

# Prevalence of rubella serum antibody in autoimmune diseases

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## ABSTRACT

**Introduction:** The association between infections and autoimmune diseases (AID) has been well described in the medical literature. Several infectious agents have been implicated as inducers of autoimmune responses, such as Parvovirus B19, Epstein-Barr virus, cytomegalovirus, and hepatitis viruses. **Patients and methods:** We examined 1,173 sera from patients with 14 different AID and 238 sera from geographically matched healthy controls, for evidence of prior infection with rubella. All samples were tested for the presence of serum antibodies against rubella using the Bio-Rad BioPlex 2200 system. **Results:** As a group, patients with AID had a higher prevalence of IgM anti-rubella antibodies as compared to healthy controls (11.7% versus 5.4%;  $P = 0.001$ ). The prevalence of IgM anti-rubella antibodies was significantly higher in 5/14 AID, namely in patients with giant cell arteritis (33.3%), primary biliary cirrhosis (24%), antiphospholipid syndrome (20.6%), polymyositis (16%), and inflammatory bowel disease (16%). A similar prevalence of IgM anti-rubella antibodies was detected among controls from different countries. A high prevalence of IgG anti-rubella antibodies was detected among patients with AID (89.9%) and controls. **Conclusion:** The increased prevalence of IgM anti-rubella antibodies in AID suggests a possible role for rubella in the etiopathogenesis of several AID.

**Keywords:** rubella, antibodies, autoimmune diseases.

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## INTRODUCTION

The association of infections with the emergence of autoimmunity has been well described in the medical literature.<sup>1-5</sup> Viral infections may promote autoimmunity via several different and sometimes combined mechanisms. These mechanisms include: direct cytolysis of virus-infected cells; induction of autoimmune responses to “altered self antigens”; molecular mimicry resulting in the activation of autoreactive T and/or B cells by viral antigens; and bystander activation of autoreactive T cells that may be driven by viral superantigens. In addition, viruses may induce disturbances of the finely tuned balance between

T regulatory cells (Tregs) and autoreactive T cells in favor of the autoreactivity, either by specific infection or destruction of Treg or by expansion of autoreactive T cells, and viruses may lead to the activation of innate immunity by triggering toll-like receptors. It could well be that multiple mechanisms need to act in concert to result in disease onset.<sup>1-6</sup>

Several viruses have been implicated as possible initiators or promoters of autoimmune responses. Viral infections commonly produce transient autoimmune responses, most commonly directed against blood cells, accompanied by transient elevation in autoantibodies titers. Parvovirus B19, Epstein-Barr virus (EBV), cytomegalovirus (CMV), and hepatitis viruses

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are notoriously associated with the induction of autoimmune hemolytic anemia, thrombocytopenia, and arthritis.<sup>1-6</sup> For the vast majority of infected patients, the clinical symptoms and antibody titers are transient, and decline over several weeks.

Rubella and congenital rubella syndrome (CRS) are caused by rubella virus (RV). Rubella infection usually presents as low-grade fever accompanied by skin rash, conjunctivitis, sore throat, headache, myalgia, anorexia, nausea, tender lymphadenopathy, and soft palate petechiae.<sup>7</sup> Joint involvement is a common manifestation of rubella. Arthritis or arthralgias occurs in up to 50% of adult females, developing soon after the onset of the rash. Transplacental transmission of RV to the fetus during organogenesis leads to severe congenital malformations collectively referred to as CRS.<sup>8</sup> These include sensorineural hearing loss, ocular abnormalities, congenital heart disease, and central nervous system abnormalities.<sup>7</sup>

Delayed-onset of type-1 diabetes (T1DM) and thyroid abnormalities may develop in the first and second decades of life and represent the most well-known association of rubella with autoimmune diseases (AID).<sup>7-10</sup> Genetic studies demonstrated that patients with CRS who developed T1DM had a significant increase in HLA-DR3 and decreased HLA-DR2 haplotype, which are characteristic of autoimmunity.<sup>7,11</sup> In addition, autoantibodies against insulin and islet cells and T-cell abnormalities have been demonstrated in CRS subjects who developed T1DM.<sup>10-13</sup>

