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Genetic basis for variation of vaccine response: our studies with rubella vaccine

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Abstract

Congenital rubella syndrome still occurs throughout the world despite an effective vaccine being used in developed countries. Heat and light lability, as well as contraindications in immunocompromised persons, limit the use of the vaccine. An improved, more durable and less reactive rubella vaccine such as a peptide or subunit vaccine would address these unmet needs. We have sought to identify the genetic factors that influence both humoral and cell-mediated immunity. Specifically, we have examined genetic polymorphisms and their associations with variations in the immune response to rubella vaccine. Our previous work with twins has identified substantial heritability with rubella vaccine antibody response. We have since identified human leukocyte antigen associations, with both humoral (class II) and cellular (class I) immunity. Our preliminary work with genetic determinants in cytokines and their receptors have offered tantalising leads as well. Now, having recruited a larger cohort to combine with our previous sample, we lay out in this paper our specific aims for a larger, more comprehensive study of the genetic associations with rubella vaccine response and components of both humoral and cellular immunity.

Keywords

Antibody formation; cytokines; HLA antigens; immunity; cellular; polymorphism; genetic; rubella; rubella vaccine

Congenital rubella syndrome still occurs throughout the world despite an effective vaccine being used in developed countries.^{1,2} The live viral vaccine that we routinely administer to children, both in the USA and in Europe, suffers from heat lability, which thwarts its transportation and storage in developing countries.³ Use of the current vaccine is also limited because of evidence, albeit weak, of a relationship with chronic arthritis in adult vaccine recipients, especially women.^{4,5} Investigations into the genetic causes of variation

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in rubella immunity may not only point to an alternative vaccine that addresses these problems, but also serve as a model for understanding viral immunity in general.⁶

The virus

The rubella virus was first isolated in 1962.^{2,7} It is a togavirus belonging to the genus *Rubivirus*, closely related to the group A arboviruses including the eastern and western equine encephalomyelitis virus. The rubella virus is an enveloped, single-stranded RNA virus with a single antigen or serotype. It does not cross-react with other togaviruses.

Rubella infection

Humans are the only host for rubella viral infections.^{2,8} The virus is transmitted primarily through respiratory droplets. It replicates in the nasopharynx and of 12–23 days, with an average of 18 days, the viral infection spreads to other organs systems including the skin, which results in a rash. The nasopharynx sheds the virus then for 1–2 weeks, peaking at 1–5 days.

As stated before, the disease only occurs in humans.² Between 20% and 50% of those infection have mild symptoms or none at all. For the child, the infection results in a transient, erythematous rash, conjunctivitis, coryza, postauricular and suboccipital lymphadenopathy and low-grade fever, with nausea. ‘Rubella’ comes from the Latin meaning ‘little red’,² the Italian term being rosolia. Around the world, many call rubella German measles because German physicians in the mid 18th century first described the disease. Originally, physicians considered rubella infection to be a mild form of measles or scarlet fever, and as a result they called it third disease, in the same way that they called parvovirus B19 disease fifth disease.

Complications of infection include arthralgia and arthritis, which are rare in children but occur in up to 70% of adults, particularly women. Rarely, the infection may also result in haemorrhagic manifestations, Guillain–Barré syndrome or encephalitis.

Congenital rubella syndrome

The childhood infection is mild and transient, requiring no special treatment.² The concern results from maternal infection early in pregnancy, resulting in a 90% likelihood of congenital rubella syndrome or fetal death. Maternal infection results in a viral infection of the placenta and fetus resulting in maldevelopment of the ears, eyes, heart and brain. This then can lead to lifelong deafness, blindness and mental retardation. Currently, 110 000 cases occur each year around the world, primarily in developing countries.¹ The cost is enormous due to the permanent disabilities that result.

Epidemiology of congenital rubella syndrome

The disease is seasonal. In temperate zones in the northern hemisphere, the illness occurs in late winter and spring.^{2,9} The occurrence is highly variable, with epidemics occurring every 5–9 years. The rates are highest among unvaccinated child-bearing women. The susceptibility in child-bearing women, however, varies widely, for unknown reasons. For example, less than 5% women of Kuwait are susceptible, but more than 60% of women in Panama are.

