Influenza vaccination in primary and secondary Immunodeficiencies
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Studies of cell-mediated immune responses to influenza vaccination in systemic lupus erythematosus

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ABSTRACT

Introduction Both antibody and cell-mediated responses are involved in the defense against influenza. In systemic lupus erythematosus (SLE) patients a decreased antibody response to subunit influenza vaccine has been demonstrated. However, cell-mediated responses have not yet been assessed.

Methods Fifty-four SLE patients and healthy HC (HC) received subunit influenza vaccine. Peripheral blood mononuclear cells and sera were obtained before and one month after vaccination. Cell-mediated responses to A/H1N1 and A/H3N2 were evaluated using interferon (IFN)γ ELISpot and flow cytometry. Antibody responses were measured using the hemagglutination inhibition test.

Results Prior to vaccination, SLE patients had fewer IFNγ spotforming cells against A/H1N1 compared to HC and a lower frequency of IFNγ+CD8+ T-cells. After vaccination, the numbers of IFNγ spotforming cells increased in both patients and HC, though it remained lower in patients. Also frequencies of CD4+ T-cells producing tumor necrosis factor α and interleukin-2 were lower in patients after vaccination, compared to HC. As expected for a subunit vaccine, vaccination did not induce a CD8+ T-cell response. For A/H3N2-specific responses, results were comparable. Diminished cell-mediated responses to influenza vaccination were associated with the use of prednisone and/or azathioprine. Patients showed a lower increase in A/H1N1-specific and A/H3N2-specific antibody titers after vaccination, as compared to HC.

Conclusion In addition to a decreased antibody response, cell-mediated responses to influenza vaccination are diminished in SLE patients, which may reflect effects of the concomitant use of immunosuppressive drugs. This may render these patients more susceptible for (complicated) influenza infections.
INTRODUCTION
Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by a remitting and relapsing course. SLE patients have an increased risk of infection, due to both intrinsic disturbances of immune responses and use of immunosuppressive drugs which are often needed to control disease activity. Indeed, infection-related morbidity and mortality occur more frequently in SLE patients [1].

For influenza, infection-related morbidity and mortality are increased in immunocompromised patients [2]. As influenza infection has a high incidence, with an estimated 5-20% of the general population infected annually [3], influenza vaccination is a clinically relevant issue in SLE patients. Influenza vaccination of SLE patients is safe, as it has been shown that influenza vaccination does not induce disease activity [4]. Annual vaccination in SLE is therefore recommended [5].

In the immune response to influenza, both antibody and cell-mediated responses, comprised of CD4+ and CD8+ T-cells, are involved. In SLE, antibody responses to influenza vaccination are diminished [6], but T-cell-mediated responses have not been assessed. The latter are relevant, as it has been shown that in certain groups, such as the elderly, cell-mediated responses to influenza vaccination can be a marker of clinical protection, independent from antibody responses [7]. The most frequently used vaccine formulations are split virus or subunit vaccines. With these vaccines, antigens are primarily presented via MHC II, which induces CD4+ T-cell stimulation [8]. However, they are incapable of inducing MHC class I restricted CD8+ T-cell responses [9]. In addition, subunit vaccines, in contrast to split virus and whole virus vaccines, do not contain any of the internal proteins that may more readily (re)activate influenza-specific CD8+ T-cells.

In SLE, decreased T-helper (Th) recall responses to influenza A and tetanus toxoid antigens have been reported in a subset of patients, as measured by interleukin (IL)-2 production upon stimulation. This decreased function could not be accounted for by the use of immunosuppressives alone, and was shown to be associated with disease activity [10]. In addition, lower levels of cell-mediated cytotoxicity against target T-cells infected with influenza A and B have been found in SLE patients [11].

Based on these data, we hypothesized that SLE patients have lower CD4+ T-cell responses to subunit influenza vaccine and lower CD8+ T-cell recall responses to influenza antigens than HC. Cell-mediated responses against influenza in SLE, prior to and following vaccination, were evaluated. In addition, antibody responses were evaluated, and vaccine safety was recorded.
METHODS

Study population

Patients were eligible for the study when they fulfilled at least four of the American College of Rheumatology criteria for SLE [12]. Exclusion criteria were pregnancy and the presence of an indication for yearly influenza vaccination based on concomitant disease according to international guidelines [13]. A control group of healthy individuals was included that was age and sex matched to the vaccinated SLE patients. Pregnancy was an exclusion criterion for participation as HC.

