Influenza A/H1N1 vaccination of patients with SLE: can antimalarial drugs restore diminished response under immunosuppressive therapy?


Abstract

Objective. To assess the efficacy and safety of pandemic 2009 influenza A (H1N1) in SLE under different therapeutic regimens.

Methods. A total of 555 SLE patients and 170 healthy controls were vaccinated with a single dose of a non-adjuvanted preparation. According to current therapy, patients were initially classified as SLE No Therapy (n = 75) and SLE with Therapy (n = 480). Subsequent evaluations included groups under monotherapy: chloroquine (CQ) (n = 105), prednisone (PRED) ≥ 20 mg (n = 76), immunosuppressor (IS) (n = 95) and those with a combination of these drugs. Anti-H1N1 titres and seroconversion (SC) rate were evaluated at entry and 21 days post-vaccination.

Results. The SLE with Therapy group had lower SC compared with healthy controls (59.0 vs 80.0%; P < 0.0001), whereas the SLE No Therapy group had equivalent SC (72 vs 80.0%; P = 0.18) compared with healthy controls. Further comparison revealed that the SC of SLE No Therapy (72%) was similar to the CQ group (69.5%; P = 0.75), but it was significantly reduced in PRED ≥ 20 mg (53.9%; P = 0.028), IS (55.7%; P = 0.035) and PRED ≥ 20 mg + IS (45.4%; P = 0.038). The concomitant use of CQ in each of these later regimens was associated with SC responses comparable with SLE No Therapy group (72%): PRED ≥ 20 mg + CQ (71.4%; P = 1.00), IS + CQ (65.2%; P = 0.54) and PRED ≥ 20 mg + IS + CQ (57.4%; P = 0.09).

Conclusion. Pandemic influenza A H1N1/2009 vaccine response is diminished in SLE under immunosuppressive therapy and antimalarials seems to restore this immunogenicity.

Trial registration. www.clinicaltrials.gov, NCT01151644.

Key words: systemic lupus erythematosus, vaccine, infection, influenza, antimalarials, disease-modifying anti-rheumatic drugs, immunosuppressive, H1N1 vaccination, immune response, efficacy, prevention.

Introduction

Infections in SLE are considered to be important causes of morbidity/mortality [1–3], and vaccination is the most effective preventive measure to control virus dissemination and to reduce associated complications [4]. Pandemic influenza is of particular concern for these immunosuppressed patients due to its additional catastrophic nature, as was reported for the influenza H1N1 (Spanish flu, 1918), A/H2N2 (Asian influenza, 1957),
A/H3N2 (Hong Kong flu, 1968) and H1N1 (Swine flu, 1976; Russian flu, 1977) strains [5]. In 2009, a new pandemic A/California/7/2009 (H1N1) virus emerged, requiring major public health efforts [6]. Its spread throughout the world prompted the decision that the Northern Hemisphere’s 2010–11 and the Southern Hemisphere’s 2011 trivalent seasonal influenza vaccines must contain the A/California/7/2009 (H1N1)-like virus [7]. Immuno-compromised patients have indications to receive this vaccine according to the European League Against Rheumatism [8, 9] and the 2010 Recommendations of the Advisory Committee on Immunization Practices [10], but the role of therapy and the vaccine’s immunogenicity in SLE are still unclear.

Vaccine immune response in SLE may be subduced as a consequence of immunological changes intrinsic to this immune-mediated disease [8, 9, 11]. Therefore, the inclusion of a sizeable number of lupus patients not receiving therapy seems to be a necessary condition to appropriately define the influence of the disease on immunogenicity, a condition not met by previous studies [12–21]. In addition, the concomitant use of multiple therapies is a common feature of SLE, which reinforces the need for an adequate population to determine the effect of drugs on the pandemic influenza A H1N1/2009 vaccine response to avoid the pitfalls of subgroup analysis. In fact, the anti-H1N1 influenza serum antibody response in SLE patients under different immunosuppressive drugs is still controversial [8, 11], with some evidence of decreased efficacy for AZA [13, 16, 20] and glucocorticoids [14, 16, 17] and scarce data for other commonly used drugs [12, 14, 18, 19].

