Original Article

Immune Response to the Mumps Virus in Iranian Unvaccinated Young Adults

Maryam Keshavarz1,2, Abbass Shafiee2, Mohammad Hossein Nicknam1, Pardis Khosravani1, Ahmad Yousefi2, and Maryam Izad1*

1Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran; 2Department of Human Viral Vaccines, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization, Karaj; and 3Department of Stem Cells and Development Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

SUMMARY: Although the first mumps vaccine was licensed more than 50 years ago, the vaccine was added to Iran’s Expanded Program on Immunization in 2003. Therefore, the majority of Iranians born before 2003 are unvaccinated, and their immunity is the result of natural infection. In order to evaluate cellular responses against the mumps virus following natural infection, we investigated 90 Iranian unvaccinated adults aged 20–30 years. Mumps specific memory CD4+ and CD8+ proliferation and frequency of cytotoxic lymphocyte CD8+ and CD107a were evaluated using flow cytometry. Our results showed that 33 subjects were seronegative, but 28 of them showed degranulation of CD8+ T lymphocytes and expression of CD 107a, as well as proliferation of CD4 and CD8 T cells, in response to mumps antigen stimulation. In all seropositive subjects, degranulation of cytotoxic T lymphocytes and proliferation of CD4+ and CD8+ T lymphocytes was detected. Proliferation of T cells and degranulation of CD8 T cells in seropositive subjects was higher than in seronegative subjects. We conclude that natural mumps infection and subclinical reinfection could induce good protection, but the severe complications associated with mumps infections suggest the need for mumps vaccination. Natural boosters because of the prevalence of the wild-type virus may help with maintenance of immunity in populations with high vaccine coverage.

INTRODUCTION

Mumps is generally a mild disease experienced during early childhood, manifested by inflammation of salivary glands and fever; however, it can cause more serious complications, including orchitis, pancreatitis, thyroiditis, and aseptic meningitis, which may result in hearing loss. Furthermore, mumps is more likely cause severe complications in adults (1,2).

Although the first mumps vaccine was licensed in 1967 (3), the mumps vaccine was added to Iran’s Expanded Program on Immunization (EPI) in 2003. It is administered as 2 doses of the measles, mumps, and rubella (MMR) vaccine, at 12 and 18 months of age (4). Mumps is endemic in Iran and low immunity against mumps in Iranian adults, due to the absence of MMR vaccine before 2003, is a result of natural infection. The level of disease-specific antibody has been conventionally used to measure protective immunity, because neutralizing antibodies likely play an important role as part of the first line of defense during reinfection (5,6). Individuals lacking humoral immunity are able to recover from viral infections, however, those with defects in cell mediated immunity seem to be susceptible to persistent viral infections (7–9).

Mumps is still one of the great problem of health in developing countries, despite the availability of safe and effective vaccines (10–14), and the wild-type mumps virus continues to cause outbreaks throughout the world (15). In 2006, a mumps outbreak occurred in the United States, although the majority of the patients aged 18–24 years being vaccinated with 2 doses of mumps vaccine. The high attack rates among vaccinated young adults raises questions about the durability of protective immunity against the mumps virus provided by the vaccine (12,14).

Whereas published reports concerning the efficacy of the mumps vaccine describe efficacy over 90% (15), levels of antibodies to mumps decline rapidly within years of vaccination, after an initial peak (16). It has been reported that natural mumps infection, in contrast, confers lifetime protection (17). However, reinfection to mumps virus for those having sustained a wild-type infection can be occurred (18). Subclinical natural booster infections play an important role in the maintenance of protective antibody levels after vaccination. Based on mathematical models, in vaccinated populations that do not periodically encounter a circulating wild-type virus, antibody titers decrease continually to non-protective levels (19).

Mumps vaccination, combined with exposure natural infection, can induce protective levels of antibody and cell mediated immunity (20). However, protection has been traditionally evaluated using antibody titers, and the extent of the decline of cellular immune response after vaccination or natural infection has not been measured adequately. Several techniques are available to
evaluate cell-mediated immunity, such as the 3H-thymidine incorporation assay, 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and hemagglutination test (unpublished results), and antigen protein concentrations were estimated using the NanoDrop 2000c spectrophotometer (Thermo Fisher, Waltham, MA, USA).