Study design

SLE patients and healthy controls (HC) were included from October to December 2005. Before entry, patients were randomized in a ratio of 2:1 to receive an influenza vaccination or to serve as non-vaccinated patient control. At entry (visit 1), patients randomized for vaccination and all HC were vaccinated. Patients and HC were followed up at 28 days (visit 2) and three to four months after inclusion (visit 3). PBMC were isolated from vaccinated participants at visits 1 and 2 (see below). At each visit blood was drawn, and serum was stored at −20°C until use. Also, SLE disease activity index (SLEDAI) [14] was recorded and patients were asked to mark a visual analogue score (VAS) for disease activity on a scale of 0-10, 0 indicating no activity and 10 indicating the highest activity. Information on influenza vaccination in the previous year was obtained. Adverse effects to vaccination were recorded using a standardized questionnaire which included: itching, pain, erythema, induration at the site of vaccination, shivers, myalgia, fever, headache, nausea, arthralgia, diarrhea, use of an analgesic/antiphlogistic drug. The study was approved by the institutional medical ethics committee, and informed consent was obtained from all participants.

Influenza vaccine

A single dose of a trivalent subunit influenza vaccine (Influvac®, 2005-2006, Solvay Pharmaceuticals, Weesp, the Netherlands), containing A/New Caledonia/20/99 [H1N1], A/NewYork/55/2004 [H3N2] and B/Hong Kong/330/2001, was administered intramuscularly.

Isolation, storage and thawing of PBMC

PBMC were isolated from heparinized venous blood by density-gradient centrifugation on Lymphoprep (Axis-Shield, Oslo, Norway) immediately after blood was drawn. PBMC were frozen in RPMI 1640 (Cambrex BioScience, Verviers, Belgium) supplemented with 10% fetal calf serum (FCS), 50 µg/ml of gentamicin (Gibco,
Paisley, UK) and 10% dimethylsulfoxide. PBMC were stored in liquid nitrogen until use. Pre- and postvaccination samples, from a SLE patient and a matched control, were simultaneously thawed and batch-processed. A minimum cell viability of 90%, evaluated by trypan blue staining, was required. Preceding ELISpot assays, PBMC were rested, by overnight incubation at 37° C. Cells were counted before plating, using an automated cell counter (Beckman Coulter, Fullerton, CA, USA).

**Influenza antigens used in assays of cell-mediated responses**

β-propiolactone whole inactivated virus (WIV) of A/New Caledonia/20/99 (H1N1) and A/Hiroshima/52/2005 (H3N2) were used to stimulate PBMC. A/Hiroshima/52/2005 is a very closely related antigenic variant of the vaccine strain A/NewYork/55/2004.

**Interferon (IFN)γ ELISpot assay**

Nitrocellulose plates (Nunc, Rochester, NY, USA) were coated overnight at 4° C with 50 µl anti-human IFNγ, 15 µg/ml per well (Mabtech, Nacka Strand, Sweden). Plates were washed and blocked with culture medium (CM; RPMI supplemented with 50 µg/ml gentamicin and 10% FCS) for one hour at room temperature (RT). Subsequently, 2 x 10^5 PBMC were added per well, in 200 µl, and incubated in CM at 37° C with WIV of A/H1N1 and A/H3N2, at a final concentration of 5 µg total viral protein/ml. Concanavalin A stimulation, 5 µg/ml, was used as a positive control and a negative control consisted of PBMC in CM alone. Stimulation tests were performed in duplo. After 48 hours plates were washed with phosphate buffered saline (PBS), and 50 µl of 1 µg/ml biotinylated anti-human IFNγ (Mabtech) was added per well for 3 hours at RT. Next, plates were washed again, and 50 µl 1:1000 streptavidin-alkaline phosphatase (Mabtech) per well was added for 1.5 hours at RT. Plates were washed and 100 µl BCIP/NBT plus substrate (Mabtech) was added per well for 10 minutes. Finally, plates were washed with tap water. After drying, spots were counted using an automated reader (automated ELISpot video-analysis system, Sanquin, Amsterdam, The Netherlands). Results are referred to as IFNγ spotforming cells, as IFNγ-producing CD4+ and CD8+ T-cells as well as natural killer (NK) cells, following WIV stimulation, have been described [15].