With regard to chloroquine (CQ), this drug was recently suggested as a promising candidate to improve immune response to vaccines [22, 23]. Indeed, it has been demonstrated that treatment with CQ improves primary CD8+ T-cell stimulation by soluble ovalbumin in experimental models [24] and enhances human memory CD8+ T-cell response against HBV antigens [25]. This improved cellular immune response may ultimately increase antibody production through more efficient support for B cells, as previously demonstrated for virosomal vaccines [26]. Reinforcing this possibility, an increase in meningococcal and diphtheria vaccine responses was observed in individuals under CQ chemoprophylaxis [27–29].

In SLE, the only three studies evaluating the influence of HCQ in seasonal [15] and pandemic [20, 30] influenza immunization revealed an effective response, but the very limited samples evaluated preclude a definitive conclusion about their findings. The present report was therefore designed to prospectively assess the impact of lupus disease and therapy in the pandemic influenza A H1N1/2009 vaccine response in a large SLE cohort.

**Patients and methods**

This study initially selected 638 lupus patients who were included in a large, prospective rheumatic disease cohort conducted at a single site in São Paulo, Brazil (Rheumatology Division, Hospital das Clínicas da Universidade de São Paulo), between March and April 2010, and who completed the study, described in detail elsewhere [31]. The study was approved by the local institutional review board (Comissão de Ética para Análise de Projetos de Pesquisa—CAPesq HCMUSP, #114/10), and all participants signed the informed consent. The trial was registered at clinicaltrials.gov under NCT01151644.

**Patients**

Adult (≥18-year-old) SLE patients (ACR criteria) [32] regularly followed at the outpatient Lupus Clinic, Rheumatology Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, were initially recruited by letter to participate in the Public Health influenza A H1N1/2009 vaccine campaign at the immunization centre of our hospital.

The exclusion criteria were previous known infection with influenza A (H1N1) 2009, an anaphylactic response to vaccine components or to eggs, an acute infection resulting in a fever of >38 °C at the time of vaccination, a history of Guillain–Barré syndrome or demyelination syndromes, previous vaccination with any live vaccine 4 weeks before the study or any inactivated vaccine 2 weeks before the study, a 2010 seasonal influenza vaccination, a blood transfusion within 6 months, hospitalization or failure to complete the protocol.

The study included two phases: entry and a follow-up period of 21 days with a diary card. A clinical and laboratory evaluation was performed, including disease activity parameters according to the SLEDAI [33], and a blood sample was obtained from each participant immediately before vaccination and 21 days after vaccination.

Five hundred and fifty-five patients fulfilled the inclusion criteria and completed the two study phases. Their charts were extensively reviewed for additional clinical and treatment data. SLE manifestations were defined as cutaneous disease (malar or discoid rash, oral ulcers or photosensitivity), articul involvement (arthralgia or erosive arthritis involving two or more peripheral joints), neuropsychiatric disease (psychosis, depression, seizure or peripheral neuropathy), renal disease (persistent proteinuria >0.5 g/24 h, presence of cellular casts of red or white blood cells or mixed, persistent haematuria >10 red blood cells per high power field and five leucocytes per high power field, excluding infection or stones), cardiopulmonary disease (serositis, myocarditis, restrictive lung disease or pulmonary hypertension) and haematological complications (haemolytic anaemia, leucopenia with a white blood cell count <4000/mm³, lymphopenia <1500/mm³ on two or more occasions or thrombocytopenia with a platelet count <100 000/mm³ in the absence of drugs).

**Controls**

One hundred and seventy age- and sex-matched healthy subjects who came to this centre seeking vaccination in response to a Public Health national campaign were invited to participate as a control group with the same...
The H1N1 vaccination immunoresponse was evaluated by determining the levels of antibodies by haemagglutination inhibition. Anti-H1N1 titre was determined by influenza HIA. The percentages of seroprotection (SP) (titre $\geq 1:40$) and seroconversion (SC) (a pre-vaccination titre $< 1:10$ and a post-vaccination HIA titre $\geq 1:40$ or pre-vaccination titre $\geq 1:10$ and a $\geq 4$-fold rise post-vaccination), geometric mean titres (GMTs) and factor increases (FIs) in GMTs were calculated.