Before vaccine use in non-epidemic years in the USA, congenital rubella syndrome occurred at a rate of 0.1–0.2 cases per thousand live births;² during epidemic years, rates ranged from 1 to 4 cases per 1000. An epidemic lasting from 1964 to 1965 led to the rapid adoption of

the vaccine after its development. During that season, 12.5 million persons contracted rubella, resulting in 2000 cases of encephalitis, 11 250 abortions, and 20 000 cases of congenital rubella syndrome. The 20 000 cases resulted in more than 11 000 cases of deafness, 3580 blind children and 1800 children with mental retardation. Since the adoption of routine childhood vaccination in 1980 in the USA, there has been a dramatic reduction in congenital rubella syndrome, and the country now has 5 or 6 cases per year.

The rubella vaccine

To prevent congenital rubella syndrome, the World Health Organization recommends two approaches.¹⁰ The first approach is to vaccinate all children, with or without mass campaigns, combined with surveillance for disease, to assure eventual immunity in females of child-bearing age. The second approach is to vaccinate females of child-bearing age including adolescent girls, women in the child-bearing years or both.

The vaccine used throughout the world is a live attenuated virus, specifically from a strain named RA27/3.2,¹⁰ Japan and China using other strains. Manufacturers propagate the vaccine in either WI-38 or MRC-5 human diploid cells. The vaccines have proven safe and effective. Between 92% and 98% of vaccine recipients develop measurably protective levels of immunity. In the field, studies estimate the efficacy of this vaccine at 86–97%.

Immunogenicity studies, including our own, show clear-cut evidence of both humoral and cell-mediated immunity following rubella vaccination. Of Canadian children originally vaccinated at 12 months of age, 92% demonstrated seropositivity by enzyme immunoassay when reimmunised at age 14 years. Seventy-four per cent had evidence of cell-mediated immunity as demonstrated by lymphocyte proliferation. Other studies indicate lower rates of cell-mediated immunity.

Current vaccine limitations

The current vaccine does, however, have its limits. The live vaccine in combination with measles has light and heat lability, resulting in cold-chain issues.³ The live vaccine is also contraindicated in pregnant females and immunocompromised individuals, further limiting its use.²

As implied, there is a measurable primary and secondary failure rate. In one outbreak, 9.8% of all those vaccinated who had been 5 years before developed reinfection. In a resurgence of congenital rubella syndrome in California in 1990, 43% of cases of congenital rubella syndrome occurred among mothers with a history of rubella vaccination. In addition, reinfection clearly risks contagions. Those with subprotective levels of antibodies can become reinfected, this reinfection resulting in viraemia and subclinical infection. The shedding spreads the disease as well as the risk for congenital rubella syndrome.

Finally, the vaccine causes both arthralgias and arthritis in 10–40% of susceptible females. Of note, some develop persistent joint reactions.^{4,5}

Our long-term mission

For these reasons, we posit that peptide or subunit vaccine, perhaps adjuvanted to elicit the appropriate cytokine milieu, should serve well as a more durable, less reactive vaccine against rubella. Our current studies would inform the development of a peptide-based vaccine. We have sought to identify the genetic factors that influence both humoral and cell-mediated immunity. We have therefore examined genetic polymorphisms that either improve or worsen the body's response to the vaccine

Much of acquired immunity depends on peptide binding in the human leukocyte antigen (HLA) groove, and the success of that binding depends on HLA allele-specific polymorphisms. Previously, our work in a group of 346 healthy children found associations with rubella vaccine response and HLA. Now we are recruiting a second cohort to confirm these associations as well as expand our work with the consideration of the influence of homozygosity of HLA on the rubella vaccine-induced immune response.

A wide spectrum of cytokine genes and secreted protein products are likely to play a role in immunity to rubella. These include interferon- α (IFN- α), IFN- β and IFN- γ as well as interleukins (IL) 2, 4, 5, 6, 10 and 12p35, tumour necrosis factor- α and granulocyte-macrophage colony-stimulating factor. We also consider polymorphisms in cytokine receptors genes. These include the IL-2 receptor α , β and γ , IL-4 receptor, IL-6 receptor, IL-6 signal transducer, IL-10 receptor α and β , IL-12 receptor $\beta 1$ and $\beta 2$, IFN- α receptor 1 and 2, IFN- γ receptor 1 and 2, tumour necrosis factor receptor superfamily members 1A and 1B, and colony-stimulating factor 2 receptors, both α and β .

Our previous work

Our previous work has demonstrated substantive heritability for a rubella vaccine.¹¹ We enrolled 100 pairs of twins aged 1–21 years. Forty-five pairs were monozygotic and 55 dizygotic. Each pair was presumed to share the same timing of vaccination as well as administration, storage and most likely lot, as well as pre- and post-vaccination exposures from shared households, daycare and school. Within this cohort, we found a variance in rubella antibody levels due to genetic effects that we calculated at 0.13. The heritability – the ratio of genetic variance to total variance – was 45.7% with a *p*-value of 0.003.

