Glucocorticoid: Major Factor for Reduced Immunogenicity of 2009 Influenza A (H1N1) Vaccine in Patients with Juvenile Autoimmune Rheumatic Disease

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ABSTRACT. *Objective.* To assess the immunogenicity and safety of non-adjuvanted influenza A H1N1/2009 vaccine in patients with juvenile autoimmune rheumatic disease (ARD) and healthy controls, because data are limited to the adult rheumatologic population.

Methods. A total of 237 patients with juvenile ARD [juvenile systemic lupus erythematosus (JSLE), juvenile idiopathic arthritis (JIA), juvenile dermatomyositis (JDM), juvenile scleroderma, and vasculitis] and 91 healthy controls were vaccinated. Serology for anti-H1N1 was performed by hemagglutination inhibition assay. Seroprotection rate, seroconversion rate, and factor-increase in geometric mean titer (GMT) were calculated. Adverse events were evaluated.

Results. Age was comparable in patients and controls (14.8 ± 3.0 vs 14.6 ± 3.7 years, respectively; p = 0.47). Three weeks after immunization, seroprotection rate (81.4% vs 95.6%; p = 0.0007), seroconversion rate (74.3 vs 95.6%; p < 0.0001), and the factor-increase in GMT (12.9 vs 20.3; p = 0.012) were significantly lower in patients with juvenile ARD versus controls. Subgroup analysis revealed reduced seroconversion rates in JSLE (p < 0.0001), JIA (p = 0.008), JDM (p = 0.025), and vasculitis (p = 0.017). Seroprotection (p < 0.0001) and GMT (p < 0.0001) were decreased only in JSLE. Glucocorticoid use and lymphopenia were associated with lower seroconversion rates (60.4 vs 82.9%; p = 0.0001; and 55.6 vs 77.2%; p = 0.012). Multivariate logistic regression including diseases, lymphopenia, glucocorticoid, and immunosuppressants demonstrated that only glucocorticoid use (p = 0.012) remained significant.

Conclusion. This is the largest study to demonstrate a reduced but adequate immune response to H1N1 vaccine in patients with juvenile ARD. It identified current glucocorticoid use as the major factor for decreased antibody production. The short-term safety results support its routine recommendation for patients with juvenile ARD. ClinicalTrials.gov; NCT01151644. (J Rheumatol First Release Nov 15 2011; doi:10.3899/jrheum.110721)

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Infection remains a leading cause of morbidity and mortality in patients with juvenile autoimmune rheumatic diseases (ARD). The combined immunosuppressive effects of the disease itself and its treatment render the individual more susceptible to infections. Further, intercurrent infections may contribute to rheumatic disease flares[1,2,3]. The recent

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pandemic caused by the new influenza A H1N1/2009 virus led to a higher incidence of hospitalization and death than the annual rates associated with the seasonal influenza viruses[4], especially in immunosuppressed patients. Of note, vaccination is the most effective measure to control the spread of the virus and to reduce associated morbidity and mortality.

Based on concerns that influenza A H1N1/2009-like viruses would continue to circulate during the next influenza season, the 2010 Recommendations of the Advisory Committee on Immunization Practices stated that all children and adolescents aged between 6 months and 18 years should receive the trivalent seasonal influenza vaccine containing the A/California/7/2009(H1N1)-like virus[5]. According to these recommendations, vaccination is particularly important for patients at increased risk for severe complication, including those with chronic conditions, such as juvenile ARD, particularly in patients under immunosuppressive therapy[5].

However, a point of concern is whether the immune response to this vaccine is significantly impaired by rheumatic disease itself and/or its treatment. To date, no study had evaluated the efficacy and safety of the influenza A H1N1/2009 vaccine in patients with juvenile ARD. A few studies with small populations evaluated the immune response to other vaccines in these patients[6,7,8]. Kanakoudi-Tsakalidou, et al showed a satisfactory antibody response to the seasonal influenza immunization in patients with juvenile rheumatic diseases under immunosuppressive therapies[6]. In contrast, studies on immunosuppressed non-rheumatic children and adolescents, such as those with cancer and after kidney transplant, revealed a limited response to the influenza A H1N1/2009 vaccine[9,10].

