁺ T cells did not differ significantly between vaccinated and naturally immune adults.

Our 2 study populations may have been exposed to circulating mumps virus that served to boost their mumps-specific immunity without producing evidence of clinical disease. Exposure to circulating mumps would be expected to be rare in the United States, but those living in other countries may be exposed more regularly. The contribution of this boosting to the immune responses in our study could not be evaluated but may have accounted for the higher IFN-γ responses in the naturally immune individuals. Our study was also limited by sample size and the fact that none of the subjects had been recently immunized; thus, we could not measure the kinetics of the immune response to mumps vaccine. The aim of our study was to determine whether mumps-specific immunity was present years after the last mumps vaccination. Given that in the recent US mumps outbreak the highest age-specific rate among 18–24-year-olds, many of whom had received 2 doses of mumps vaccines years before reexposure [1, 2], our population aimed to mimic this cohort. An important finding of our study was that immunity was still present several years after the second dose of mumps vaccine in the few subjects evaluated.

In our study, all adults tested were positive for mumps-specific IgG at least 10 years after their second dose of MMR vaccine or after natural mumps virus infection; we did not test for neutralizing antibodies, however, so the functional status of the subjects’ humoral immunity was not evaluated. Potentially, B cell immunity may have poor neutralizing ability several years after mumps immunization despite adequate T cell immunity. In this situation, an individual may be protected from clinical disease but contribute to viral transmission through subclinical infection. Questions have been raised about the contribution of subclinical transmission in the recent US outbreak [2].

Mumps-specific cell-mediated immunity is expected to play a crucial role in protecting vaccinated adults from infection after exposure to wild-type mumps virus. The present study shows that mumps vaccination induces memory T cell responses that can be detected by in vitro testing. The presence of T cell immunity years after 2 doses of mumps vaccine in the population tested argues against significant waning of immunity, or secondary vaccine failure. Responses in vaccinated adults were compared with those in naturally immune individuals, who are expected to achieve lifelong immunity after mumps virus infection. The immune responses in vaccinated adults were comparable to those seen for natural immunity to mumps, with the exception of IFN-γ release, which has unknown clinical relevance. If further mumps outbreaks occur, information about cellular immunity to mumps will be helpful, to determine whether there are correlations with protection as measured by the current assays and in relation to functional antibodies.

References