In a previous cohort of 346 students 11–18 years of age, all attending schools in Rochester, who had had two and only two documented doses of measles–mumps–rubella (MMR) vaccine given in the USA, we examined the overall response rate to rubella.¹² We looked at rubella-specific gamma immunoglobulin (IgG) antibody levels using the Enzygnost anti-rubella virus IgG enzyme immunoassay system (Dade Behring, Marburg, Germany), with a sensitivity of 100.0% and specificity of 98.5%. Here we found 99.4% to be seropositive, with an average IgG titre of 38.63 IU/ml. We also looked at the lymphocyte proliferation assay for rubella, and we measured an average stimulation index (SI) of 2.29.

In that study, we found only a modest correlation between humoral and cell-mediated immunity after making a log transformation of stimulation indices.¹² The correlation coefficient was 0.04, with a 95% confidence interval (CI) of 0.07–0.14. In the same cohort, we found higher correlations with humoral and cell-mediated immunities with measles (0.13; 95% CI 0.03–0.24) and mumps (0.12; 95% CI 0.02–0.23).

We also sought evidence of correlation across the viral components of the MMR vaccine with antibody levels. We found an interclass correlation coefficient representing the correlation across the three antibody levels of 0.39, with a 95% CI of 0.33–0.46. The pair-wise Pearson correlation coefficients were 0.45 between rubella and measles antibody, 0.40 between rubella and mumps, and 0.33 between measles and mumps, all with *p*-values less than 0.001.

HLA and rubella immunity

We examined the association between HLA genes and immunity, both humoral and cell-mediated, against rubella in the same cohort.¹² Again, using the Dade Behring enzyme immunoassay to measure antibodies, we found no evidence of an association between class I HLA alleles and humoral immunity. Using the lymphocyte proliferation assay, we did,

however, find some evidence of an association with class I HLA and cell-mediated immunity. The median SI was 2.29 overall, but we found positive associations with HLA-B*3503 [SI of 3.0 ($p = 0.031$)] and with HLA-Cw*1502 [SI of 3.19 ($p = 0.035$)]. In contrast, the HLA-B*3901 allele had a negative association with an SI of 1.34 ($p = 0.066$).

We also found associations with HLA class II alleles and humoral immunity.¹³ The median antibody level for the cohort was 38.63 IU/ml, but for those subjects carrying the HLA-DPB1 locus, we found a global p -value of 0.032. DPB1*0301 was associated with a median antibody level of 22.79 IU/ml ($p = 0.024$), DPB*0401 a median of 42.39 ($p = 0.016$), DPB1*1301 a median of 25.85 IU/ml ($p = 0.050$) and DPB1*1501 a median of 66.10 IU/ml ($p = 0.032$). We found no association with the DQB1 (global $p = 0.817$) or DRB1 (global $p = 0.993$) loci. Furthermore, we found no associations between cell-mediated immunity and HLA class II, including DPB1 (global $p = 0.129$), DQB1 (global $p = 0.061$) and DRB1 (global $p = 0.056$).¹³

We then looked at HLA haplotypes using an expectation maximisation algorithm to infer haplotypes.¹⁴ We found no haplotypes associated with humoral immunity, but we did find three haplotypes associated with variation in cell-mediated immunity. One haplotype was DRB*13/14-DQB*06-DPB*03 ($p < 0.001$), a second was DRB*04-DQB*03-DPB*03 ($p = 0.002$), and a third was DRB*07-DQB*02-DPB*11 ($p = 0.003$).

Homozygosity of the HLA genes has been associated with poor immune response to both vaccines and infectious agents; our previous work found this with measles vaccine.¹⁵ Children with lower antibody level after a single dose of measles vaccine seem to demonstrate higher rates of HLA homozygosity. This effect disappeared after a second dose of measles vaccine.¹⁶ In our cohort of 346 subjects, all having had two doses of rubella, we found a different pattern. In this study, homozygosity at HLA-DPB1 increased the antibody level after two doses. Homozygosities at other genes had no measurable effect on either humoral or cellular immunity to the rubella vaccine.

Cytokines and their receptors

We have preliminary results from 118 recipients of two rubella doses regarding immune response and single nucleotide polymorphisms (SNPs) in the genes of the cytokines and cytokine receptors. We tested a total of 58 SNPs from six cytokine and cytokine receptor genes for IL-2, IL-4, IL-10, IL-12A, IL-12B and IFN- γ . These represent both T-helper cell type 1 and 2 cytokines.