**Material and Methods**

**Study population:** Ninety volunteers aged 20–30 years were included in this study; all were unvaccinated because they were born before the introduction of routine administration of 2 doses of the live attenuated MMR vaccine as part of EPI (24). All participants completed a questionnaire about their age, usage of immune-suppressive drugs, and interaction during the prior 3 weeks with persons who had measles, mumps, or rubella.

The study was performed in the Immunology Department of Tehran University of Medical Sciences, Iran (2014–2015). The research protocol was approved by the Ethical Committee of Tehran University of Medical Sciences, Iran.

**Serological analysis:** Mumps IgG level for all plasma samples was measured using GenWay Mumps IgG Antibody ELISA Kit (specificity and sensitivity = 95%; San Diego, CA, USA). According to the manufacturer, individuals with mumps IgG levels of 11 or greater were seropositive, whereas those with lower levels were considered seronegative.

**Mumps antigen preparation:** Mumps antigen was prepared using an Iranian mumps virus strain, RS-12 (25) that has been described elsewhere (26). Briefly, Vero cell monolayers infected with mumps virus at a multiplicity of infection of 0.01 were incubated at 37°C. Vero-infected cells were harvested at maximum cytopathic effect (≥70%) after 2 freeze-thaw periods. Vero culture lysates were pooled and treated with Tween 80-ether. After centrifugation at 3,000 × g for 2 h at 4°C, Vero-infected lysates were aliquoted in equal volume and stored at −70°C. Hemagglutinin level of the in-house mumps antigen was evaluated by a hemagglutination test (unpublished results), and antigen protein concentrations were estimated using the NanoDrop 2000c spectrophotometer (Thermo Fisher, Waltham, MA, USA).

**Antibodies and reagent:** Conjugated monoclonal antibodies (mAbs) CD3 allophycocyanin (APC)-Cy7, CD8, DAPI (4’,6-diamidino-2-phenylindole), CD107a APC, CD4 phycoerythrin (PE) Cy7, CD8 PE Cy7, and costimulatory mAbs for CD28 and CD49d, were obtained from Biologend (San Diego, CA, USA). Fluorescent dye 5, 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) was obtained from Life Technologies, Molecular Probes (Eugene, OR, USA). FACS Lysing Solution was obtained from BD Biosciences, Immunocytometry System (San Jose, CA, USA).

**Cytotoxicity assay based on CD107a expression:** Cytotoxicity of CD8 lymphocytes express CD107a molecules in response to recognizing of their specific antigens. Surface expression of CD107a in CD8+ T lymphocytes and NK cells is associated with a loss of intracellular perforin, and result in killing the target (27). In order to determine degranulation by cytotoxic CD8+ T lymphocyte following specific antigenic stimulation, we used whole blood samples submitted by healthy young volunteers, with or without protective mumps IgG levels.

Antigen-specific activation of whole blood has been explained elsewhere (28). Briefly, sodium heparinized whole blood was incubated with costimulatory mAb to CD28 and CD49d at 1 µg/ml each, SEB (Staphylococcus aureus, enterotoxin type B; positive control) at 10 µg/ml and the homemade mumps antigen at various concentrations (5, 7, 10, and 15 µg/ml). Stimulation was detected to be most effective at concentrations of 10 and 15 µg/ml of mumps antigen; tests were therefore performed using 10 µg/ml of mumps antigen. The culture tubes were incubated in a 37°C 5% CO2 incubator for 5 h. After the incubation time, 1/10 volume of 20 mM EDTA was added to the cultures. Culture tubes were vortexed vigorously and incubated for 15 min at room temperature. Then erythrocytes were lysed and leukocytes were fixed using 10 ml of FACS Lysing Solution. Leukocytes were leaving in FACS Lysing Solution and directly frozen at −80°C or suspended in phosphate buffer saline (PBS) containing 10% dimethyl sulfoxide (DMSO) and 1% bovine serum albumin (BSA). According to the manufacturer, individuals with mumps IgG levels of 11 or greater were seropositive, whereas those with lower levels were considered seronegative.

