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SPECIAL ARTICLE

Autoimmune response following influenza vaccination in patients with autoimmune inflammatory rheumatic disease

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Vaccines have undoubtedly brought overwhelming benefits to mankind and are considered safe and effective. Nevertheless, they can occasionally stimulate autoantibody production or even a recently defined syndrome known as autoimmune/inflammatory syndrome induced by adjuvants (ASIA). There is scarce data regarding autoimmune response after seasonal/influenza A (H1N1) vaccine in patients with autoimmune inflammatory rheumatic disease (AIRD). The objective of our study was therefore to determine autoimmune response in a large group of AIRD patients vaccinated against seasonal and/or H1N1 influenza. We conducted a prospective cohort study with a 6-month follow-up. Two-hundred and eighteen patients with AIRD (50 vaccinated against seasonal influenza, six against H1N1, 104 against both, 58 non-vaccinated controls) and 41 apparently healthy controls (nine vaccinated against seasonal influenza, three against H1N1, 18 against both, 11 non-vaccinated controls) were included. Blood samples were taken and screened for autoantibodies [antinuclear antibody (ANA), anti-extractable nuclear antigen (anti-ENA), anticardiolipin (aCL) IgG/IgM antibodies, anti-beta 2-glycoprotein I (anti-β2GPI)] at inclusion in the study, before each vaccination, 1 month after the last vaccination and 6 months after inclusion. For non-vaccinated participants (patients and healthy controls) blood samples were taken at the time of inclusion in the study and 6 months later. We report that after the administration of seasonal/H1N1 vaccine there were mostly transient changes in autoantibody production in AIRD patients and in healthy participants. However, a small subset of patients, especially ANA-positive patients, had a tendency towards anti-ENA development. Although no convincing differences between the seasonal and H1N1 vaccines were observed, our results imply that there might be a slight tendency of the H1N1 vaccine towards aCL induction. Although seasonal and H1N1 vaccines are safe and effective, they also have the potential to induce autoantibodies in selected AIRD patients and healthy adults. Follow-up of such individuals is proposed and further research is needed. *Lupus* (2012) **21**, 175–183.

Key words: autoantibodies; autoimmunity; H1N1 influenza; patients; seasonal influenza; vaccination

Introduction

Patients with autoimmune inflammatory rheumatic disease (AIRD) are at increased risk of influenza infection.^{1–3} As annual vaccination remains the most effective way of preventing influenza and its complications, the latest European League Against

Rheumatism (EULAR) recommendations suggest that inactivated influenza vaccine should be strongly considered for patients with AIRD.⁴ Since the emergence of the influenza A (H1N1) pandemic in 2009, several studies regarding the efficacy and safety of the influenza vaccination in AIRD patients did not find a significant vaccine-related harm⁵ and showed satisfactory seroconversion and seroprotection rates.⁶

Although the pathogenetic mechanisms that underlie autoimmune response remain an intriguing and largely unresolved issue, the importance of

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molecular mimicry has been emphasized^{7–9} and linked to an autoimmune response following influenza vaccination.^{10,11} The influenza vaccination has been associated with autoimmune adverse events, most frequently the Guillain–Barré syndrome,¹² but also microscopic polyangiitis and rheumatoid vasculitis in adults.^{13,14} Moreover, the H1N1 influenza vaccination has recently been linked to thrombotic thrombocytopenic purpura,¹⁵ while another report demonstrated increased antiphospholipid (aPL) antibodies, including seroconversion following seasonal influenza vaccination in healthy subjects.¹⁶

Although several studies evaluated protective antibody development and assessed clinical disease flare-ups in vaccinated AIRD patients,^{6,17–21} data concerning the autoimmune response in these patients is scarce. Tarján and colleagues established that repeated influenza vaccination in clinically stable systemic lupus erythematosus (SLE) patients may result in increased production of anti-beta 2-glycoprotein I (anti-β2GPI) and could increase the risk of thrombotic manifestations.²² While influenza vaccination did not affect the clinical expression of SLE in two other studies, a transient production of various autoantibodies, including anticardiolipin (aCL) antibodies,^{23,24} was observed.

The objective of this study was to address the issue of autoantibody production following seasonal and/or pandemic influenza vaccination in a large group of AIRD patients.