**Flow cytometry**

For stimulations, 1.0-1.5 x 10^6 PBMC were cultured in 200 µl CM, in 5 ml polypropylene round-bottom FalconTM tubes (Becton Dickinson and Company (BD), Franklin Lakes, NJ, USA). Staphylococcal enterotoxin B, at 5 µg/ml, (SEB, Sigma-Aldrich, Saint Louis, MO, USA) was used as a positive control. WIV A/New Caledonia (H1N1) and WIV A/Hiroshima (H3N2) were used at final concentrations of 1 µg of total
viral protein/ml. WIV and negative control (CM only) cultures were incubated in the presence of 10 µg/ml anti CD28/CD49 (BD). Cells were incubated for 18h at 37° C, the final 16 hours in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich). Following incubation, 10 µl 40mM EDTA in PBS was added, tubes were vortexed and incubated for 10 minutes, to facilitate resuspending. Next, 2 ml FACS lysing solution (BD) was added for 10 minutes. Cells were spun down and washed in PBS-1% bovine serum albumin. Subsequently cells were permeabilized in 500 µl PERM-2 (BD) for 10 minutes in the dark in the presence of pacific blue and orange (Invitrogen, Carlsbad, CA, USA), in a different combination for each stimulus, to enable fluorescent T-cell barcoding [16]. PBS-20% FCS was added for 5 minutes. Cells were washed and pooled per PBMC sample. Next, anti-CD3-FITC, anti-CD4-PE-Cy7, anti-CD8-PercP, anti-CD69-APC-Cy7, anti-IFN-γ-Alexa 700, anti-tumor necrosis factor (TNF)α-APC and anti-interleukin (IL)-2-PE (all from BD) were added, following the manufacturer’s instruction. After incubation for 30 minutes at RT, cells were washed and immediately analyzed on a LSR II flow cytometer (BD). Data for at least 1 x 10^6 CD3+ cells were collected. Using the Win-List software package (Verity Software House, Topsham ME, USA), positively and negatively stained populations were gated and Boolean gating was applied. First, lymphocytes were gated by CD3 expression and sideward scatter patterns. Next, CD4+ and CD8+ T-cell populations were gated as CD4+CD8- or CD4-CD8+, respectively. Then, cells from different stimulation tubes were separated in a pacific blue/orange plot. Finally CD69+/− cytokine+/− quadrants were set for the different stimuli simultaneously, according to the negative and positive controls. Percentages of antigen-specific cells were expressed as the percentage of CD69+ cytokine+ CD4+ or CD8+ T-cells within the total CD4+ or CD8+ T-cell population.

Antibody response to influenza

For quantitative detection of anti-influenza antibodies the hemagglutination inhibition assay (HIA) was employed, following standard procedures [17]. Influenza A/New Caledonia/20/99 [H1N1] and A/NewYork/55/2004 [H3N2] were provided by Solvay Pharmaceuticals (Weesp, the Netherlands). Seroconversions were defined as a fourfold rise in titer one month after vaccination, and seroprotection was defined as a titer ≥40. Titers <10 (below detection level) were assigned a value of 5 for calculation purposes [18].

Statistical analysis

Data were analyzed using SPSS 14 (SPSS Inc., Chicago, IL, USA). Titers were log transformed prior to testing of geometric mean titers. For comparisons of T-cell cytokine responses, Mann-Whitney U test and Wilcoxon signed rank test were
used. All T-cell frequencies reported are after background subtraction of the frequency of the identically gated population of cells from the same sample stimulated without antigen. For correlations, Spearman’s rho was used. Age was normally distributed and tested with Student’s t-test. For all other variables Fisher’s exact test and Mann-Whitney U test were used where appropriate. A two sided P value <0.05 was considered statistically significant. No adjustments for multiple testing were made given the explorative design of the study.