The aim of our study was to evaluate the immunogenicity and safety of influenza A H1N1/2009 vaccine in patients with juvenile ARD compared to healthy controls.

MATERIALS AND METHODS

Patients and controls. A total of 237 outpatients routinely followed at the Pediatric Rheumatology Unit and the Rheumatology Division of Clinics Hospital, São Paulo, with juvenile ARD were included. All patients fulfilled the international classification criteria as follows: for juvenile systemic lupus erythematosus (JSLE)[11], juvenile idiopathic arthritis (JIA)[12], juvenile scleroderma (JScl)[13], juvenile dermatomyositis (JDM)[14], Behçet disease[15], Takayasu arteritis[16], granulomatosis with polyangiitis (previously denoted Wegener granulomatosis)[16], polyarteritis nodosa[16], and Henoch-Schönlein purpura or Kawasaki disease[17]. A total of 91 age-matched healthy subjects were concomitantly included in the control group. All participants were ≥ 9 and ≤ 21 years old, and exclusion criteria included previous proven infection by influenza A H1N1/2009; anaphylactic response to vaccine components or to egg; previous vaccination with any live vaccine 4 weeks before or any inactivated vaccine 2 weeks before the study; 2010 seasonal influenza vaccination; acute infection resulting in fever over 38°C at the time of vaccination; Guillain-Barré syndrome or demyelinating syndromes; blood transfusion within 6 months; and hospitalization.

Study design. This was a prospective, open study conducted between March 2010 and April 2010. All patients with juvenile ARD were invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the immunization center of the same hospital. Healthy volunteers who came to this center seeking vaccination in response to the national public health campaign were included in the control group. This protocol was approved by the local institutional review board, and informed consent was obtained from all participants. The study was registered with clinicaltrials.gov under NCT01151644.

A single intramuscular dose (0.5 ml) of H1N1 A/California/7/2009-like virus vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur) was administered to all participants. Patients and controls were evaluated on the day of vaccination (from March 22 to April 2) and after 3 weeks. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

Vaccine. A novel monovalent, non-adjuvanted, inactivated, split-virus vaccine was supplied by Butantan Institute/ Sanofi Pasteur (São Paulo, Brazil). The vaccine contained an inactivated split influenza virus with 15 μg hemagglutinin antigen equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. Embryonated chicken eggs were employed using the same standard techniques for the production of seasonal, trivalent, inactivated influenza vaccine. The vaccine was presented in 5-ml multidose vials with thimerosal (45 μg per 0.5-ml dose) as a preservative.

Hemagglutination inhibition assay. Antibody levels against H1N1 A/California/7/2009-like virus were evaluated using the hemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute.

Sera were tested for antibodies to the H1N1 A/California/7/2009 influenza strain supplied by Butantan Institute. Sera were tested at an initial dilution of 1:10, and at a final dilution of 1:2560. For the purposes of calculations, negative titers had an assigned value of 1:5, and titers > 1:2560 a value of 1:2560. Samples were tested in duplicate, and geometric mean values were used in the analysis.

Virus concentrations were previously determined by hemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring nonspecific inhibitors from the sera as described[18].

The immunogenicity endpoints after vaccination were the seroprotection rate (titer ≥ 1:40), seroconversion rate (prevaccination titer < 1:10 and postvaccination HIA titer ≥ 1:40 or prevaccination titer ≥ 1:10 and postvaccination titer ≥ 4-fold increase), geometric mean titers (GMT), and factor-increase in GMT (ratio of GMT after vaccination to GMT before vaccination).

Safety assessment. At the day of vaccination, parents were given a 21-day personal diary card containing the following list of predefined adverse events: local reactions (pain, redness, swelling, and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea, and nasal congestion). Participants were asked to give "yes/no" responses for each side effect and to return their diary cards at the second evaluation day (21 days after vaccination). Adverse events that were not on the list were also reported.

All local reactions were considered related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analyzed by the investigators to determine causality. Severe side effects were defined as those requiring hospitalization or death.

Statistical analysis. The sizes of the juvenile ARD population and controls gave the study a power of analysis > 95%.