More recent studies have questioned the association of CRS with the development of these autoantibodies.<sup>14</sup> Experimental evidence for a possible causal relationship between rubella infection and T1DM can be derived from the experimental model of T1DM in hamsters, where infection of neonatal hamsters with rubella induces diabetes.<sup>15</sup> As no signs of cytopathological effects can be observed after infection of human islets cells by rubella,<sup>16</sup> one of the most accepted mechanisms for injury is the immune mechanism via molecular mimicry. This is supported by findings of cross-reactivity of T cells from CRS patients affected by T1DM, between rubella virus peptides and GAD protein determinants.<sup>17</sup> Additional features of CRS, such as the late-onset pulmonary and skin disease in infants, lymphocytic infiltration seen in infant pancreas at autopsy, and in some individuals the detection of pancreatic islet cell antibodies and insulin autoantibodies, all support a role for rubella infection on the development of T1DM.<sup>7,12,13,17,18</sup> Thyroid autoimmunity is also more common in the CRS,<sup>19,20</sup> providing further support for the concept of rubella-associated autoimmunity.

Due to its many teratogenic effects leading to CRS, rubella infection is a major public health topic. The best strategy to prevent rubella and CRS is vaccination, which has been

endorsed by the WHO, and is currently applied all over the world. A single dose of rubella vaccine confers immunity in 95% or more of recipients at 12 months of age or older.

Until now, the exposure to rubella in the past has been evaluated only in small groups of patients with selected AID. In this study we recruited a large cohort of patients with several AID and evaluated their sera for evidence of prior exposure to rubella, and its possible implications.

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## PATIENTS

Serum samples were collected from 1,173 patients with 14 different AID. These included rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), inflammatory bowel disease (IBD), polymyositis, systemic sclerosis (SSc), primary biliary cirrhosis (PBC), Sjögren's syndrome (SS), multiple sclerosis (MS), autoimmune thyroid disease (Graves disease and Hashimoto's thyroiditis), vasculitides including giant cell arteritis (GCA), and pemphigus vulgaris (PV). The control sera were collected from 238 geographically matched healthy controls with similar age and gender distribution. This study was approved by the local Ethical Committees and fulfilled the ethical guidelines of the Declaration of Helsinki (Edinburgh, 2000).

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## METHODS

All sera were tested for the presence of serum antibodies (IgM and IgG) against rubella using the Bio-Rad BioPlex 2200 system® (Hercules, CA, USA). The BioPlex 2200 is a fully automated, random-access analyzer built on a synthesis of multiplex, magnetic beads, and flow cytometry technologies. At the core of the technology are 25 different populations of 8- $\mu$ m magnetic beads, which are dyed with two fluorophores for classification purposes. Each bead is coated with specific proteins, according to the different assay being tested, thus representing a different target antigen. Briefly, colored beads coated with different antigens were mixed together, along with the patient's sample and sample diluent and then allowed to incubate for 20 min at 37 °C.

After a wash cycle, different isotypes of antihuman antibodies, according to the different assay, conjugated to phycoerythrin (PE) were added to the dyed bead and allowed to incubate for 10 min at 37 °C. After removal of excess conjugate, the bead mixture was passed through the detector that identifies the beads based on the fluorescence of the dyes. The amount of antibody bound to the bead was determined by the fluorescence of PE.

Raw data were initially measured as the relative fluorescence intensity and then converted to the fluorescence ratio using a pre-dyed internal standard bead, which is included in every bead set to normalize the detector signal. A series of calibrators were analyzed along with the patient samples to convert fluorescence ratios to antibody concentration units. Two additional control beads were also included in all incubations. A serum verification bead and a blank bead were added to verify the addition of serum to the reaction vessel and the absence of significant nonspecific binding, respectively. Elevated titers of IgG anti-rubella antibodies were determined at 10 IU/mL according to the manufacturer instructions. At the time the tests were performed, the BioPlex kit for IgM was still in developmental stages and was not commercially available. As such, we decided on a cut-off value of two standard deviations above the geographically matched normal control group to define results as positive. The technology applied in this work had already been published and evaluated prior to this study, including in our previous works as well as in other publications.<sup>21,22</sup>

All data are expressed as means  $\pm$  confidence interval. The statistical analysis was performed by Mann-Whitney *U* test.  $P < 0.05$  was considered significant. The Matlab program (Mathworks, Natick, MA, USA) was used for all statistical analyses.