**Mumps antigen preparation:** Mumps antigen was prepared using an Iranian mumps virus strain, RS-12 (25) that has been described elsewhere (26). Briefly, Vero cell monolayers infected with mumps virus at a multiplicity of infection of 0.01 were incubated at 37°C. Vero-infected cells were harvested at maximum cytopathic effect (≥70%) after 2 freeze-thaw periods. Vero culture lysates were pooled and treated with Tween 80-ether. After centrifugation at 3,000 × g for 2 h at 4°C, Vero-infected lysates were aliquoted in equal volume and stored at −70°C. Hemagglutinin level of the in-house mumps antigen was evaluated by a hemagglutination test (unpublished results), and antigen protein concentrations were estimated using the NanoDrop 2000c spectrophotometer (Thermo Fisher, Waltham, MA, USA).

**Antibodies and reagent:** Conjugated monoclonal antibodies (mAbs) CD3 allophycocyanin (APC)-Cy7, CD8, DAPI (4’,6-diamidino-2-phenylindole), CD107a APC, CD4 phycoerythrin (PE) Cy7, CD8 PE Cy7, and costimulatory mAbs for CD28 and CD49d, were obtained from Biologend (San Diego, CA, USA). Fluorescent dye 5, 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) was obtained from Life Technologies, Molecular Probes (Eugene, OR, USA). FACS Lysing Solution was obtained from BD Biosciences, Immunocytometry System (San Jose, CA, USA).

**Cytotoxicity assay based on CD107a expression:** Cytotoxicity of CD8 lymphocytes express CD107a molecules in response to recognizing of their specific antigens. Surface expression of CD107a in CD8+ T lymphocytes and NK cells is associated with a loss of intracellular perforin, and result in killing the target (27). In order to determine degranulation by cytotoxic CD8+ T lymphocyte following specific antigenic stimulation, we used whole blood samples submitted by healthy young volunteers, with or without protective mumps IgG levels.

Antigen-specific activation of whole blood has been explained elsewhere (28). Briefly, sodium heparinized whole blood was incubated with costimulatory mAb to CD28 and CD49d at 1 µg/ml each, SEB (Staphylococcus aureus, enterotoxin type B; positive control) at 10 µg/ml and the homemade mumps antigen at various concentrations (5, 7, 10, and 15 µg/ml). Stimulation was detected to be most effective at concentrations of 10 and 15 µg/ml of mumps antigen; tests were therefore performed using 10 µg/ml of mumps antigen. The culture tubes were incubated in a 37°C 5% CO2 incubator for 5 h. After the incubation time, 1/10 volume of 20 mM EDTA was added to the cultures. Culture tubes were vortexed vigorously and incubated for 15 min at room temperature. Then erythrocytes were lysed and leukocytes were fixed using 10 ml of FACS Lysing Solution. Leukocytes were leaving in FACS Lysing Solution and directly frozen at −80°C or suspended in phosphate buffer saline (PBS) containing 10% dimethyl sulfoxide (DMSO) and 1% bovine serum albumin (BSA), and then placed at −80°C. Equivalent results were obtained by the 2 freezing methods. Alternatively, leukocytes were suspended in PBS and stained for CD3, CD8, and CD107a. Finally, leukocytes were washed in PBS and fixed with 1% paraformaldehyde. The CD107a expression on CD8 T cells was analyzed using BD FACS Aria II with FACS Diva software (BD Bioscience).

**Antigen-specific CD4 and CD8 T cell CFSE proliferation assay:** To determine the most effective concentration of CFSE, peripheral blood mononuclear cells were labeled with 0.5, 1, 1.5, 2.5, and 5 µmol CFSE. The best results were obtained using 2.5 µmol CFSE. Peripheral blood mononuclear cells were suspended in PBS at a concentration of 1 × 10^6/ml and labeled with 2.5 µmol CFSE in PBS for 15 min at 37°C. CFSE-labeled PBMCs were cultured with RPMI1640 containing 10% heat-inactivated fetal calf serum, 1 mM L-glutamine, and 1% penicillin-streptomycin alone (negative control), phytohemagglutinin (PHA, 5 µg/ml; positive control), or mumps antigen (10 µg/ml) at
Table 1. The geometric mean of mumps specific degranulation and proliferation of T lymphocytes

|                | Mean of proliferation index (PI) | History of mumps infection, n |
|----------------|---------------------------------|-------------------------------|
|                | CD8 T cell  | CD4 T cell  | Positive | Unknown |
| Seropositive   | 1.42 (0.12–2.96) | 1.63 (1.44–1.82) | 1.77 (1.44–2.10) | 30 |
| Seronegative   | 1.12 (0.70–1.50) | 1.55 (1.22–1.88) | 1.63 (1.35–1.90) | 14 |