The immunogenicity and safety analyses were descriptive, and 2-sided 95% CI were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for hemagglutination inhibition titers. For prednisone and immunosuppressant drug use, seroprotection rate, seroconversion rate, and adverse events, Fisher's exact test was used. GMT were compared between each subgroup of patients with juvenile ARD and the control group using a 2-sided Student t test or Mann-Whitney U test on the log_{10}-transformed titers. The factor-increase in GMT was also calculated for all participants. Spearman's correlation was used to compare the log_{10}-transformed titers and log_{10}-transformed factor-increase with gluco-

corticoid dose. Multivariate logistic regression analysis was performed using seroconversion rate as the dependent variable and the variables with p < 0.2 in the univariate analyses as independent variables (JSLE, JIA, JDM, primary vasculitis, glucocorticoid use, concomitant glucocorticoid and immunosuppressant use, and lymphopenia). All tests were 2-sided, and significance was set at a p value < 0.05.

RESULTS

In total, 237 patients with the following juvenile ARD were studied: 99 JSLE, 93 JIA, 18 JDM, 11 JScl [5 systemic sclerosis (SSc) and 6 localized scleroderma], and 16 primary vasculitis (5 Henoch-Schönlein purpura, 3 Takayasu arteritis, 3 granulomatosis with polyangiitis, 3 polyarteritis nodosa, 1 Kawasaki disease, and 1 Behçet disease), and 91 healthy controls (Table 1).

Patients and controls were comparable regarding mean current age (14.8 ± 3.0 yrs vs 14.6 ± 3.7 years, respectively; p = 0.47), with a predominance of females among patients with ARD (66% vs 52%; p = 0.02). Mean disease duration was 5.8 ± 3.7 years. Ninety patients (38%) were taking glucocorticoids, with a mean dose of prednisone 17.4 ± 14.2 mg/day (0.36 ± 0.32 mg/kg/day), and mean glucocorticoid duration of 43.1 ± 34.5 months, and 84.5% of patients had a diagnosis of JSLE. Sixty percent (60.3%) of patients were treated with immunosuppressive agents, and more than half (51.7%) were under methotrexate (MTX) therapy (Table 1).

Influenza A H1N1/2009 vaccine immunogenicity. Seroprotection and seroconversion rates of patients and controls are shown in Table 2. At baseline, seroprotective antibody titer ≥ 1:40 was seen in 22.4% (n = 53) of patients with juvenile ARD and 20.9% (n = 19) of controls (p = 0.882; Table 2). After 21 days, the vaccine seroprotection rate was 81.4% (95% CI 76.5%–86.4%) in patients with juvenile ARD, sig-

nificantly lower than in controls (95.6%; 95% CI 91.4%–99.8%; p = 0.0007). Moreover, following vaccination, the seroconversion rate was significantly lower in patients with juvenile ARD compared to controls [74.3% (95% CI 68.7%–79.9%) vs 95.6% (95% CI 91.4%–99.8%); p < 0.0001]. As for immunogenicity in each rheumatic disease, seroprotection rates prior to vaccination were comparable between patients and controls. The postvaccination seroprotective rate was lower in patients with JSLE compared to controls (p < 0.0001), and a tendency of a reduced rate was observed in those with primary vasculitis (p = 0.067). Of note, seroconversion rates were reduced in patients with JSLE (p < 0.0001), JIA (p = 0.008), JDM (p = 0.025), and primary vasculitis (p = 0.017) compared to controls (Table 2).

The GMT values in patients with juvenile ARD and controls are illustrated in Table 3. GMT after immunization [147.2 (95% CI 119.7–181.1) vs 250.8 (95% CI 196.3–320.3); p = 0.011] and the factor-increase in GMT [12.9 (95% CI 10.7–15.7) vs 20.3 (95% CI 15.6–26.4); p = 0.012] were significantly lower in the ARD group compared to the control group. Disease evaluations for specific patient subgroups revealed lower GMT after immunization and also a lower factor-increase in GMT only in patients with JSLE compared to controls (p < 0.0001; Table 3).