Serological determinations

Serum samples were stored at $-70^\circ$C until use. Anti-dsDNA antibody titres were detected by ELISA with purified antigen using a commercially available kit (INOVA Diagnostics Inc., San Diego, CA, USA). Serum levels of C3 complement fractions were measured by RID (SIEMENS Health Care, Marburg, Germany).

Statistical analysis

The sample size was chosen practically rather than statistically because of the need to obtain robust estimates of vaccine immunogenicity in lupus patients. The large sample size of the lupus population and controls gave the study a power analysis $\geq 80\%$.

The analyses were descriptive, with the calculation of two-sided 95% CIs, assuming binomial distributions for dichotomous variables and a log-normal distribution for haemagglutination inhibition titres. For categorical variables, statistical summaries included the rates of SC and SP; these rates were compared using Fisher’s exact test. Every subgroup had its haemagglutination inhibition GMT calculated before vaccination and 21 days after vaccination. The FI in GMT (i.e. the ratio of the titres after vaccination to the titres before vaccination) was also obtained. Log-transformed FI was compared between subgroups of SLE patients using a two-sided Student’s $t$-test with an $\alpha$-level of 0.05.

Results

Among the 638 lupus patients initially vaccinated, 555 (87%) completed the study and comprised the patient group. SLE patients and controls had similar ages [36.7 (12.2) vs 38.7 (13.2) years old; $P = 0.28$] and frequencies of female sex (92.6 vs 90.6%; $P = 0.41$). The mean disease duration was 13.0 (8.9) years, and the frequencies of current/previous SLE manifestations were cutaneous (67.9%), articular (60.5%), neuropsychiatric (13.9%), renal (41.4%), cardiopulmonary (16.6%) and haematological (33.5%).

At entry, the overall analysis of patients’ therapies revealed 75 (13.5%) without drugs; 350 (63%) under CQ diphosphate (all using 250 mg/day), with 105 as mono-therapy (18.9%); 303 under glucocorticoids (54.6%), with a current mean prednisone (PRED) dose of 7.7 (11.3) mg/day, 16% with doses $> 20$ mg/day and 3.8% with doses $> 40$ mg/day; and 286 (51.5%) under immunosuppressors (ISs) with the following distribution among them: 115 (20.7%) AZA, 87 (15.7%) MMF, 65 (11.7%) MTX and 19 (3.4%) i.v. CYC. Exclusive use of ISs was
identified in 95 patients: 38 (6.8%) AZA [mean dose of 127.0 (50.1) mg/day], 30 (5.4%) MMF [mean dose of 2.26 (0.85) g/day] and 27 (4.9%) MTX [mean dose of 16.4 (5.7) mg/week].

Vaccine immunoresponse

Before immunization, SP rates were comparable in the SLE group and healthy controls (P = 0.36). Three weeks after vaccination, significantly lower SP [64.7% (95% CI 60.7%, 68.7%) vs. 84.1% (95% CI 78.6%, 89.6%); P < 0.0001] and SC rates [60.7% (95% CI 56.7%, 64.8%) vs. 80.0% (95% CI 74.0%, 86.0%); P < 0.0001] were observed in SLE compared with the healthy controls. The FI in GMTs after immunization were significantly lower in SLE compared with the healthy control group [8.0 (95% CI 7.1, 9.0) vs. 14.4 (95% CI 11.7, 17.6); P < 0.0001].

Effect of disease and therapy on immune response

SLE without any therapy (the SLE No Therapy group) had equivalent post-vaccine SP [74.7% (95% CI 64.8%, 84.5%) vs. 84.1% (95% CI 78.6%, 89.6%); P = 0.10] and SC rates [72.0% (95% CI 61.8%, 82.1%) vs. 80.0% (95% CI 74.0%, 86.0%); P = 0.18] compared with the healthy control group (Table 1). In contrast, the SLE with Therapy group had significantly lower SP [63.1% (95% CI 58.8%, 67.4%) vs. 84.1% (95% CI 78.6%, 89.6%); P < 0.0001] and SC rates [59.0% (95% CI 54.6%, 63.4%) vs. 80.0% (95% CI 74.0%, 86.0%); P < 0.0001] compared with controls (Table 1). The FI in GMTs of the SLE No Therapy group was comparable with the healthy control group [11.7 (95% CI 8.6, 15.9) vs. 14.4 (95% CI 11.7, 17.6); P = 0.28], whereas the SLE with Therapy group had significantly lower levels [7.6 (95% CI 6.6, 8.6); P < 0.0001].