**Table 1**  
Prevalence of IgM and IgG for rubella in autoimmune diseases

European AID	No. of patients evaluated (IgM)	IgM levels > 1.8 IU/mL % (n)	P	No. of patients evaluated (IgG)	IgG levels > 10 IU/mL % (n)	P
APS	97	20.6% (20)	< 0.001	96	97.9% (94)	NS
SLE-APS	60	11.6% (7)	NS	62	98.4% (61)	NS
Vasculitides	32	9.4% (3)	NS	31	90.3% (28)	NS
GCA	21	33.3% (7)	< 0.001	29	96.5% (28)	NS
IBD	119	15.9% (19)	0.015	115	93.9% (108)	NS
PM	100	16% (16)	0.02	98	95.9% (95)	NS
Graves	70	5.7% (4)	NS	70	98.6% (69)	NS
Hashimoto	50	10% (5)	NS	50	90% (45)	NS
PV	29	13.8% (4)	NS	29	96.5% (28)	NS
PBC	66	24.2% (16)	< 0.001	69	88.4% (61)	NS
SSc	78	12.8% (10)	NS	80	93.7% (75)	NS
Columbian AID	No. of patients evaluated (IgM)	IgM levels > 2.6 IU/mL % (n)	P	No. of patients evaluated (IgG)	IgG levels > 10 IU/mL % (n)	P
SLE	119	6.7% (8)	NS	290	90% (261)	NS
SS	82	4.8% (4)	NS	84	78.6% (66)	NS
RA	152	8.5% (13)	NS	187	93% (174)	NS
MS	98	2% (2)	NS			

## RESULTS

The 1,173 patients with AID and their respective controls came from two distinct groups, Western Europeans and Colombian patients, and each group was analyzed separately. Among the 722 Western Europe patients, the AID occurred as follow: primary APS (97), SLE and secondary APS (60), ANCA-positive vasculitides (32), GCA (21), IBD (80 Crohn's disease and 39 patients had ulcerative colitis), polymyositis (100), Hashimoto's thyroiditis (50), Graves disease (70), PV (29), PBC (66), and SSc (78). Among the 451 Colombian patients, there were 152 patients with RA, 119 with SLE, 98 with MS, and 82 with SS.

Table 1 summarizes the prevalence of IgM and IgG antibodies to rubella in the different AID and according to geographic origin. Of notice, the prevalence of anti-rubella IgM among all the controls, from the two distinct geographical areas, was low, and did not significantly differ (5.1% and 5.7%  $P = 0.3$ ). In contrast, as a group, patients with AID had a higher prevalence of IgM antibodies to rubella, 138/1173 patients (11.7%) as compared to 13/238 (5.4%) among all matched healthy controls ( $P = 0.001$ ).

The prevalence of IgM anti-rubella antibodies was significantly higher in 5/14 different AID, namely in patients with GCA (33.3%), PBC (24%), APS (20.6%), polymyositis

(16%), and IBD (15.9%) as compared to their respective controls. A similar high prevalence of IgG antibodies against rubella was detected among patients with AID (89.9%) and among the controls, albeit with differences between countries. The prevalence was significantly higher in European healthy controls as compared to Colombian controls (95.9% *versus* 89.3%;  $P = 0.01$ ).

## DISCUSSION

Rubella infection has been associated with several autoimmune features including transient hematological disturbances, joint diseases, and late-onset T1DM and thyroid abnormalities.<sup>7</sup> As rubella vaccination became the standard of care in the western world, we sought to explore the possible link between rubella past exposure, either by natural infection or by vaccination, with several AID. This is the first study to evaluate the prevalence of rubella past exposure in a very large cohort of patients with a wide spectrum of AID.

Rubella virus (RV) is a single-stranded RNA virus, which has a central core, the nucleocapsid is composed of polypeptide C (C protein), and is surrounded by a lipid-containing envelope. The outer envelope contains several copies of two virus-specific polypeptides (E1, E2), which are important for viral virulence and immunity.<sup>7</sup> Following invasion of the epithelium of the nasopharynx, the RV spreads hematogenously (primary viremia) to regional and distant lymphatics and replicates in the reticuloendothelial system. This is followed by a secondary viremia, when the RV can be recovered from different body sites including lymph nodes, urine, cerebrospinal fluid (CSF), synovial fluid, and lungs. Individuals may acquire the infection from a completely asymptomatic patient or from an individual shedding the virus during the incubation period.