Further analysis of the influence of therapy on immunogenicity revealed a lower percentage of seroconversion among patients using glucocorticoids compared to those without this medication (60.4% vs 82.9%; p = 0.0001). There was no difference in rates for seroprotection (p = 0.247) or seroconversion (p = 0.279) between patients taking prednisone < 20 mg/day and those taking ≥ 20 mg/day. However, a trend for lower GMT and factor-increase in GMT after vaccination was observed among patients taking prednisone > 20 mg/day [49.4 (95% CI 28.9–84.7) vs 95.2 (95% CI 63.4–143.1), p = 0.076, and 5.3 (95% CI 3.4–8.3) vs 9.3 (95% CI 6.6–13.2), p = 0.054, respectively]. Also, a significant negative correlation was observed regarding glucocorticoid dose and $\log_{10}$-transformed titers (r = –0.36, p < 0.0001), as well as glucocorticoid dose and $\log_{10}$-transformed factor-increase of GMT (r = –0.30, p < 0.0001).

Concerning immunosuppressant use, no differences in the seroconversion rate (76.4% vs 75.5%; p = 0.763), seroprotection rate (80.4% vs 83%; p = 0.733), or GMT [130.3 (95% CI 99.3-170.8) vs 177.4 (95% CI 129.7-242.6); p = 0.151] were observed comparing patients taking and not taking these drugs. The specific analysis of MTX, azathioprine, cyclosporine, mycophenolate mofetil, leflunomide, and cyclophosphamide revealed no effects on seroconversion and seroprotection (p > 0.05) in patients taking and not taking these drugs. A reduced postvaccination GMT was observed only for patients taking azathioprine (p = 0.019) and mycophenolate mofetil (p = 0.01). Concomitant use of immunosuppressive therapy and glucocorticoid resulted in a

Table 1. Distributions of rheumatic diseases and therapies in 237 patients. Data are the mean ± SD or n (%).

Feature	
Disease	
Juvenile systemic lupus erythematosus	99 (41.8)
Juvenile idiopathic arthritis	93 (39.2)
Juvenile dermatomyositis	18 (7.6)
Juvenile scleroderma	11 (4.6)
Primary vasculitis	16 (6.8)
Treatment	
Prednisone	90 (38)
Dose, mg/day	17.4 ± 14.2
Dose, mg/kg/day	0.36 ± 0.32
Dose ≥ 20 mg/day	36 (40)
Duration of glucocorticoid therapy, mo	43.1 ± 34.5
Immunosuppressant	143 (60.3)
Methotrexate	74 (51.7)
Azathioprine	43 (30.1)
Cyclosporine	23 (16.1)
Mycophenolate mofetil	13 (9.1)
Leflunomide	6 (4.2)
Cyclophosphamide	3 (2.1)

Table 2. Seroprotection and seroconversion rates of influenza A (H1N1) 2009 vaccine in patients with rheumatic disease and controls.

| | N | Seroprotection Rate (titer $\geq$ 1/40) | | Seroconversion Rate, % (95% CI) |
		Before Immunization, % (95% CI)	After Immunization, % (95% CI)	
Control	91	20.9 (12.6–29.3)	95.6 (91.4–99.8)	95.6 (91.4–99.8)
JARD	237	22.4 (17.1–27.7)	81.4 (76.5–86.4)*	74.3 (68.7–79.9)*
JSLE	99	20.2 (12.3–28.1)	73.7 (65.0–82.4)*	63.6 (54.1–73.1)*
JIA	93	20.4 (12.2–28.6)	88.2 (81.6–94.8)	82.8 (75.1–90.5)*
JDM	18	38.9 (16.4–61.4)	83.3 (66.1–100.5)	77.8 (58.6–97.0)*
JScl	11	27.3 (1.0–53.6)	90.9 (73.9–107.9)	90.9 (73.9–107.9)
Primary vasculitis	16	25.0 (13.8–46.2)	81.3 (62.2–100.4)	75 (53.8–96.2)*

* $p < 0.05$. JARD: juvenile autoimmune rheumatic diseases; JSLE: juvenile systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; JDM: juvenile dermatomyositis; JScl: juvenile scleroderma.