The comparison of the SLE No Therapy and SLE with Therapy groups revealed a significantly higher SC rate (P = 0.032) and a trend towards a higher SP rate (P = 0.052) in the former group (Table 1). Likewise, a lower FI in GMTs was detected in the SLE with Therapy group compared with SLE No Therapy (P = 0.013). The SLE No Therapy group had significantly lower mean SLEDAI scores [2.2 (3.5) vs. 3.3 (4.0); P = 0.0042] and higher mean lymphocyte counts [1628 (759) vs. 1407 (723)/mm³; P = 0.007] compared with the SLE with Therapy group, but they had similar mean leucocyte counts [5797 (2338) vs. 6142 (2632)/mm³; P = 0.34].

Of note, SLE patients with CQ monotherapy had similar post-vaccination SP [78.0% (95% CI 70.1%, 85.9%); P = 0.60] and SC [96.5% (95% CI 63.0%, 78.3%); P = 0.75] rates compared with the SLE No Therapy group and significantly higher SP rates before immunization (P = 0.0076) (Table 2). FIs in GMTs were comparable in the CQ and SLE No Therapy groups [8.8 (95% CI 7.0, 11.2) vs. 11.7 (95% CI 8.6, 15.9); P = 0.21]. These two groups had similar mean SLEDAI scores [2.8 (3.4) vs. 2.2 (3.5); P = 0.11], mean lymphocyte counts [1417 (665) vs. 1628 (759)/mm³; P = 0.45] and mean leucocyte counts [6505 (2961) vs. 5797 (2338)/mm³; P = 0.22].

SLE patients using steroids [mean PRED dose of 14.1 (12.0) mg/day] had a significantly lower SC rate after vaccination (P = 0.04) and FI in GMT [7.3 (95% CI 5.5, 9.6); P = 0.04] and a trend towards a lower SP rate (P = 0.051) compared with the SLE No Therapy group (Table 2). A significantly lower SC rate [53.9% (95% CI 42.7%, 65.1%); P = 0.028] and a reduced FI in GMT [6.7 (95% CI 4.8, 9.4); P = 0.018] were observed in SLE patients using > 20 mg/day of PRED compared with the SLE No Therapy group. In contrast, the concomitant use of PRED > 20 mg/day and CQ (PRED > 20 + CQ) resulted in a comparable SC rate [71.4% (95% CI 47.7%, 95.0%); P = 1.00] (Table 2) and FI in GMT [8.8 (95% CI 6.2, 12.5); P = 0.49] compared with that observed in the SLE No Therapy group. These two groups of patients (PRED > 20 and PRED > 20 + CQ) had comparable mean PRED doses [27.8 (10.6) vs. 24.3 (7.5) mg/day; P = 0.25], mean SLEDAI scores [2.9 (3.7) vs. 4.1 (6.2); P = 0.90], mean lymphocyte counts [1485 (697) vs. 1050 (473)/mm³; P = 0.11] and mean leucocyte counts [6195 (3173) vs. 4741 (1970)/mm³; P = 0.11].

SLE patients using ISs had significantly lower SC [55.7% (95% CI 45.7%, 65.7%); P = 0.035], SP [58.9% (95% CI 49.0%, 68.8%); P = 0.037] and FI in GMT [7.2 (95% CI 5.4, 9.5); P = 0.041] after vaccination compared with the SLE No Therapy group (Table 2). Further analysis revealed that SLE patients with AZA had a significantly lower SC rate (P = 0.024) and a trend towards a lower SP rate after vaccination (P = 0.053) compared with the SLE No Therapy group (Table 2). MTX also had a reduced SC rate (P = 0.03) and a trend towards a lower SP rate after vaccination (P = 0.051) compared with the SLE No Therapy group.