Rubella major manifestations include skin rash, conjunctivitis, sore throat, headache, myalgia, fever, tender lymphadenopathy, and soft palate petechiae. The rash disappears as the humoral immune response develops, parallel also to termination of viremia.<sup>7</sup> Arthritis or arthralgias are common features during or after rubella infection. The arthritis is frequently symmetrical, polyarticular, and most commonly involves metacarpal and proximal interphalangeal joints of the hand, followed by the wrists, knees, and ankles.<sup>7</sup> The arthritis is usually self-limited,<sup>7</sup> however massive effusions may accompany arthritis, and prolonged or relapsing joint symptoms may follow exposure to RV or to rubella vaccine.<sup>23</sup> As RV may directly infect synovial cells,<sup>24</sup> and as arthritis appears concomitantly with the antibody response, immune complexes are probably involved in the pathogenesis of rubella arthritis.<sup>7</sup>

Rare complications of RV infection include encephalitis, post-infectious encephalopathy, Guillain-Barré polyradiculitis, thrombocytopenia purpura and hemolytic anemia. In the CRS, patients typically are afflicted by sensorineural hearing loss, ocular abnormalities, congenital heart disease, and central nervous system abnormalities.<sup>7</sup> Other findings in CRS include hepatosplenomegaly, jaundice, hepatitis, bone lesions, anemia, and thrombocytopenia purpura, and late-onset manifestations consist of thyroid abnormalities and T1DM.<sup>7</sup> These later manifestations support a possible role for RV in the induction of autoimmune disorders.

Most rubella infections lead to long-lasting immunity mediated by antibodies. Humoral and cell-mediated responses are produced against all three structural proteins (C-protein and gp E1 and E2). The capsid protein and E1 and E2 gp contain major virus-neutralizing B-cell and T-helper cell epitopes.<sup>25–29</sup> As RV has been isolated from patients with prolonged rubella-associated arthritis with apparently adequate antibody responses,<sup>30</sup> several authors have suggested that antibodies alone may sometimes be insufficient to eradicate RV, heightening the important contribution of capsid-specific cytotoxic T cells to the elimination of RV.<sup>29</sup> The method of choice for the diagnosis of rubella is the detection of rubella-specific IgM antibodies in a single serum sample, or a significant (> 4-fold) rise in rubella-specific IgG antibody titer between the acute and convalescent serum specimens.<sup>31</sup>

In the present study, a significant increase in anti-rubella IgM antibodies was detected in five AID, namely in APS, GCA, IBD, polymyositis, and PBC patients. These results support the importance of infection exposure in AID.

Several authors have demonstrated an association of rubella exposure, either by natural infection or by vaccination, with autoimmune manifestations such as autoimmune hemolytic anemia, autoimmune thrombocytopenia purpura, arthritis, and arthralgia.<sup>23,32–35</sup> Joint involvement has been demonstrated especially in females.<sup>32–35</sup> Specifically the rubella vaccine has been associated with the development of transient arthralgia (~ 25%), acute arthritis (< 10%), and even rarely with chronic arthritis.<sup>23,36,37</sup> Female gender, older age, prior seronegativity, and certain HLA types appear to be risk factors.<sup>23</sup>

Some authors have suggested that prior rubella infection may be associated with RA and with juvenile RA.<sup>38–40</sup> In a past analysis of antibody profile against rubella in selected groups of patients with juvenile arthritis, rubella vaccine associated-arthritis, SLE and adult RA, antibody titers to rubella in SLE and RA patients were similar to controls, but levels were significantly increased in vaccine-associated arthritis and juvenile arthritis. In addition, in the juvenile group, rubella antigens were detected in the synovial fluid in 33% of patients, leading

the authors to suggest a possible role for rubella infection at least in a proportion of juvenile arthritis patients.<sup>40</sup> This notion has been reinforced by a recent case reported by Korematsu et al.,<sup>41</sup> where a young girl developed abruptly a severe and life-threatening relapse of systemic type juvenile idiopathic arthritis after a prolonged remission, five days after rubella vaccination. The abrupt onset and the temporal relationship to vaccination suggest that molecular mimicry was a plausible mechanism for the severe disease reactivation. Other studies failed to provide conclusive evidence for the role of the rubella in the etiopathology of juvenile RA.<sup>42</sup>