Table 3. Geometric mean titers and factor-increases in the geometric mean titer after influenza A (H1N1) 2009 vaccination in patients with juvenile autoimmune rheumatic disease and controls.

| | N | Geometric Mean Titer | | Factor-increase in Geometric Mean Titer (95% CI) |
		Before Immunization, % (95% CI)	After Immunization, % (95% CI)	
Control	91	12.4 (9.7–15.7)	250.8 (196.3–320.3)	20.3 (15.6–26.4)
JARD	237	11.4 (9.7–13.3)	147.2 (119.7–181.1)*	12.9 (10.7–15.7)*
JSLE	99	10.9 (8.5–13.9)	91.1 (66.0–125.8)*	8.4 (6.3–11.2)*
JIA	93	10.8 (8.4–13.8)	217.2 (159–296.7)	20.2 (14.8–27.5)
JDM	18	15.3 (8.9–26.3)	201.6 (95.4–425.8)	13.2 (7.2–24.1)
JScl	11	12.1 (6.0–24.2)	181.5 (70.2–469.4)	15.0 (6.3–35.9)
Primary vasculitis	16	14.1 (6.8–29.2)	182.2 (68.1–487.4)	12.9 (5.9–28.2)

* $p < 0.05$. JARD: juvenile autoimmune rheumatic diseases; JSLE: juvenile systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; JDM: juvenile dermatomyositis; JScl: juvenile scleroderma.

lower seroconversion rate compared to patients without immunosuppressive or glucocorticoid therapy (64.8% vs 78.3%; p = 0.0352).

In the analysis of lymphocyte count, patients with juvenile ARD with lymphopenia (lymphocyte count < $1000/mm^3$) showed a significantly lower seroconversion rate compared to those without this complication (55.6% vs 77.2%, respectively; p = 0.012).

Multivariate logistic regression was performed to determine possible deleterious factors for the seroconversion rate [i.e., disease (JSLE, JIA, JDM, primary vasculitis), lymphopenia (lymphocyte count < $1000/mm^3$), or glucocorticoid use or concomitant glucocorticoid and immunosuppressant]. Only glucocorticoid use remained significant (OR 0.20, 95% CI 0.06–0.70, p = 0.012; Table 4). Reinforcing this finding, a significant negative correlation was observed between glucocorticoid dose and log_{10}-transformed titers (r = –0.36, p < 0.0001), as well as between glucocorticoid dose and log_{10}-transformed factor-increase of GMT (r = –0.30, p < 0.0001).

Vaccine safety. Local and systemic adverse events reported within 21 days of vaccination are summarized in Table 5.

Table 4. Multivariate logistic regression analyses including current treatment and lymphopenia as independent variables for seroconversion in patients with juvenile autoimmune rheumatic diseases after influenza A (H1N1) 2009 vaccination.

Variable	OR (95% CI)	p
JSLE	0.36 (0.039–3.33)	0.368
JIA	0.45 (0.05–3.83)	0.47
JDM	0.51 (0.05–5.70)	0.586
Primary vasculitis	0.60 (0.05–7.21)	0.691
Glucocorticoid use	0.20 (0.06–0.70)	0.012
Concomitant use of glucocorticoid plus immunosuppressant	2.71 (0.90–8.20)	0.077
Lymphopenia	0.61 (0.27–1.38)	0.235

JSLE: juvenile systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; JDM: juvenile dermatomyositis.

Local itching was reported exclusively by patients with juvenile ARD (p = 0.003). The only systemic reaction more frequently observed in patients was arthralgia (13.1% vs 2.2% in controls; p = 0.002), with a median duration of 1 (range 1–9) days and median time of appearance after vac-

Table 5. Adverse events following influenza A (H1N1) 2009 vaccination in patients with juvenile autoimmune rheumatic diseases (JARD) and controls. Data are n (%).