### Table 1: SP and SC rates of influenza A (H1N1) 2009 vaccine in SLE and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SP rate (titre ≥1/40), % (95% CI)</th>
<th>SC rate, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before immunization</td>
<td>After immunization</td>
</tr>
<tr>
<td>SLE</td>
<td>555</td>
<td>8.6 (6.3, 11.0)</td>
<td>64.7 (60.7, 68.7)*</td>
</tr>
<tr>
<td>SLE with Therapy</td>
<td>480</td>
<td>9.2 (6.6, 11.8)</td>
<td>63.1 (58.8, 67.4)*</td>
</tr>
<tr>
<td>SLE No Therapy</td>
<td>75</td>
<td>5.3 (0.3, 10.4)</td>
<td>74.7 (64.8, 84.5)</td>
</tr>
<tr>
<td>Control</td>
<td>170</td>
<td>11.2 (6.4, 15.9)</td>
<td>84.1 (78.6, 89.6)</td>
</tr>
</tbody>
</table>

*P < 0.0001 vs control; **P < 0.05 vs SLE No Therapy.
Therapy group (Table 2). MMF induced a significantly lower SC rate ($P=0.003$) and SP rate after vaccination ($P=0.01$) compared with the SLE No Therapy group. The association of ISs and CQ (IS + CQ) disclosed similar SC [65.2% (95% CI 51.5%, 79.0%); $P=0.58$] rates to those found in the SLE No Therapy group (Table 2). A higher mean SLEDAI score was identified in IS + CQ compared with ISs ($P=0.01$) compared with the SLE No Therapy group. The same combination with concomitant use of CQ (PRED + CQ) achieved an SC rate [57.4% (95% CI 44.2%, 70.6%); $P=0.09$] and FI in GMT [9.9 (95% CI 6.6%, 14.7%); $P=0.27$, respectively]. The two groups of patients (PRED >20 + IS and PRED >20 + IS + CQ) had comparable mean PRED doses [45.4% (95% CI 24.6, 66.2); $P=0.038$] and FI in GMT [6.6%, 14.7%]; $P=0.41$) and mean lymphocyte count [1339 (624)/mm$^3$; $P=0.41$] and mean leucocyte count [7210 (1525)/mm$^3$; $P=0.55$] were alike among groups.

Nineteen SLE patients were using CYC, but the majority ($n=16$) were also using CQ diphosphate. The apparent low SP [3.6 (95% CI 3.8, 7.2); $P=0.034$, vs 7.2 (95% CI 6.6%, 14.7%); $P=0.58$] and mean lymphocyte count [1525 (6125)/mm$^3$; $P=0.27$, respectively].

Patients under the combination of PRED >20 mg/day and ISs (PRED >20 + IS) showed a significantly lower SC rate [45.4% (95% CI 24.6, 66.2); $P=0.038$] and FI in GMT [6.6%, 14.7%]; $P=0.41$] and mean lymphocyte count [1339 (624)/mm$^3$; $P=0.41$] and mean leucocyte count [7210 (1525)/mm$^3$; $P=0.55$] were alike among groups.

Regarding lupus safety, no significant differences were observed among SLEDAI scores before and 3 weeks after immunization [3.2 (3.9) vs 2.8 (3.2); $P=0.62$], and no patients had new major organ involvement. The frequency of positive anti-dsDNA (47.2 vs 44%; $P=0.33$) and the mean C3 levels [100.5 (30.5) vs 100.3 (30.8) mg/dl; $P=0.70$] were comparable before and after vaccination.

No severe side effects were reported in any groups over the 3 weeks of follow-up. Minor local reactions (8.6 vs 17.6%; $P=0.017$) and mild systemic reactions (18.3 vs 27.6%; $P=0.012$) were less frequently observed in SLE than in controls, in particular, influenza-related symptoms such as headache ($P=0.013$), myalgia ($P=0.015$), sore throat ($P=0.0008$), rhinorhoea ($P=0.0016$) and nasal congestion ($P=0.027$) (Table 3). No severe side effects were observed in SLE patients or controls.