Methods

Participants

We conducted a prospective cohort study with a 6-month follow-up (from October–December 2009 to April–June 2010). In total 218 patients with AIRD [176 with rheumatoid arthritis (RA), 14 with psoriatic arthritis (PsA), 16 with ankylosing spondylitis (AS), two patients with mixed connective tissue disease (MCTD), one patient with Still's disease, one patient with dermatopolymyositis, two patients with polymyalgia rheumatica, three patients with juvenile rheumatoid arthritis (JRA), three patients with SLE] and 41 apparently healthy controls were included in the study.

All participants were initially offered vaccination against seasonal and/or pandemic influenza and were fully informed of the aims of the study and the risks involved before providing written consent. They were surveyed regarding intercurrent infections, previous vaccinations, vaccine reactions and

allergies to vaccine components at inclusion in the study. The study protocol was approved by the Ethics Committee of the Slovenian Ministry of Health and was conducted according to the principles outlined in the Declaration of Helsinki. (version VI, originally adopted in 1964).

Protocol

Patients were vaccinated against seasonal influenza at inclusion, received the first H1N1 vaccine 3–5 weeks later and the second H1N1 vaccine after another 3–5 weeks. Blood samples were taken from the antecubital vein and the presence of autoantibodies was assessed at inclusion (before the first vaccination), before each subsequent vaccination, 1 month after the last vaccination and 6 months after inclusion. For non-vaccinated patients and healthy controls, blood samples were taken at the time of inclusion in the study and 6 months later.

Vaccines

Those participants who wished to be vaccinated against seasonal influenza received a single intramuscular dose (0.5 ml) of commercially available vaccine (Sanofi Pasteur SA, Lyon, France), which contained 15 µg of inactivated purified surface fragments from the three different strains of the influenza virus [A/Brisbane/59/2007 (H1N1)-like strain (A/Brisbane/59/2007 (IVR-148), A/Brisbane/10/2007 (H3N2)-like strain (A/Uruguay/716/2007 NYMC X-175C) and B/Brisbane/60/2008-like strain (B/Brisbane/60/2008)] according to the recommendations of the World Health Organization (WHO) for the winter season 2009–2010. Other vaccine ingredients were up to 15 µg formaldehyde, up to 0.25 ml sodium phosphate-buffered isotonic sodium chloride solution, 1 µg thiomersal (sodium-ethylmercuric-thiosalicylate), Triton® X-100, trace amounts of sucrose and neomycin.

Each 0.5 ml dose of the commercially available H1N1 vaccine (GlaxoSmithKline plc, Brentford, Middlesex, UK) contained 3.75 µg of inactivated purified surface fragments from A/California/7/2009 (H1N1)v-like strain (X-179A). The vaccine contained an adjuvant, adjuvant system 03 (AS03; squalene, 10.69 mg), dl-alpha-tocopherol (11.86 mg) and polysorbate 80 (4.86 mg). Healthy participants received a single 0.5 ml intramuscular dose and patients received two 0.5 ml doses at least 3 weeks apart. Immunocompromised patients received two doses of anti-H1N1 vaccine in line with the 2009 guidelines produced by the Slovene National Institute of Health.

Autoantibodies

The autoantibody analysis was performed blinded from the clinical data as previously described.¹⁶ Briefly, all participants were screened for the presence of antinuclear antibodies (ANA), antibodies against extractable nuclear antigens (anti-ENA), aCL IgG/IgM antibodies, anti- β 2GPI IgG/IgM/IgA antibodies before and after each vaccination. Additionally, some participants were screened for lupus anticoagulant (LA). ANA were detected by a standard indirect immunofluorescence technique on HEP-2 (human laryngeal tumour) cells (Immuno Concepts, Sacramento, California, USA). A titre of 1:80 or higher was considered positive. Anti-ENA antibodies [anti-Ro, anti-La, anti-Sm, anti-SL, anti-U1-ribonucleoprotein (U1-RNP), anti-Jo1, anti-Scl 70, anti-PM/Scl, anti-Ku and anti-proliferating cell nuclear antigen (PCNA)] were detected by standard counterimmunoelectrophoresis using rabbit thymus and human spleen extracts as the antigen substrates.²⁵ aCL IgG/IgM and anti- β 2GPI IgG/IgM/IgA antibodies were determined by an in-house enzyme-linked immunosorbent assay (ELISA) as previously described.^{26–29} The LA was detected by one-stage clotting assay, using the simplified dilute Russell's viper venom time (dRVVT) test, and confirmation was performed by the dRVVT test with a high phospholipid concentration (Dade Behring GmbH, Marburg, Germany) to neutralize the possible LA effect. The normalized ratio of both tests was calculated and values of 1.2 or higher indicated the presence of LA.