RESULTS

Patient characteristics

Eighty SLE patients gave informed consent to participate and were randomized: 54 for the vaccination group and 26 for the non-vaccination group. Two patients initially randomized for the non-vaccination group were excluded (due to pregnancy and withdrawal, respectively). Patient groups did not differ in sex, age, and medication use. More patients in the vaccination group had received an influenza vaccination the year before as compared to patients not receiving vaccination and HC (table 1).

Cell-mediated responses against A/H1N1 and A/H3N2 were measured in a subset

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics and disease parameters</th>
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<tbody>
<tr>
<td>SLE patients</td>
</tr>
<tr>
<td>Non-vaccinated (n=24)</td>
</tr>
<tr>
<td>Vaccinated (n=54)</td>
</tr>
<tr>
<td>HC Vaccinated (n=54)</td>
</tr>
<tr>
<td>Sex, males</td>
</tr>
<tr>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>10 (18.5%)</td>
</tr>
<tr>
<td>11 (20.4%)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
</tr>
<tr>
<td>45.5 (11.5)</td>
</tr>
<tr>
<td>44.8 (13.6)</td>
</tr>
<tr>
<td>43.1 (10.9)</td>
</tr>
<tr>
<td>Influenza vaccination in previous year</td>
</tr>
<tr>
<td>9 (37.5%)</td>
</tr>
</tbody>
</table>
| 34 (63.0%)                                             *
| 3 (5.6%) **                                            |
| Without immunosuppressives                             |
| 5 (20.8%)                                              |
| 5 (9.3%)                                               |
| N/A                                                    |
| Prednisone                                             |
| 10 (41.7%)                                             |
| 28 (51.9%)                                             |
| N/A                                                    |
| median (range), in users (mg/day)                      |
| 6.25 (2.5–15)                                          |
| 5 (1.25–15)                                            |
| N/A                                                    |
| Hydroxychloroquine                                     |
| 10 (41.7%)                                             |
| 30 (55.6%)                                             |
| N/A                                                    |
| median (range), in users (mg/day)                      |
| 400 (200–800)                                          |
| 400 (200–1000)                                         |
| N/A                                                    |
| Azathioprine                                           |
| 6 (25%)                                                |
| 17 (31.5%)                                             |
| N/A                                                    |
| median (range), in users (mg/day)                      |
| 87.8 (50–125)                                          |
| 125 (75–200)                                           |
| N/A                                                    |
| Other immunosuppressive drugs                          |
| 0 (0%)                                                 |
| 6 (11.1%) #                                            |
| N/A                                                    |
| SLEDAI, median (range)                                 |
| t=0                                                    |
| 2 (0–8)                                                |
| 2 (0–12)                                               |
| N/A                                                    |
| VAS, median (range)                                    |
| t=0                                                    |
| 2.2 (0.5–6)                                            |
| 1.6 (0–6.6)                                            |
| N/A                                                    |

SLE, systemic lupus erythematosus; HC, healthy controls; SLEDAI, systemic lupus erythematosus disease activity index; VAS, visual analogue score; N/A, not applicable

# Methotrexate, 5 patients used 15 mg per week, 1 used 25 mg per week; * p < 0.05 (vaccinated SLE patients versus non-vaccinated SLE patients); ** p < 0.001 (HC versus vaccinated SLE patients)
of vaccinated SLE patients (n=38) and HC (n=38), matched for age and sex. This subset was based on availability of a matched control and proper acquisition of PBMC prior to and one month following vaccination. Mean age (SD) in this subgroup was 43.4 years (10.2); 24% were male.

**Lower prevaccination cell-mediated responses to A/H1N1 and A/H3N2 in SLE patients**

In ELISpot, prior to vaccination, SLE patients had fewer IFNγ spotforming cells against A/H1N1 and A/H3N2 as compared to HC (figure 1). In flow cytometry, the frequency of CD4+TNFα+ T-cells upon A/H1N1 stimulation was lower in SLE patients than in HC (figure 2, A). SLE patients also had a lower frequency of IFNγ+CD8+ T-cells upon A/H1N1 stimulation as well as lower frequencies of IFNγ- and TNFα-producing CD8+ T-cells upon A/H3N2 stimulation (figure 3, A and B).