RESULTS

Mumps IgG level: The geometric mean concentration of mumps IgG was 16.4 IU/ml (95% confidence interval [CI], 13.71–19.24). Fifty seven of the volunteers had a protective level (≥11 IU/ml) of mumps IgG, and 33 had a nonprotective (<11 IU/ml) level of IgG.

Mumps virus proliferation responsive to CD8 and CD4 T cells in seropositive and seronegative individuals: Mumps-specific CD8 and CD4 T cell proliferation was measured in seropositive and seronegative subjects who would traditionally have been considered non-immune subjects. In all subjects, proliferation of CD4+ T lymphocytes was higher than CD8+ T lymphocytes. The geometric mean of the proliferation index of CD4+ and CD8+ T lymphocytes were 1.72 (95% CI, 1.50–1.94) and 1.60 (95% CI, 1.45–1.75), respectively. Proliferation of both CD4 and CD8 T cells in seropositive subjects was higher than that in seronegative subjects, but the difference was not significant. A summary of the results is shown in Table 1. A non-significant diversity of intensity in proliferative response was detected in seropositive and seronegative subjects. Fig. 1 shows different levels of CD8 T cell proliferation in 2 seropositive subjects.

Evaluation of mumps-specific cytotoxic T lymphocyte based on CD107a expression: CD107a is a member of the LAMP family. LAMP-1 molecules are highly glycosylated membrane proteins that presented on the luminal side of the vesicle and are involved in protecting the cellular membrane from enzymatic lysing by the lytic enzymes of granules. Following degranulation, CD107a molecules express on the extracellular membrane of the effector cells and thus may protect the extracellular membrane of the effector cell (21–23). Expression of CD 107a molecules was introduced as a marker of CD8 T-cell degranulation following antigen specific stimulation. Therefore, we used CD107a expression to evaluate mumps-specific CD8+ T lymphocytes in seronegative or seropositive subjects. The geometric mean frequency of degranulation of CD8+ T lymphocyte that expressed CD107a was 1.25% of the total (95% CI, 0.70–1.61). Frequency of CD107a+ CD8+ T lymphocytes in seropositive volunteers was higher than seronegative individuals (Table 1). Fig. 2 shows the expression of CD107a in CD8+ cytotoxic T lymphocytes (CTLs) in 4 volunteers, positive control, and unstimulated negative control.

Cell mediated response against mumps in seronegative and seropositive subjects: Thirty three volunteers were seronegative, but 28 of them showed expression of CD107a and T cell proliferation in response to mumps antigen stimulation. Therefore, the other seronegative cases were actually non-immune against mumps infection. Seropositive subjects showed various intensities of mumps-specific cellular immune response. Positive cell mediated response against mumps was measured in some of the seronegative and all seropositive subjects. Fig. 2 shows mumps-specific degranulation of CD8+ T lymphocytes in 3 seropositive subjects.
Our findings showed that there was a high level of heterogeneity in mumps-specific immune responses among the unvaccinated population. Levels of antigen-specific antibody have traditionally been used to evaluate immunity against infectious agents and efficacy of vaccines. Therefore, in the present study, mumps IgG levels were evaluated. Our findings showed that over 50% of the subjects had protective levels of mumps IgG and thus conventionally were immune against mumps. Recent findings about the recovery of individuals with impaired humoral systems from viral infections, such as mumps, arise consideration of maintenance of cell-mediated immunity in viral infections (8,16). Therefore, in the present study, mumps-specific cellular immunity in seronegative and seropositive Iranian young adults was investigated. The possibility of natural booster reinfections is very high because mumps is endemic in Iran, and wild-type virus periodically circulates in the population.