Statistical analysis

Statistical analysis was performed using SPSS version 14 (SPSS, Chicago, Illinois, USA) and the statistical framework R, version 2.12.1 (R Development Core Team, Institute for Statistics and Mathematics, WU Vienna, Austria). The evaluation of autoantibody induction upon vaccination was determined using Fisher's exact test. A positive response was assumed when the titres or the levels of autoantibodies increased or if new autoantibodies developed. Results were considered statistically significant if $p < 0.05$.

Results

Participants

Within the AIRD group 160 subjects (117 women, 43 men, mean age 53.2 ± 14) chose to be vaccinated

either against seasonal influenza (50 participants), H1N1 influenza (six participants; four patients vaccinated only once and two patients who were revaccinated) or both (104 participants; for H1N1 influenza 41 patients were vaccinated only once and 61 were revaccinated). Fifty-eight patients who decided to participate but did not wish to be vaccinated were included as non-vaccinated patient controls (49 women, nine men, mean age 56.14 ± 12). Within the healthy adult group 30 were vaccinated either against seasonal (nine participants) or H1N1 influenza (three participants) or both (18 participants) (22 women, eight men, mean age 37.9 ± 13), while 11 were included as the non-vaccinated healthy controls (10 women, one man, mean age 39.8 ± 6).

ANA titres and type of immunofluorescence

Before the first vaccination 96 out of 160 (60%) patients in the vaccinated group were negative for ANA. A month after seasonal influenza vaccination 96 out of 139 (69.1%) were without change, 9 out of 139 (6.4%) patients had ANA titre seroconversion and 11 out of 139 (7.9%) had an increased ANA titre (by one dilution in nine cases and by two dilutions in two cases). There was a substantial proportion of patients (23 out of 139; 16.5%) who became ANA negative [16 out of 139 (11.5%)] or remained seropositive, but with reduced ANA titre (seven out of 139; 5%). Reductions in ANA titre were by one (six cases) or two dilutions (one case). Changes in ANA immunofluorescence type were observed in 29 out of 139 (20.86%), speckled and homogenous being the most prevalent types detected.

Approximately 1 month after being vaccinated against pandemic influenza, ANA titres increased for at least one dilution in 12 out of 96 (12.5%) (seven seroconverted and five increased their ANA titre by one dilution) and decreased in 8 out of 96 (8.1%) (six became negative and two had a one-dilution decrease in the ANA titre). The ANA titre increased in 11 out of 69 (16%) patients after the second pandemic influenza vaccination (seven seroconversions and four increases by one dilution). Five became seronegative and four had decreased ANA titre (one dilution).

At the end of the 6-month follow-up, 106 out of 160 (66.5%) had no changes in ANA titres and 123 out of 160 (76.88%) showed no changes in ANA immunofluorescence type. Twenty patients (12.50%) had higher ANA titres while in 35

(22%) the ANA titres decreased. In 19 out of 160 (11.8%) patients ANA disappeared and in 10 out of 160 (6.3%) patients ANA appeared de novo. In 37 out of 160 patients (23.12%) there were changes in ANA immunofluorescence type.

In the healthy adult group 22 out of 30 (73.3%) were negative for ANA at the beginning of the study. A month after being vaccinated against seasonal influenza we observed changes in ANA titres for one dilution in five out of 27 (16.67%), and in three out of 27 (11.1%) of them ANA developed de novo. Five out of 27 (16.67%) presented with changes in immunofluorescence type, speckled being the most prevalent type. In three out of 27 (11.1%) ANA titres decreased.

Six months after the inclusion into the study 25 out of 30 (83.33%) healthy vaccinated participants had no changes in ANA titres or type of

immunofluorescence. ANA titres were elevated in three out of 30 (10%) participants (two after vaccination against seasonal influenza and one after both vaccines had been administered) and decreased in three out of 30 (10%), while five participants (16.67%) had changes in ANA immunofluorescence patterns. Changes in ANA titres are presented in Figure 1 (A–D).