**Lower cell-mediated responses to A/H1N1 and A/H3N2 in SLE patients following influenza vaccination**

Following vaccination, 68.4% of SLE patients and 71.1% of HC showed a rise in IFNγ spotforming cells against A/H1N1; for A/H3N2 60.5% of patients and 73.7% of HC showed a rise. Rises were similar in SLE patients and HC. After vaccination, the number of IFNγ spotforming cells remained lower in SLE patients, compared to HC (figure 1).

**Figure 1.** Enzyme-linked immunospot assay of interferon (IFN)γ spot-forming cells per 2 x 10^5 peripheral blood mononuclear cells in patients with systemic lupus erythematosus (SLE) and healthy controls (HC) in response to A/H1N1 and A/H3N2 stimulation before vaccination (t=0 days) and 4 weeks after vaccination (t=28 days). Results are corrected for responses in unstimulated cultures from the same sample. Bars show the median and interquartile range.
Following vaccination, A/H1N1-specific IFNγ-producing CD4+ T-cells increased in 66.7% of SLE patients and 65.7% of HC. Similarly, A/H1N1-specific TNFα-producing CD4+ T-cells increased in 61.1% of patients and 71.4% of HC. In 71.4% of HC, also IL-2-producing CD4+ T-cells increased (figure 2, B). For A/H3N2, 60% of SLE patients and 61.8% of HC showed an increase in IL-2+CD4+ T-cells following vaccination; 73.5% of HC showed an increase in TNFα*CD4+ T-cells as well (figure 2, B). So, in SLE patients the response to vaccination was restricted to a more limited cytokine profile. Moreover, SLE patients reached lower frequencies of TNFα- and IL-2-producing CD4+ T-cells against A/H1N1 compared to HC (p = 0.014 and p = 0.034, respectively).

As was expected, neither SLE patients nor HC showed changes in percentages of A/H1N1- and A/H3N2-specific CD8+ T-cells upon vaccination. Accordingly, post-vaccination, similar differences in influenza-specific CD8+ T-cells were observed as prevaccination (data not shown).

**Adequate responses of CD4+ and CD8+ T-cells following SEB stimulation in SLE patients**

Upon SEB stimulation, SLE patients and HC showed similar frequencies of IFNγ-, TNFα- and IL-2-producing CD4+ T-cells (figure 4, A) and CD8+ T-cells (figure 4, B). This indicated that T-cells from SLE patients were generally capable of adequate cytokine responses.

**Lower antibody response to influenza vaccination in SLE patients**

Prior to vaccination, SLE patients had a higher GMT against A/H1N1 as compared

![Figure 2. CD4+ T-cell responses against A/H1N1 and A/H3N2. Frequencies of cytokine-producing CD4+ T-cells upon stimulation with A/H1N1 (A) and A/H3N2 (B) in patients with SLE and healthy control subjects, before vaccination and 4 weeks after vaccination. Results are corrected for responses in unstimulated (Unstim.) cultures from the same sample. Bars show the median and interquartile range. SEB = staphylococcal enterotoxin B; IL-2 = interleukin-2 (see figure 1 for other definitions).](image)
with HC. One month postvaccination, SLE patients and HC reached comparable GMTs to each vaccine strain. However, the fold-increases following vaccination were lower in SLE patients for the A/H1N1 and A/H3N2 strains. Three to four months after vaccination, titers had decreased in both SLE patients and HC; GMTs remained comparable. SLE patients had a lower seroconversion rate for A/H1N1 than HC (p = 0.001), but for A/H3N2 seroconversion rates in SLE and HC were similar. Prior to vaccination, seroprotection rates were comparable in SLE patients and HC. One month after vaccination SLE patients had a lower seroprotection rate against the A strains compared with HC, which was significant for A/H3N2 (p = 0.032). Three to four months after vaccination seroprotection levels had dropped in SLE patients as well as HC to comparable levels (table 2).

Taken together, the antibody response in SLE patients was, moderately, decreased. This was further substantiated by results in serologically naïve SLE patients and HC (prevaccination titer <10). For A/H1N1, 5 of 11 (46%) SLE patients showed such a seroconversion, versus 20 of 25 (80%) HC (p = 0.056); for A/H3N2 this occurred in 1 of 7 (14%) SLE patients versus 18 of 22 (82%) HC (p = 0.003). Finally, we analyzed whether immunosuppressive medication influenced antibody responses. No such influence was found (data not shown).