Moreover, in vitro CD4+ and CD8+ lymphoproliferation against mumps virus was observed in all individuals with protective mumps IgG levels. However, proliferation of CD4+ and CD8+ T lymphocytes was detected in some individuals with non-immune mumps IgG levels. Similar findings are available in the report by Ovsyannikova et al. which describes that cases lacking protective levels of IgG showed in vitro proliferation of T lymphocytes in response to measles virus (26). We observed proliferation of CD4 and CD8 T cells in seronegative subjects; however, it was lower in seropositive subjects.

In individuals with a high rate of vaccination, specific lymphoproliferation response against mumps virus was detected. There was no significant correlation between CD4+ or CD8+ lymphoproliferation responses and anti-mumps IgG levels (p<0.005).

In 2012, Jacobson et al. reported that no significant correlation was observed between cellular and humoral responses against the measles virus (29). Similar results were expected for mumps virus because both the measles and mumps viruses belong to the Parainfluenza family. However, there is evidence of a significant correlation with virus-specific humoral and cellular immune response of some viruses such as rubella virus, a member of the Togaviridae family (30).

CD107a was introduced as a marker of degranulation of CD8+ T lymphocyte and NK cells following stimulation and its expression correlates with cytokine secretion, and cell-mediated lysis of target cells (21,27). CD107a expression correlates with cytotoxicity of NK cells and CTLs and may be a sensitive marker for cytotoxic activity determination (22,23). Based on recent findings about CD107a+, CD8+ T lymphocytes that expressed CD107a in response to mumps stimulation can be considered as mumps-specific CTLs. Therefore, we analyzed expression of CD107a using flow cytometric assay. In all of the seropositive subjects, degranulation of mumps-specific CD8 CTLs was observed, and all volunteers showed proliferation of CD4+ and CD8+ T lymphocytes. Similar findings were reported by Jokinen et al., who found mumps antigen-specific lymphoproliferative response in 100% of subjects that naturally acquired immunity to the mumps virus (16). In research performed by Hanna-Wakim et al., 80% of naturally immune individuals had a positive stimulation index (15). Cell-mediated immunity to mumps in vaccinated individuals was lower than that in naturally immune adults (15,16). There was a significant correlation between frequency of CTLs and proliferation of CD8+ T lymphocytes.

We conclude that vaccination for mumps infection and the related complications is important for providing immunity against mumps, in addition to the natural infection due to circulation of wild-type virus, which is important in maintenance of immunity.

Acknowledgments We thank Razi Vaccine and Serum Research Institute for their assistance and support during the study.

Conflict of interest None to declare.

REFERENCES
1. Plotkin SA, Wharton M. Mumps vaccine. In: Plotkin SA, Orenstein WA, editors. Vaccines, 3rd ed. Philadelphia, PA: W.B. Saunders Company; 1999. p. 267-292.
2. Date AA, Kyaw MH, Rue AM, et al. Long-term persistence of mumps antibody after receipt of 2 measles-mumps-rubella (MMR) vaccinations and antibody response after a third MMR vaccination among a university population. J Infect Dis. 2008;197:1662-8.
3. Avijgan M, Moghni M, Hafziz M, et al. Immunogenicity and efficacy of the Hoshino strain of mumps (MMR Vaccine) in Iran. Iranian Red Crescent Med J. 2011;13:1-5.
4. Corbin V, Beytout J, Auclair C, et al. Shift of the 2009–2011
measles outbreak from children to adults: an observational review at the University Hospital of Clermont-Ferrand, France. Infection. 2013;41:1157-61.

5. Avijan M, Hafizi M, Moghni M, et al. Immunogenicity and efficacy of Hoshino strain of mumps vaccine in Iran; two years study. East Afr J Public Health. 2011;8:88-91.

6. Namaei MH, Ziae M, Naseh N. Congenital rubella syndrome in infants of women vaccinated during or just before pregnancy with measles-rubella vaccine. Indian J Med Res. 2008;127:551-4.

7. Albertyn C, van der Plas H, Hardie D, et al. Silent casualties from the mumps outbreak in South Africa. S Afr Med J. 2011; 101:313-6.