A comparison between the vaccinated patient group and the vaccinated healthy adult group 1 or 6 months after vaccination revealed no statistically significant differences in ANA changes ($p=0.56$ or 0.58 , Fisher's exact test), but within the patient group a statistically significant elevation in ANA titres was observed ($p=0.04$, Fisher's exact test) if vaccinated female patients were considered. There was no statistical significance if both sexes were considered.

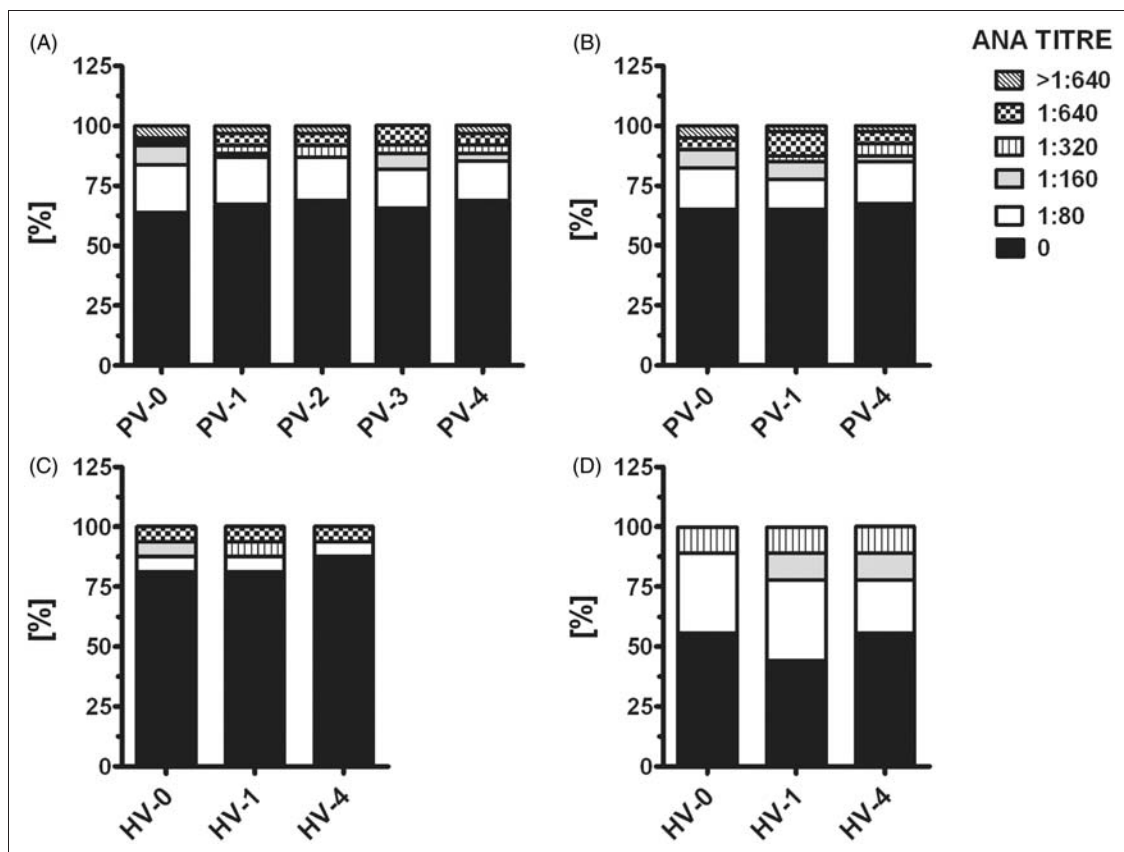


Figure 1 Percentage ANA titres in vaccinated patients and healthy controls. ANA titres exhibited a tendency to increase after the seasonal as well as the influenza A (H1N1) vaccine. Presented is the titre dynamics from inclusion in the study until 6 months later. (A) patients vaccinated against seasonal and pandemic influenza ($N=61$ for all columns; only the same patients are included throughout), (B) patients vaccinated only against seasonal influenza ($N=50$ for all columns; only the same patients are included throughout), (C) healthy vaccinated individuals against seasonal and pandemic influenza ($N=16$ for all columns; only the same patients are included throughout), (D) healthy vaccinated only against seasonal influenza ($N=9$ for all columns; only the same patients are included throughout). HV: healthy vaccinated participants, PV: vaccinated patients, 0: before vaccination, 1: 1 month after seasonal influenza vaccination, 2: 1 month after H1N1 influenza vaccination, 3: 1 month after H1N1 revaccination, 4: 6 months after the first vaccination.