Table 2. Seroprotection rates against the A strains before and after vaccination

<table>
<thead>
<tr>
<th>Vaccine Strain</th>
<th>Seroprotection Rate</th>
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<tbody>
<tr>
<td>A/H1N1</td>
<td>SLE: 20% (p = 0.056)</td>
</tr>
<tr>
<td>A/H3N2</td>
<td>SLE: 10% (p = 0.003)</td>
</tr>
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Correlations between changes in IFN\(\gamma\) spotforming cells following vaccination and seroconversions in both patients with SLE and control subjects

Changes in IFN\(\gamma\) spotforming cells following vaccination correlated with seroconversions in both SLE patients and HC. The change in IFN\(\gamma\) spotforming cells against

![CD8+ T cell responses against A/H1N1 and A/H3N2. Frequencies of cytokine-producing CD8+ T-cells prior to vaccination upon stimulation with A/H1N1 (A) and A/H3N2 (B) in patients with SLE and healthy control subjects. Results are corrected for responses in unstimulated cultures from the same sample. Bars show the median and interquartile range. For interleukin-2 (IL-2) production following stimulation with A/H3N2 in patients with SLE, both the median and the interquartile range were 0. See Figure 1 for other definitions.](image)
A/H1N1, measured by ELISpot, correlated positively with seroconversion against A/H1N1 (r = 0.311, p = 0.058 for HC; r = 0.348; p = 0.032 for SLE patients; r = 0.339, p = 0.003 for all vaccinees). For A/H3N2 such a correlation was observed in HC (r = 0.318, p = 0.052), but not in SLE patients. No correlations were observed between CD4+ T-cell cytokine responses and antibody responses in HC or SLE patients.

Prior vaccination did not influence cell-mediated responses, but did lower antibody responses

In a subanalysis, SLE patients (n=13) and HC (n=35) who were not vaccinated in the previous year, were evaluated. Groups did not differ in age; mean age (SD) was 40.2 (8.9) years in SLE patients and 44.5 (9.6) in HC (p = 0.164). In the IFNγ-ELISpot assay, SLE patients had fewer spotforming cells prior to vaccination against A/H1N1 (p = 0.023) and A/H3N2 (p = 0.034) than HC. After vaccination similar differences were found, though these did not reach significance (p = 0.125 for A/H1N1 and p = 0.051 for A/H3N2). Also flow cytometry results showed a tendency towards a restricted CD4+ T-cell response in SLE (data not shown).

In this subanalysis no differences in antibody responses (GMTs, fold increases of GMTs, seroconversion and seroprotection rates) were found between SLE patients and HC (data not shown). In addition, a comparison was made between SLE patients who were vaccinated the previous year (n=20) versus those who were not (n=34). Vaccination in 2004 led to a higher prevaccination GMT against A/H1N1 (26.6 versus 10.5; p = 0.001) and, subsequently, lowered seroconversion rate (27% versus 75%; p = 0.001).

Figure 4. CD4+ and CD8+ T-cell responses to staphylococcal enterotoxin B (SEB). Frequencies of cytokine-producing CD4+ T-cell (A) and CD8+ T-cells (B) in patients with systemic lupus erythematosus (SLE) and healthy controls (HCS) upon stimulation with SEB in prevaccination samples. Results are corrected for responses in unstimulated cultures from the same sample. Bars show the median and interquartile range. IFNγ=interferon-γ; TNF=tumor necrosis factor; IL-2=interleukin-2.

A

B
The use of prednisone and/or azathioprine was associated with lower cell-mediated responses to influenza vaccination

Patients using prednisone and/or azathioprine (PRED/AZA; n=22) were compared to patients who did not use these drugs (n=16). In this subanalysis, no differences were noted prior to vaccination. Following vaccination, patients on PRED/AZA had fewer IFNγ spotforming cells against A/H1N1 and A/H3N2 (p <0.004 and p <0.007, respectively) and lower frequencies of A/H1N1-specific IFNγ-, TNFα- and IL-2-producing CD4+ T-cells (p <0.004, p <0.033 and p <0.036, respectively) as well as A/H3N2-specific IFNγ-producing CD4+ T-cells (p <0.023). No differences in CD8+ T-cell responses to A/H1N1 and A/H3N2 were observed (data not shown). In patients not using prednisone and/or azathioprine, cell mediated responses to influenza vaccination were not significantly lower than in HC (data not shown).