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**Table 2** SP and SC rates of influenza A (H1N1) 2009 vaccine in systemic lupus patients according to therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SP rate (titre $\geqslant$ 1/40), (95% CI)</th>
<th>Before immunization</th>
<th>After immunization</th>
<th>SC rate, (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE No Therapy</td>
<td>75</td>
<td>5.3 (0.3, 10.4)</td>
<td>74.7 (64.8, 84.5)</td>
<td>72.0 (61.8, 82.1)</td>
<td></td>
</tr>
<tr>
<td>CQ</td>
<td>105</td>
<td>19.0 (11.5, 26.5)*</td>
<td>78.0 (70.1, 85.9)</td>
<td>69.5 (60.7, 78.3)</td>
<td></td>
</tr>
<tr>
<td>PRED</td>
<td>99</td>
<td>8.8 (2.6, 13.3)</td>
<td>59.6 (49.9, 69.2)</td>
<td>56.6 (46.8, 66.3)*</td>
<td></td>
</tr>
<tr>
<td>PRED $\geqslant$ 20</td>
<td>76</td>
<td>7.9 (1.8, 13.9)</td>
<td>63.1 (52.2, 73.9)</td>
<td>53.9 (42.7, 65.1)*</td>
<td></td>
</tr>
<tr>
<td>PRED $\geqslant$ 20 + CQ</td>
<td>14</td>
<td>7.1 (-6.3, 20.6)</td>
<td>71.4 (47.7, 95.0)</td>
<td>71.4 (47.7, 95.0)</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>95</td>
<td>3.0 (-3.0, 6.4)</td>
<td>58.9 (49.0, 68.8)*</td>
<td>55.7 (45.7, 65.7)*</td>
<td></td>
</tr>
<tr>
<td>AZA</td>
<td>38</td>
<td>2.6 (-2.4, 7.6)</td>
<td>55.2 (39.4, 71.0)</td>
<td>50.0 (34.1, 65.9)*</td>
<td></td>
</tr>
<tr>
<td>MTX</td>
<td>27</td>
<td>3.7 (-3.4, 10.8)</td>
<td>51.8 (32.9, 70.6)</td>
<td>48.1 (29.2, 66.2)*</td>
<td></td>
</tr>
<tr>
<td>MMF</td>
<td>30</td>
<td>3.3 (-3.0, 9.7)</td>
<td>46.6 (28.7, 64.4)*</td>
<td>40.0 (22.5, 57.5)*</td>
<td></td>
</tr>
<tr>
<td>IS + CQ</td>
<td>46</td>
<td>6.5 (-6.6, 13.6)</td>
<td>67.4 (53.8, 80.9)</td>
<td>65.2 (51.5, 79.0)</td>
<td></td>
</tr>
<tr>
<td>PRED $\geqslant$ 20 + IS</td>
<td>22</td>
<td>4.5 (-4.1, 13.1)</td>
<td>59.1 (38.6, 79.6)</td>
<td>45.4 (24.6, 66.2)*</td>
<td></td>
</tr>
<tr>
<td>PRED $\geqslant$ 20 + IS + CQ</td>
<td>54</td>
<td>5.5 (-6.0, 11.6)</td>
<td>63.0 (50.1, 75.9)</td>
<td>57.4 (44.2, 70.6)*</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.05$ vs SLE No Therapy.