8. Griffin DE, Lin WH, Pan CH. Measles virus, immune control, and persistence. FEMS Microbiol Rev. 2012;36:649-62.

9. McQuaid S, Cosby SL. An immunohistochemical study of the distribution of the measles virus receptors, CD46 and SLAM, in normal human tissues and subacute sclerosing panencephalitis. Lab Invest. 2002;82:403-9.

10. Noorbakhsh S, Ashotoani F, Rimaz S, et al. Mumps meningoencephalitis in pediatric ward of Rasool Akram Hospital in Tehran, Iran, 1999–2000. Med J Islamic Republic Iran. 2004;18:123-6.

11. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. N Engl J Med. 2008;358:1580-9.

12. Dayan GH, Rubin S. Mumps outbreaks in vaccinated populations: are available mumps vaccines effective enough to prevent outbreaks? Clin Infect Dis. 2008;47:1458-67.

13. Centers for Disease Control and Prevention (CDC). Mumps outbreak on a university campus--California, 2011. MMWR Morb Mortal Rep. 2012;61:986-9.

14. Park DW, Nam MH, Kim JY, et al. Mumps outbreak in a highly vaccinated school population: assessment of secondary vaccine failure using IgG avidity measurements. Vaccine. 2007;25:4665-70.

15. Hanna-Wakim R, Yasukawa LL, Sung P, et al. Immune responses to mumps vaccine in adults who were vaccinated in childhood. J Infect Dis. 2008;197:1669-75.

16. Jokinen S, Osterlund P, Julkunen I, et al. Cellular immunity to mumps virus in young adults 21 years after measles-mumps-rubella vaccination. J Infect Dis. 2007;196:861-7.

17. Peltola H, Kulkarni PS, Kapre SV, et al. Mumps outbreaks in Canada and the United States: time for new thinking on mumps vaccines. Clin Infect Dis. 2007;45:459-66.

18. Crowley B, Afzal M. Mumps virus reinfection--clinical findings and serological vagaries. Commun Dis Public Health. 2002;5:311-3.

19. Mossong J, Nokes DJ, Edmunds WJ, et al. Modeling the impact of subclinical measles transmission in vaccinated populations with waning immunity. Am J Epidemiol. 1999;150:1238-49.

20. Gans H, Yasukawa L, Rinki M, et al. Immune responses to mumps and mumps vaccination of infants at 6, 9, and 12 months. J Infect Dis. 2001;184:817-26.

21. Altier G, Malenfant JM, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. J Immunol Methods. 2004;294:15-22.

22. Huth TK, Brenchley JM, Ashton KJ, et al. Natural killer cell cytotoxic activity: measurement of the apoptotic inducing mechanisms. Clin Exp Med Sci. 2013;1:373-86.

23. Akbas E, Kucuksezerci UC, Bilgic S, et al. Relationship between CD107a expression and cytotoxic activity. Cell Immunol. 2009;254:149-54.

24. Zarei SM, Gouya MM, Azad TM, et al. Successful control and impending elimination of measles in the Islamic Republic of Iran. J Infect Dis. 2011;204 Suppl 1:S305-11.

25. Sassani A, Mirchamsy H, Shafi Y, et al. Development of a new live attenuated mumps virus vaccine in human diploid cells. Biologicals. 1991;19:203-11.

26. Ovsyannikova IG, Dhiman N, Jacobson RM, et al. Frequency of measles virus-specific CD4+ and CD8+ T cells in subjects seronegative or highly seropositive for measles vaccine. Clin Diagn Lab Immunol. 2003;10:411-6.

27. Betts MR, Brenchley JM, Price DA, et al. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. J Immunol Methods. 2003;281:65-78.

28. Suni MA, Picker LJ, Maino VC. Detection of antigen-specific T cell cytokine expression in whole blood by flow cytometry. J Immunol Methods. 1998;212:89-98.

29. Jacobson RM, Ovsyannikova IG, Vierkant RA, et al. Independence of measles-specific humoral and cellular immune responses to vaccination. Hum Immunol. 2012;73:474-9.

30. Lambert ND, Haralanvieve IH, Ovsyannikova IG, et al. Characterization of humoral and cellular immunity to rubella vaccine in four distinct cohorts. Immunol Res. 2014;58:1-8.