In our large cohort of SLE and RA patients, we could not demonstrate an increased prevalence of rubella past exposure, or increased rubella antibody titers. Our results are in agreement with recent studies which did not demonstrate an increased risk for chronic arthropathy among women vaccinated against rubella.<sup>23,35,43</sup> Interestingly, in the subgroup of neuropsychiatric lupus patients, our group has previously reported elevated titers of rubella IgM antibodies, which had a marginal correlation with psychosis and depression.<sup>44</sup>

Our analysis revealed an increased prevalence of anti-rubella IgM in GCA (33.3%). We could not find previous studies on past rubella exposure in GCA patients. Maybe because rubella is usually an infection affecting the young and GCA a disease of the more elderly, this association has not been explored. Our preliminary results should encourage further studies to confirm and explore this possible link.

The association of rubella and liver disease has been documented by several authors.<sup>45-47</sup> In the past, significant elevated titers of antibodies against rubella have been detected in patients with chronic persistent hepatitis, chronic active hepatitis, and occasionally in acute hepatitis.<sup>45-47</sup> More recently Kalvenes et al.<sup>48</sup> demonstrated a high antibody response to rubella in active autoimmune chronic hepatitis patients, using three distinct antigen-antibody systems, illustrating an enhanced response to rubella structural proteins in these patients. But with the use of radioimmunoprecipitation assays (RIPA), rubella antibody titers were not increased in PBC patients. These results in PBC patients are in contrast to the other groups of liver diseases, and also with the findings of the present study, and may reflect differences in the population studied and/or in assay technology.

It has been well documented that several infections can be accompanied by increases in circulating antiphospholipid antibodies, including rubella infection.<sup>49,50</sup> We found a significant prevalence of IgM against rubella in primary APS patients (20.6%), but not among patients with secondary APS (SLE and APS). To our knowledge, this is the first reported association between rubella exposure and primary APS, and as such merits

further evaluation. In patients with secondary APS, titers of IgM to rubella were not increased, similar to patients with SLE only.

In the present study we detected elevated titers of IgM to rubella in a significant percentage of patients with polymyositis (16%). We found only one study performed thirty years ago which evaluated antibody titers to viral infections, including rubella, among 20 polymyositis children.<sup>51</sup> In contrast to our results, in this early study performed in children, rubella titers were not elevated. But not only the studies differ in sample size and patients' age, but studies performed in the 70's probably included large percentages of non-vaccinated people, and maybe non-exposed people to rubella patients. These differences may explain the discrepancy between results.

In our IBD patients' group we detected a significant percentage of patients (16%) with increased IgM titers against rubella. Several studies have been conducted addressing the possible relationship between rubella exposure or MMR vaccination and IBD.<sup>52-54</sup> Most of these studies were epidemiological, and were based on documentation of vaccination or disease, and one study evaluated IgG titers for rubella. All these studies did not find any association between past exposure to measles, mumps or rubella either by natural infection or by vaccination, with the development of ulcerative colitis or Crohn's disease. Similarly, in our cohort of IBD patients we could not find differences in IgG levels to rubella compared to controls, but IgM titers were significantly elevated. This finding should be confirmed in future studies.

In our large cohort of patients with a wide spectrum of AID, we could not find significant differences in IgG levels against rubella, and these were similar almost across all the diseases and the controls. The high prevalence of IgG seropositivity to rubella reflects the success of the global effort to eradicate CRS by worldwide vaccination. Interestingly, comparing the two control groups, we found that the prevalence was significantly higher in European healthy controls compared to Colombian controls (95.9% *versus* 89.3%;  $P = 0.01$ ). This difference can be explained by the earlier introduction of rubella vaccination and the higher coverage of vaccination in Western Europe as compared to Colombia.

Even though rubella vaccination has been available for over 30 years, and global efforts have achieved a high coverage of vaccination in the western world, as reflected by the high percentage of IgG seropositivity among our patients and controls, this study detected significant differences in the titers of IgM to rubella. A significant percentage of selected patients with AID had elevated titers of IgM to rubella. Our results add up to the accumulating evidence on the association of infections and AID, and reinforce the importance of previous exposure to infectious agents on the induction of AID.

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