Anti-ENA

Within the vaccinated patient group 142 out of 160 (88.8%) had negative anti-ENA before vaccination. A month after vaccination against seasonal influenza 139 out of 139 (100%) showed no changes in anti-ENA. At the end of the 6-month follow-up, 16 out of 160 (10%) patients showed changes in anti-ENA if compared with the time point before their first vaccination. There were constant changes in a small number of patients after vaccination, which usually meant a transient decline of semi-quantitative assessment in already existing antibodies. However, one RA and one AS patient developed antibodies of unknown specificity against human spleen extract (anti-HSE) and one RA patient against rabbit thymus extract (anti-RTE) *de novo* by month 6 (Figure 2). Moreover in one RA patient with a history of anti-Ro positive serology, anti-Ro was barely detectable before vaccination and became strongly expressed 6 months after vaccination. In eight patients, anti-ENA levels were of lower titre than initially. We also observed that in patients with positive ANA, there had been significantly more anti-ENA dynamics after vaccination ($p=0.02$, Fisher's exact test, Figure 3).

In contrast, 29 out of 30 (96.7%) healthy controls were negative for anti-ENA at the beginning of the study and remained anti-ENA negative.

aCL IgG and aCL IgM

In the vaccinated patient group, 112 out of 160 (70%) patients were negative for aCL IgG and 150 (93.8%) for aCL IgM at the beginning of the study. A reduction or rise in aCL was considered important if levels changed for at least one class (arbitrary units: 0–4 negative, 5–10 mildly positive, 11–30 medium positive, >30 highly positive). One month after the seasonal influenza vaccination nine out of 112 (8%) previously aCL IgG-negative patients presented with elevated aCL IgG and six out of 150 (4%) with elevated aCL IgM antibodies. aCL IgG were reduced in 27/139 (19.6%) and aCL IgM in 4/139 (2.9%) patients. There was a further decrease of aCL IgG after the pandemic influenza vaccination in 19/96 (19.8%) and an increase in 31/96 (32.3%) patients, whereas aCL IgM decreased further in 8/96 (8.3%) and rose in 11/96 (11.5%). 111/160 (69.4%) patients showed no change in aCL IgG 6 months after the first vaccination, in 8/160 (5%) patients aCL IgG were higher, and lower in 41/160 (25.6%). As for aCL IgM, 6 months after the first vaccination they were unchanged in 145/160 (91%), increased in 6/160 (3.8%) and decreased in 8/160 (5%) patients.

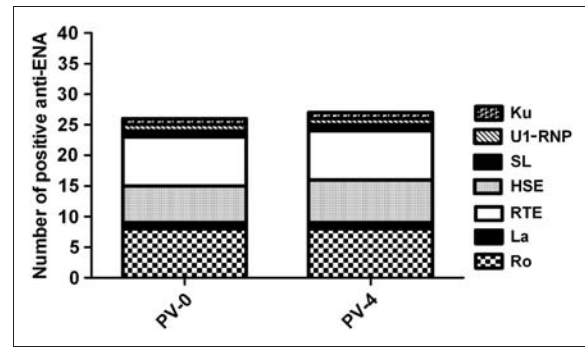


Figure 2 Anti-ENA antibodies at inclusion in the study and 6 months after the first vaccination. We found persistent changes in anti-ENA in a limited number of patients. As shown here, two patients developed *de novo* anti-HSE and one patient anti-RTE by visit 4. HSE: anti-ENA antibodies detected by a standard counterimmunoelectrophoresis using human spleen extract as the antigen, RTE: anti-ENA antibodies detected by a standard counterimmunoelectrophoresis using rabbit thymus extract as the antigen, PV: vaccinated patients, 0: before vaccination ($n=160$), 4: 6 months after the first vaccination ($n=160$). ENA: extractable nuclear antigen, HSE: human spleen extract, RTE: rabbit thymus extract, U1-RNP: U1-ribonucleoprotein.