Three SNPs (rs3213093, rs2421047 and rs3212227) within the IL-12B cytokine gene were associated with variation in rubella vaccine-specific cell-mediated immunity ($p = 0.02$). These intronic SNPs were associated with an allele-dose-related increase in cell-mediated immunity (Table 1). Among the 58 SNPs representing cytokine receptor genes, three SNPs (rs1870063, rs2305743 and rs11914) were also associated with rubella-specific cell-mediated immunity. Two SNPs were from IL-12 receptor β 1 and one from IFN- γ receptor 1 (Table 2). Thus, SNPs present within IL-12 β cytokine and IL-12 receptor β 1 and IFN- γ receptor 1 cytokine receptor genes were associated with cellular response to rubella vaccine in an allele dose-related manner.

We also performed a study on T-cell cytokine production in healthy children after rubella vaccination.¹⁷ We tested 106 subjects previously vaccinated with measles, mumps and rubella who were 14–17 years of age. Subjects' peripheral blood mononuclear cells (PBMCs) were purified from heparinised blood. These PBMCs were cultured with and without live rubella vaccine (Meruvax, Merck). We used the Wistar RA27/3 strain of rubella

virus at 75 plaque-forming units per millilitre and incubated the stimulated PBMCs for 5 days.

We then tested the supernatants by enzyme-linked immunosorbent assay for secreted cytokines such as IFN- γ and IL-10. The IFN- γ had a median level of 23.3 pg/ml with an interquartile range from -7.0 to 91.3 pg/ml; 71% of subjects showed a measurable response. IL-10 had a median of 291 pg/ml with an interquartile range of 197.8–355.6 pg/ml, and 99% of study subjects had a response. The correlation between IFN- γ and IL-10 was only 0.26. Our results were not associated with age or gender.

We then sought to test for associations between HLA alleles and either IFN- γ or IL-10 secretion. We found no association with HLA class I or II and rubella-induced IL-10 cytokine secretion. We did find an association with IFN- γ . We found a global p value of 0.03 with the HLA-A locus and levels of IFN- γ . We found three allele-specific associations. The first was HLA-A*0201, associated with an elevated level of IFN- γ (median 30.79 pg/ml; $p = 0.03$). The second was HLA-A*2402, associated with a decreased level of IFN- γ (median 4.62 pg/ml; $p = 0.04$). The third was HLA-A*6801, with a decreased level of IFN- γ (median 3.03 pg/ml; $p = 0.03$).

The work ahead

We have now recruited an additional 396 subjects. Each is 11–19 years of age, and each has previously received two and only two doses of the Merck and Co. measles MMRII. Each subject has undergone a blood draw of 100 ml. We will combine these 396 newly recruited subjects with the 342 previously studied, for a total of 738 subjects. All will undergo genotyping and immune response in vitro assays. We look forward to sharing the results of those investigations with you in the future.

Conclusion

Rubella remains a serious health condition, and the current vaccine has significant shortcomings. We believe that understanding the influence of genetic variation on the vaccine's immune response should generate a better understanding of how the vaccine works and in turn aid in the development in improved vaccines, both for rubella and for other viral diseases.

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Table 1

Cytokine gene single nucleotide polymorphisms (SNPs; $n = 58$) associated with cell-mediated immunity following rubella vaccination

SNP ID	Location	Genotype	Median SI (interquartile range)	P-value
IL-12 β rs3213093	Intron	GG/GA/AA	1.9/3.0/5.8	0.02
IL-12 β rs2421047	Intron	CC/CT/TT	1.9/3.1/5.8	0.02
IL-12 β rs3212227	3'UTR	AA/AC/CC	1.9/3.2/5.8	0.02

A, adenine; C, cytosine; G, guanine; T, thymine; SI, stimulation index; UTR, untranslated region.

Both rs3212227 and rs3212227 are tagged SNPs.

Table 2

Cytokine receptor gene single nucleotide polymorphisms (SNPs; $n = 58$) associated with cell-mediated immunity following rubella vaccination

SNP ID	Location	Genotype	Median SI (interquartile range)	<i>p</i> -value
IL-12 receptor β 1 rs1870063	Intron	GG/GA/AA	2.1/2.4/2.8	0.02
IL-12 receptor β 1 rs2305743	Intron	CC/CT/TT	2.0/2.6/3.0	0.02
IFN- γ receptor 1 rs11914	S350S	TT/TG/GG	2.4/1.9/17.5	<0.01

A, adenine; C, cytosine; G, guanine; T, thymine; SI, stimulation index.

Both rs3212227 and rs3212227 are tagged SNPs.