No increase in disease activity following influenza vaccination, but more adverse effects in SLE than in HC

Prior to inclusion (Table 1), and during follow-up, vaccinated and non-vaccinated patient groups did not differ in SLEDAI and VAS scores. At visit 2, median (range) SLEDAI scores were 2 (0–13) in vaccinated SLE patients versus 2 (0–8) in non-vaccinated patients and at visit 3 these were 2 (0–10) versus 2 (0–4), respectively. For VAS scores, median (range) scores at visit 2 were 1.4 (0–8.1) in vaccinated SLE patients versus 2.1 (0–7.4) in non-vaccinated patients, at visit 3 these were 1.8 (0–9.4) versus 2.2 (0–8.9) respectively. Following vaccination, SLE patients more often reported itching (18% vs. 2% in HC; p <0.006), erythema (24% vs. 4%; p <0.003) and induration (30% vs. 11% p <0.026) at the site of vaccination, and arthralgia (16% vs. 4%; p <0.046). All adverse effects were mild and short-lasting.

DISCUSSION

To our knowledge, this is the first study to evaluate cell-mediated immune responses to subunit influenza vaccine in patients with a systemic autoimmune disease. To do so, we used ELISpot and flow cytometry. ELISpot is the more sensitive assay, whereas flow cytometry allows phenotyping and detection of multiple cytokines, which offers additional information on the gamma of the response [19]. Cell-mediated recall responses to influenza were lower in SLE patients. Prior to vaccination, SLE patients had considerably fewer IFNγ spotforming cells than HC against both A/H1N1 and A/H3N2. CD4+ T-cell responses to A/H1N1 were lower in SLE patients, which reached significance for TNFα-producing CD4+ T-cells. Also CD8+ T-cell responses were lower in SLE patients than in HC, for both A/H1N1 (IFNγ-production) and A/H3N2 (IFNγ and TNFα-production).
Following influenza vaccination, cell-mediated responses to influenza remained lower in SLE patients. Although both SLE patients and HC showed an increase in IFNγ spotforming cells upon vaccination, for A/H1N1 as well as A/H3N2, numbers remained lower in SLE patients. SLE patients showed an increase in cytokine-producing A/H1N1-specific and A/H3N2-specific CD4+ T-cells following vaccination, however, this increase was restricted with respect to cytokine profile compared to HC. Moreover, SLE patients reached lower frequencies of A/H1N1-specific TNFα-producing and IL-2-producing CD4+ T-cells after vaccination. As expected, we did not observe a change in cytokine-producing CD8+ T-cells following vaccination in either SLE patients or HC.

As CD4+ and CD8+ T-cell responses to SEB were normal in SLE patients, the decreased cell-mediated response to influenza vaccination could not be attributed to a decreased responsiveness of T-lymphocytes in general. Furthermore, the observed differences in cell-mediated responses were, at least largely, independent of previous influenza vaccination status as well. The degree of influenza vaccination in the previous year was higher in SLE patients, but in a subanalysis comparing previously unvaccinated SLE patients with HC, SLE patients still showed considerably lower responses. Importantly, the use of medications played a major role, as the use of prednisone and/or azathioprine was associated with lower cell-mediated responses against both A/H1N1 and A/H3N2 following vaccination.

A diminished T-helper cell response in SLE patients to influenza has been reported previously [20], as measured by IL-2 secretion in the supernatant of influenza-stimulated PBMC of unvaccinated patients. We found a decreased CD8+ T-cell recall response in SLE patients to influenza antigens, which is in accordance with a previous study [11]. WIV, as used in this study, is able to induce CD8+ T-cell responses in vivo and to reactivate memory CD8+ T-cells in vitro [21]. However, WIV might be a weaker stimulus of CD8+ T-cells as compared to live virus, due to lower antigen presentation on MHC I.