Adverse Events	JARD, n = 237	Control, n = 91	p
Local reactions	60 (25.3)	21 (23.1)	0.78
Pain	43 (18.1)	21 (23.1)	0.35
Redness	9 (3.8)	2 (2.2)	0.73
Swelling	3 (1.3)	2 (2.2)	0.62
Itching	19 (8)	0 (0.0)	0.003
Systemic reactions	84 (35.4)	27 (29.7)	0.36
Arthralgia	31 (13.1)	2 (2.2)	0.002
Fever	13 (5.5)	3 (3.3)	0.57
Headache	41 (17.3)	18 (19.8)	0.63
Myalgia	27 (11.4)	6 (6.6)	0.22
Sore throat	9 (3.8)	5 (5.5)	0.54
Cough	16 (6.8)	5 (5.5)	0.8
Diarrhea	8 (3.4)	2 (2.2)	0.73
Rhinorrhea	19 (8)	3 (3.3)	0.15
Nasal congestion	13 (5.5)	3 (3.3)	0.57

cination of 0 (range 0–12) days. No severe side effects were observed in patients or controls (Table 5).

DISCUSSION

Our study is the largest analysis in patients with juvenile ARD to demonstrate that the non-adjuvanted influenza A H1N1/2009 vaccine is safe and exhibits a reduced immunogenicity associated with glucocorticoid therapy.

This was the first report that evaluated the influenza A H1N1/2009 vaccine response in a cohort of pediatric patients with rheumatic diseases. All patients who agreed to participate were included regardless of disease activity status or current treatment, to closely represent the real-life situation. Also, all patients fulfilled the international criteria for juvenile ARD, and the study benefited from the inclusion of a large patient population, an essential requirement to accurately define vaccine immunoresponse and safety, which was not met by previous studies of seasonal influenza vaccine[6,8]. Moreover, age-matching of the control group is essential because effectiveness of vaccine has a distinct pattern in children and adolescents[19]. Our report included only patients over age 9 years, excluding younger children, who have a lesser humoral response to influenza A H1N1/2009 vaccine[19,20].

This study design provided strong evidence that the immunoresponse to influenza A H1N1/2009 vaccine was impaired in the juvenile ARD population, in contrast to previous studies on seasonal influenza vaccination[6,7,8]. In this regard, Malleson, *et al* evaluated 34 children with chronic arthritis (91% JIA) and observed similar seasonal vaccine immunogenicity in patients and 13 controls, independent of the use of prednisone or immunosuppressive agents[7]. The lack of age-matching to controls hampers the interpretation of their findings due to the inclusion of extremes of age[7]. In

addition, the adequate humoral response reported for children with JIA, JSLE, JDM, and other rheumatic diseases was also not conclusive due to overrepresentation of JIA in the sample and the lack of a healthy control group[6]. On the other hand, in the study of Ogimi, *et al*, the 49 patients with rheumatic disease and 36 with juvenile chronic diseases in the control group had unexpectedly low immunoresponses to the seasonal influenza, although it was comparable between groups[8]. Again, the inclusion of infants and the vaccination protocol used in that study may account for the impaired response that was observed[8].

Of note, our disease subgroup analysis revealed a reduced protective immunogenicity against the pandemic influenza A H1N1/2009 vaccine in all rheumatic autoimmune conditions except JScl. Similarly, we have recently observed an adequate response for this vaccine in adult patients with SSc[21], and effective humoral and cellular responses to an adjuvanted virosomal nonpandemic flu vaccine were also reported in others with this disease[22].

The immunoresponse was considerably compromised in our patients with JSLE, as indicated by the inadequate postseroprotection and postseroconversion rates, deficient increase in GMT, and low factor-increase in GMT, suggesting a more severely impaired immune state in persons with this illness that may ultimately affect the response to antigenic challenge[23]. The well-known lupus intrinsic antibody and cellular dysfunction[24] may account for this finding, which is reinforced by the observation of decreased antibody response[25] and cell-mediated response to influenza vaccination in adult SLE[26].

With regard to JIA, a diminished vaccine response, determined by the significantly lower seroconversion rate, was observed, although it was higher than that in juvenile lupus, in spite of comparable postimmunization seroprotection, GMT, and factor-increase in GMT. The preimmunization rate cannot account for this finding because it was similar to that of the control group. In contrast, previous reports suggest apparently adequate vaccine responses for seasonal influenza[8] and hepatitis[27] in persons with JIA. The inclusion of patients or controls younger than age 9 years[8,27] and 3 years old[8] precludes a definitive conclusion about their findings, as vaccine responses in these 2 age brackets are expected to be much lower than in older children.