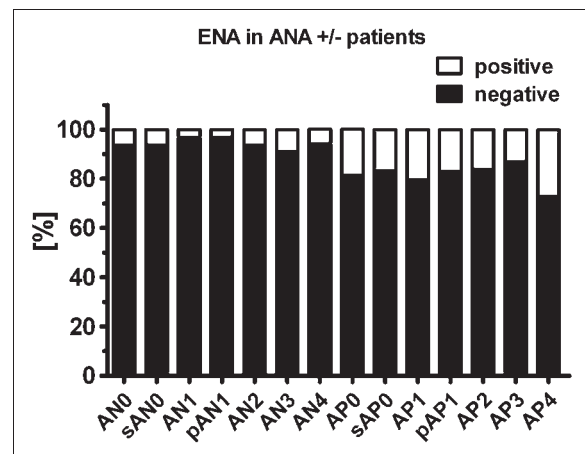


Figure 3 Percentage of positive anti-ENA in vaccinated patients with positive/negative ANA. A few cases of anti-ENA induction were observed. As shown here, anti-ENA were more prone to dynamics after vaccination in patients with positive ANA ($p=0.02$, Fisher's exact test). AN: ANA negative, AP: ANA positive, sAN: ANA-negative patients vaccinated against seasonal influenza, pAN: ANA-negative patients vaccinated against H1N1 influenza, sAP: ANA-positive patients vaccinated against seasonal influenza, pAP: ANA-positive patients vaccinated against H1N1 influenza, 0: before vaccination, 1: 1 month after seasonal influenza vaccination, 2: 1 month after H1N1 influenza vaccination, 3: 1 month after H1N1 revaccination, 4: 6 months after the first vaccination. ANA: antinuclear antibodies, ENA: extractable nuclear antigen.

Three RA and one Still's disease patient, who were initially aCL IgG negative, developed them de novo (one after seasonal influenza vaccination, three after being vaccinated against seasonal and pandemic influenza). Out of three patients with newly induced aCL IgM after vaccination against seasonal influenza, one female patient developed high levels of aCL IgM which persisted.

When comparing positive autoantibody response 1 month and 6 months after vaccination, there was a statistically significant decrease in the number of patients with elevated aCL IgG from month 1 to month 6 ($p = 0.022$, Fisher's exact test) (Figure 4).

In the healthy control group, 18 out of 30 (60%) participants were aCL IgG negative before vaccination, 12 out of 30 (40%) were low-positive and 30 out of 30 (100%) were aCL IgM negative. aCL IgG increased after seasonal influenza vaccination in 3/27 (11.1%) participants and decreased in 8/27 (29.6%). There was a further increase of aCL IgG in 1 person after receiving the H1N1 influenza vaccine. Six months after the inclusion into the study aCL IgG were increased in 4/30 (13.3%) participants, decreased in 9/30 (30%) and remained unchanged in 17/30 (56.7%). aCL IgM increased in one person after receiving a seasonal influenza vaccine and remained increased until 6 months after inclusion in the study.

Among the healthy participants with elevated aCL IgG, two had de novo aCL IgG, one of them after being vaccinated against pandemic influenza only and one after receiving both vaccines.

Anti- β 2GPI, anti-LA and anti-DNA

Before vaccination 152 out of 160 (95%) patients tested negative for anti- β 2GPI. The levels of anti- β 2GPI IgG became slightly positive in two (1.5%) and decreased in three (2.2%) patients after administration of the seasonal influenza vaccine. Anti- β 2GPI IgA increased for one class in four out of 137 (2.92%) patients after administration of the seasonal influenza vaccine. Six months later six (3.8%) patients had lower anti- β 2GPI IgG than initially and in one (0.6%) patient they had been raised. Anti- β 2GPI IgM levels were lower in four patients and anti- β 2GPI IgA levels were elevated in three (1.9%). Among the latter, they were highly positive in one patient. One RA patient developed new slightly positive anti- β 2GPI IgA after being vaccinated against seasonal and H1N1 influenza and one PsA patient developed new slightly positive anti- β 2GPI IgA after administration of the anti-H1N1 influenza vaccine. There was no dynamics of anti- β 2GPI in the healthy, vaccinated group.