Importantly, fewer influenza-specific PBMC in SLE may be of clinical relevance. Recently, it was shown that numbers of spotforming cells correlate with clinical protection from, culture-confirmed, influenza in young children [22]. These numbers may vary depending on antigen type and influenza strain, as median numbers in our assays were higher than in assays in which hemagglutinin (HA) or vaccine components were used [23-26], and as in the present study A/H3N2-specific cell-mediated responses were lower than A/H1N1-specific responses. WIV contains core antigens in addition to surface antigens. Also, the uptake and presentation of WIV is more efficient [27]. Both factors might contribute to higher responses to WIV compared to HA or vaccine components.
SLE patients showed normal T-cell cytokine responses to SEB. Previous reports reported a normal capacity of PBMC from SLE patients to respond to different stimuli, though diminished cell-mediated responses may be present during active disease [28-31]. As our cohort of SLE patients predominantly had quiescent disease, this may explain their normal responses to SEB. In addition, previous studies reported decreased proliferation of PBMC [32-34], whereas others found normal proliferative capacity [35], or heterogeneous results [36].

Diminished cell-mediated responses to influenza vaccination in SLE patients appear to reflect, in particular, effects of immunosuppressive drugs. Effects of previous influenza vaccinations, or natural infections, could not be completely excluded. Whether intrinsic defects are involved, such as a defective antigen-presenting cell function [37, 38], is uncertain.

In SLE, antibody production upon influenza vaccination is lower than in the general population [39]. In the present study, we too found lower antibody responses in SLE patients, as reflected by lower fold-increases in titers, a trend towards lower postvaccination GMTs and fewer seroconversions in serologically naïve SLE patients. Notably, antibody titers are the gold standard for protection and with regard to seroprotection rates, little differences were observed between SLE patients and HC. Influenza vaccination in the previous year was associated with a lower seroconversion rate to A/H1N1; both vaccines contained the same A/H1N1 strain. Effects of previous influenza vaccination on antibody responses remain subject to discussion, as some studies reported decreased antibody responses [40-42], whereas others found similar [43-45] or improved responses [46].

We evaluated relationships between antibody and cell-mediated responses, as CD4+ T-cell help is necessary for antibody responses [47]. However, we did not find a correlation between CD4+ T-cell responses and antibody responses using flow cytometry. We did observe a modest, correlation in SLE patients between changes in IFNγ spotforming cells against A/H1N1, measured by ELISpot, upon vaccination and seroconversion to A/H1N1. This suggests that in a subset of poorer responding patients both cell-mediated and antibody responses are affected. Possibly, no correlation between CD4+ T-cell responses and antibody responses was observed due to the lower sensitivity of flow cytometry as compared to ELISpot [48].

Finally, we showed that influenza vaccination did not induce disease activity over a period of three to four months. This confirms previous studies, reviewed in [49].

Our study has some limitations. First, the sample size was relatively small and multiple comparisons were made. However, a proper power analysis was not
possible as this is the first study to explore cell-mediated responses to influenza vaccination in SLE patients. Second, medication use in vaccinated SLE patients was heterogeneous. Third, more vaccinated SLE patients than HC had received an influenza vaccination in the previous year, which was of influence upon antibody responses. Fourth, there are no well-defined correlates between cell-mediated responses to influenza and the risk of influenza infection, which limits translation of our results to clinical implications. Fifth, phenotypes of cells responding in ELISpot assays are unknown. It can be speculated that NK cells are among the cells which have responded in our ELISpot assay [50].

Despite these limitations, we conclude that the combined data point towards diminished cell-mediated immune responses to influenza vaccination in a cohort of SLE patients representative for daily practice. Diminished cell-mediated responses may reflect effects of the concomitant use of immunosuppressive drugs. The antibody response to influenza vaccination is also reduced in SLE patients. Clinicians should be aware that this combined defect might increase the morbidity and mortality due to influenza virus infection, in particular in patients on prednisone and/or azathioprine. Therefore evaluation of clinical protection from influenza in SLE patients, following influenza vaccination, seems indicated in order to assess if more effective influenza vaccines, or vaccination strategies, are warranted.
Reference List


(37) Via CS, Tsokos GC, Bermas B, Clerici M, Shearer GM. T cell-antigen-presenting cell interactions