Patients with JDM had a deficient seroconversion rate, which is in accord with a report for the same vaccine in adult DM[21]. This finding may be associated with the underlying pathology of this disease, which is known to involve the humoral endotheliopathy initiated by complement deposition in intramuscular blood vessels[28].

The lower immune response to vaccine that we observed in the primary vasculitis group contrasts with the adequate response in reports concerning adult patients with granulomatosis with polyangiitis immunized with seasonal[29] and pandemic H1N1 vaccine[21]. The most likely explanation for

this discrepancy is the limited number of children with primary vasculitis analyzed in our study and the underrepresentation of granulomatosis with polyangiitis in our sample.

Alternatively, a vaccine response may be affected by immunosuppressive therapy, and we determined by multivariate analysis that glucocorticoid therapy was the main contributing factor to a reduced immunoresponse in patients with juvenile ARD. There are conflicting data regarding this drug[30], with a few reports describing no effects on influenza vaccine response in children with rheumatic diseases[6,7,8]. However, the prednisolone dose was described in only 1 of these studies, and it was quite low (0.21 ± 0.16 mg/kg), making it difficult to determine the influence of this drug on vaccine immunogenicity[8]. In contrast, others have reported an attenuated immune response to seasonal influenza vaccination in patients with SLE and asthma under glucocorticoid therapy[25,31]. Indeed, Holvast, et al found that glucocorticoid and/or immunosuppressant was associated with lower humoral and cell-mediated responses against the H1N1 strain of seasonal influenza vaccine in adult SLE[25,26].

Interestingly, in our study the seroconversion rate was not affected by the use of immunosuppressive drugs other than glucocorticoid. However, this analysis was uncertain because MTX represented more than half of the immunosuppressive drugs used, and there was a clear bias of indication by disease. In this regard, an extensive separate analysis of disease activity and drug influence in JSLE and JIA is under way. Nevertheless, previous studies with pediatric and adult rheumatic patients have suggested no deleterious effect of immunosuppressive drugs on antibody responses to seasonal influenza vaccine[6,32,33].

We observed that lymphopenia also reduced seroconversion to unadjuvanted influenza A H1N1/2009 vaccine in patients with juvenile ARD. The response to influenza vaccine depends on adequate antigen processing and presentation, and normal interaction between T and B cells and their activation[25,26]. Studies in patients infected with HIV-1 have shown that anti-influenza-specific antibody responses correlated with the CD4 T cell count[34]. Indeed, HIV-1 infected patients generated poorer responses to monovalent influenza A H1N1/2009 vaccine compared to healthy subjects[35,36].

For pandemic influenza vaccines to be licensed they must meet all 3 current immunologic standards established for seasonal vaccines, which include a percentage of seroprotection > 70%, a percentage of seroconversion > 40%, and a factor-increase in GMT > 2.5[37,38]. These criteria were established for healthy adults aged 18 to 60 years, but were also proposed to be used among the pediatric population[39]. Therefore, although our population of patients with juvenile ARD presented lower percentages of seroprotection and seroconversion and a lower factor-increase in GMT compared to healthy controls, these patients still achieved all of the 3 established immunologic thresholds, showing that the

vaccine, while being less immunogenic, was effective in protecting them.

Influenza A (H1N1) vaccine was well tolerated and safe in patients with juvenile ARD, as no serious short-term adverse event was observed. Arthralgia was a more frequent complaint of patients with juvenile ARD compared to healthy controls. Studies on influenza A/H1N1 2009 vaccine in healthy children and adolescents have not reported musculoskeletal complaints[19,20], suggesting that the occurrence of this manifestation could be related to the patient's genetic background for rheumatic disease[40].

Our study revealed a reduced but adequate immune response to the unadjuvanted influenza A H1N1/2009 vaccine in patients with juvenile autoimmune rheumatic diseases, and identified current glucocorticoid use as the major factor for decreased antibody production. The short-term safety results support routine recommendation for vaccination for patients with juvenile ARD.

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