**Table 3** Adverse events of influenza A (H1N1) 2009 vaccine in SLE and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SLE (n = 555)</th>
<th>Control (n = 170)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local reactions</td>
<td>48 (8.6)</td>
<td>30 (17.6)</td>
<td>0.017</td>
</tr>
<tr>
<td>Pain</td>
<td>43 (7.7)</td>
<td>28 (16.4)</td>
<td>0.017</td>
</tr>
<tr>
<td>Redness</td>
<td>14 (2.5)</td>
<td>6 (3.5)</td>
<td>0.43</td>
</tr>
<tr>
<td>Swelling</td>
<td>18 (3.2)</td>
<td>11 (6.4)</td>
<td>0.07</td>
</tr>
<tr>
<td>Itching</td>
<td>8 (1.4)</td>
<td>1 (0.6)</td>
<td>0.69</td>
</tr>
<tr>
<td>Systemic reactions</td>
<td>102 (18.3)</td>
<td>47 (27.6)</td>
<td>0.012</td>
</tr>
<tr>
<td>Fever</td>
<td>15 (2.7)</td>
<td>3 (1.7)</td>
<td>0.77</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>22 (3.9)</td>
<td>9 (5.3)</td>
<td>0.51</td>
</tr>
<tr>
<td>Headache</td>
<td>55 (9.9)</td>
<td>29 (17)</td>
<td>0.013</td>
</tr>
<tr>
<td>Myalgia</td>
<td>31 (5.5)</td>
<td>19 (11.2)</td>
<td>0.015</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>15 (2.7)</td>
<td>12 (7)</td>
<td>0.017</td>
</tr>
<tr>
<td>Sore throat</td>
<td>18 (3.2)</td>
<td>17 (10)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Cough</td>
<td>18 (3.2)</td>
<td>11 (6.4)</td>
<td>0.072</td>
</tr>
<tr>
<td>Rhinorhoea</td>
<td>13 (2.3)</td>
<td>14 (8.2)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>14 (2.5)</td>
<td>11 (6.4)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Data are expressed as n (%).
Discussion

This is the first study to demonstrate that CQ counterbalances the deleterious effects of immunosuppressive therapies in lupus influenza A/H1N1 immunoresponse. Despite prevailing concerns about the risks of influenza in patients with autoimmune rheumatic diseases, including SLE, and especially patients under immunosuppression, their response to vaccine is not well determined. In fact, influenza vaccination is widely recommended for patients who are immunosuppressed, such as those with SLE (European League Against Rheumatism and 2010 Recommendations of the Advisory Committee on Immunization Practices) [8–10], but also for patients infected with HIV [35] and recipients of solid organ [36] and haemopoietic stem-cell transplants [37]. However, the same immune dysfunctions that can increase the risks and consequences of influenza infection can also compromise vaccine responses and effectiveness.

The inclusion of a sizeable number of lupus patients without any therapy and of healthy controls in the present study was an essential step in determining that the disease itself does not seem to reduce the vaccine immune response, a condition that had not been met by previous reports [12–21]. However, the observed significant decrease in SP and SC of the influenza A/H1N1 vaccine in lupus patients with therapy compared with those without drugs provides clear evidence for the deleterious role of treatment in this vaccine’s antibody production.

In real-life circumstances, most lupus patients are under immunosuppression, particularly with Cs. This therapy promotes the impairment of antigen processing and has implications for the efficacy of anti-viral vaccines [38, 39], including the reduced vaccine response observed in SLE [14, 16, 17]. Regarding seasonal influenza vaccination, five studies have demonstrated impaired response [12, 13, 16, 19, 40], mainly associated with PRED doses >20 mg/day [12, 16]. According to these findings, the British Society of Rheumatology Clinical Affairs Committee [41] and the 2010 Advisory Committee on Immunization Practices [10] recommended vaccination in patients under this dose. We have confirmed and extended this observation for pandemic influenza A H1N1/2009.

With regard to the influence of ISs on seasonal influenza vaccination, AZA has been described as reducing this response, despite most patients having achieved protective levels of antibodies [13, 16, 42]. This impairment of humoral response with AZA was also observed herein and confirms the report of SLE with pandemic A H1N1/2009 influenza vaccine [20]. Our study introduces MMF as another IS that significantly reduces SP and SC in SLE patients, as is also reported for other non-rheumatological immunosuppressive conditions [43–46]. Moreover, the present study offers the first evidence in SLE that MTX negatively influences the pandemic influenza A H1N1/2009 immune response, in contrast to that observed for the seasonal influenza vaccination [16, 40]. Finally, the small representation of patients under CYC evaluated herein precluded a definitive conclusion about its possible deleterious effect on vaccination. It should be emphasized that although the vaccine response was diminished in patients with immunosuppressive drugs, the majority of these patients still have a response. Therefore the findings presented here still underline the recommendations to vaccinate all patients treated with immunosuppressive drugs.