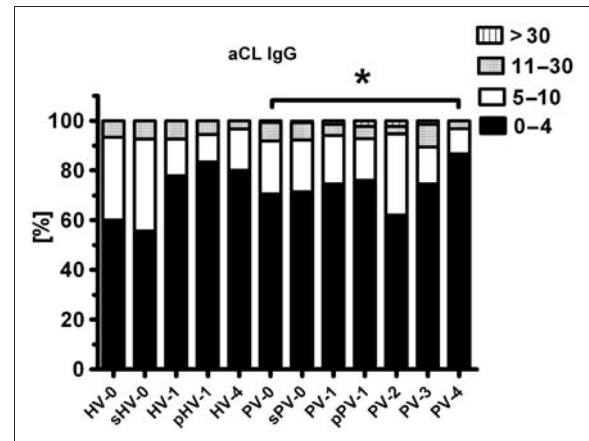


Figure 4 Percentage of aCL IgG in vaccinated patients and healthy controls. There were transient elevations in aCL IgG levels after the administration of seasonal and influenza A (H1N1) vaccine. However, 6 months after inclusion in the study there was a statistically significant decrease in aCL IgG ($*p = 0.022$, Fisher's exact test). HV: healthy vaccinated controls, PV: vaccinated patients, sHV: healthy participants, vaccinated against seasonal influenza, pHV: healthy participants, vaccinated against H1N1 influenza, sPV: patients vaccinated against seasonal influenza, pPV: patients vaccinated against H1N1 influenza, 0: before vaccination, 1: 1 month after seasonal influenza vaccination, 2: 1 month after H1N1 influenza vaccination, 3: 1 month after H1N1 revaccination, 4: 6 months after the first vaccination. 0-4: negative aCL IgG, 5-10: mildly positive aCL IgG, 11-30: medium positive aCL IgG, >30: highly positive aCL IgG. aCL: anti-cardiolipin, IgG: immunoglobulin G.

None of the patients or healthy controls had any kind of changes in anti-DNA levels (all had been negative for anti-DNA at inclusion in the study and remained negative).

The LA test was negative in 79 out of 94 (84%) vaccinated patients and in 34 out of 36 (94.66%) non-vaccinated patients at the beginning of the study and remained negative in 66 out of 82 (80.50%) vaccinated patients and in 32 out of 34 (94.12%) non-vaccinated patients 6 months later. There were no statistically significant differences between the vaccinated and the non-vaccinated group.

The influence of different vaccines

The analysis of the possible differences between the two vaccines was performed in the vaccinated patient group (Table 1). The observed changes in the number of positive autoantibodies were mostly due to transient, de novo ANA development or the transient appearance of aCL IgG or aCL IgM. Overall, long-term autoantibody response, mostly a decrease in the ANA titre, was observed in 36 out of 69 (52%) patients with autoantibody

Table 1 Autoantibody status in vaccinated patients at different time points

Visit	Number of patients (%)	Number of different autoantibodies (%)						
		0	1	2	3	4	5	6
0	160 (100)	54 (33.8)	30 (18.8)	34 (21.2)	23 (14.4)	12 (7.5)	7 (4.4)	0 (0.0)
1	139 (100)	53 (38.1)	26 (18.7)	29 (20.9)	14 (10.1)	16 (11.5)	0 (0.0)	1 (0.7)
2	96 (100)	28 (29.2)	30 (31.2)	19 (19.8)	9 (9.4)	7 (7.3)	3 (3.1)	0 (0.0)
3	69 (100)	25 (36.2)	17 (24.6)	16 (23.2)	7 (10.1)	4 (5.8)	0 (0.0)	0 (0.0)
4	160 (100)	68 (42.5)	31 (19.4)	24 (15.0)	26 (16.2)	8 (5.0)	2 (1.2)	1 (0.6)

Visit 0: before vaccination; visit 1: 1 month after seasonal influenza vaccination; visit 2: 1 month after H1N1 influenza vaccination; visit 3: 1 month after H1N1 revaccination; visit 4: 6 months after the first vaccination.

changes after seasonal influenza vaccination. Seven out of 63 (11.1%) patients vaccinated for seasonal influenza showed an increase in ANA titres or aCL upon vaccination for H1N1 influenza, but only in one of them this resulted in increased ANA titres 6 months later. There was no autoantibody dynamics in the six patients vaccinated only against H1N1 influenza.

Analysis of the autoantibody induction following vaccination revealed significantly more aCL IgG/IgM induction after the H1N1 compared to seasonal influenza vaccine ($p=0.02$ for aCL IgG and $p=0.03$ for aCL IgM, Fisher's exact test).

Discussion

This is the first study that focuses on autoimmune response after seasonal/H1N1 vaccination in a large group of patients with diverse AIRD, while the majority of previously available data was mostly limited to autoantibody response in SLE patients^{22,24,30} and healthy adults.¹⁶ Moreover, the present study was conducted in the 2009–2010 season, when two separate types of influenza vaccine (the 2009 pandemic H1N1 vaccine and the 2009–2010 seasonal influenza vaccine) were in use. Since the vaccinations took place at different times we had the opportunity to evaluate their influence on autoantibody response separately. Our results show that seasonal/H1N1 influenza vaccination caused transient changes in ANA titres, including the development of new ANA. Within the patient group there was a statistically significant elevation in ANA titres when women were considered, which could indicate not only the impact of individual differences but also of hormonal status. Two patients with RA and one patient with AS developed antibodies of unknown specificity against rabbit thymus or human spleen extracts 6 months

after vaccination. We also found an intriguing correlation within the vaccinated patient group, where ANA-positive participants experienced significantly more anti-ENA dynamics following vaccination (Figure 3). As this was mostly seen at the time point where acute vaccine effects are no longer expected, we cannot directly propose the vaccine as a causative agent.

Nevertheless, high titres of anti-Sm, anti-Sm/ribonucleoprotein (RNP), anti-Ro and anti-La were already reported in SLE patients 6 weeks after influenza vaccination.³⁰ It is also possible that the acute response after vaccination, which might have been observed with ELISA, was missed in our study since counterimmunoelectrophoresis can only detect precipitating IgG antibodies.

We noted the potential of both vaccines to induce de novo aCL IgG/IgM in susceptible subjects. Nevertheless, although a transient increase in aCL IgG after either vaccination was often observed, the long-term effect of vaccination resulted in lower aCL IgG in most patients (Figure 4). A transient increase is in accordance with previous reports,^{24,30} but a statistically significant decrease of aCL IgG 6 months later is not, and could be due to the fact that other studies evaluated only SLE patients, where there is a strong association with baseline aCL production.³¹ There were only separate cases of anti-β2GPI induction after vaccination observed in the patient group, which were far from a remarkable elevation in the levels of anti-β2GPI demonstrated by Tarján and colleagues.²² Consistent with previous reports, we observed no effect of seasonal or H1N1 vaccine on anti-DNA levels or LA.

Overall our data suggests no significant differences between the two vaccines except for their potential to induce aCL, where the H1N1 vaccine seemed to be more potent. The reason might be the oil-based and probably more effective adjuvant

squalene in the H1N1 vaccine, which is known to induce SLE-associated antibodies in mice³² or arthritis in rats.³³ However, a recent report suggested a good safety profile of a similar squalene-based vaccine.³⁴

Although vaccines are doubtlessly one of the greatest achievements of modern medicine, infrequently the vaccine itself or the adjuvant can induce the development of autoimmunity.^{10,35} Recently, different autoimmune conditions have been grouped as a single syndrome called autoimmune/inflammatory syndrome induced by adjuvants (ASIA)³⁶ characterized by clinical manifestations (myalgia, myositis, muscle weakness, arthralgia, arthritis, chronic fatigue, sleep disturbances, cognitive impairment and memory loss), which are triggered by external stimuli (infection, vaccine, etc.) and which improve on removal of the causative agent. Among the proposed minor criteria for diagnosing this syndrome is the appearance of autoantibodies.³⁶ It is therefore important not only to know the protective potential of individual vaccines, but also to understand their potential to provoke an autoantibody response.

To conclude, influenza vaccines are considered safe, effective and lifesaving in the healthy population as well as in AIRD patients. However, our data suggest that the seasonal and H1N1 influenza vaccines, which were used in the 2009–2010 season, have a potential to induce not only transient but also long-term autoantibody changes, including the development of new antibodies in some susceptible AIRD patients and healthy subjects. We recommend the follow-up of such rare individuals and suggest further research to possibly confirm true cases of ASIA syndrome.

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Conflict of Interest

None declared.

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