Remarkably, the concomitant use of CQ enhanced antibody production to the pandemic influenza A H1N1/2009 vaccine in patients under PRED ≥20 mg and/or immunosuppression. This novel beneficial effect of this drug is further supported by the observations that the mean glucocorticoid dose was comparable in these groups of patients with or without concomitant CQ therapy. Moreover, lower systemic inflammation does not seem to account for these favourable findings in view of the fact that comparable SLEDAI scores were detected among glucocorticoid or immunosuppressive groups with and without concomitant use of CQ. The mean lymphocyte count was also similar in these groups of patients with and without concurrent CQ therapy, despite previous reports that lymphopenia may influence the SC rate of the pandemic (H1N1) vaccine [47].

This finding is unique because there are no studies regarding the influenza vaccine antibody response in individuals under CQ and there are limited data for other immunizations [27–29, 48, 49]. In Nigerian children, continuous CQ chemoprophylaxis enhanced the response to meningococcal vaccines and had no depressive effects for tetanus, diphtheria, measles, poliomyelitis, typhoid or BCG [27, 28]. However, reports of reduced antibody responses to rabies, cholera and typhoid are most likely explained by their short-term simultaneous administration [48, 49]. Reinforcing the beneficial effects of CQ, an improved humoral response was reported for diphtheria vaccination (diphtheria and tetanus) with prolonged antimalarial prophylaxis [29].

As CQ treatment in lupus is usually long term, we hypothesize that the underlying mechanisms for a better immune response to the pandemic influenza H1N1/2009 vaccine associated with antimalarial therapy may be related to more efficacious generation or to the maintenance of immunological memory. In fact, we have observed a significantly higher SP rate before immunization in patients under antimalarial monotherapy compared with those without any therapy, raising the possibility of a CQ T-cell-driven broader spectrum or of a longer protection response after exposure to the first wave of the pandemic H1N1/2009 during the previous year. A recent study demonstrated that cross-presentation of soluble HBV antigens to specific CD8+ T memory cell clones was dramatically improved with CQ in hepatitis vaccine boosters [25].

The effect of CQ observed herein most likely can be extended to HCQ therapy in view of the fact that SP after pandemic A H1N1/2009 influenza vaccination in a limited number of SLE patients under this drug was suggested to meet the European Committee for Proprietary Medicinal Products criteria [20].
Regarding the short-term safety of the A/California/7/2009 (H1N1)-like virus vaccine in SLE patients, adverse effects were mild and occurred at lower frequencies compared with healthy controls. None of the SLE patients developed any major neurological manifestations, such as Guillain–Barré syndrome, convulsions, psychosis or organic brain syndromes [50]. Moreover, no significant differences were observed between SLEDAI scores and the frequencies of positive anti-dsDNA before or 3 weeks after immunization. This absence of severe side effects is probably not explained by the exclusion of participants who did not return for the second phase because all patients are still being regularly followed in our Outpatient Rheumatology Clinic, but it does not exclude the possibility that a longer observation period will reveal additional vaccine or disease-related effects.

We conclude that CQ is a promising candidate to improve the pandemic influenza A H1N1/2009 immune response in SLE, including in patients under glucocorticoid and/or immunosuppressive therapy. Further studies are necessary to determine the underlying mechanism of this antimalarial adjuvant effect and its possible use as an effective strategy for other vaccines in rheumatic diseases.

**Rheumatology key messages**

- SLE patients with other therapy had lower SP and SC rates compared with healthy population.
- Concomitant use of CQ enhanced antibody response even in SLE patients under PRED and/or ISs.

**Acknowledgements**

We thank Luciana Bastos de Queiroz Lima and Fabiana Oliveira Burgos for Adolfo Lutz Institute laboratory analysis.

**Funding:** This study was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2009/51897-5 to E.B. and #2010/10749-0 to E.B.), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #303165/2008-1 to E.B., #300248/2008-3 to C.A.A.S., #301411/2009-3 to E.B.), Federico Foundation (to E.B., C.A.A.S. and E.B.) and Butantan Foundation.

**Disclosure statement:** The authors have declared no conflicts of interest.

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