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Autoimmune response following annual influenza vaccination in 92 apparently healthy adults

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ABSTRACT

Objective: To evaluate the possibility of autoimmune responses following annual influenza vaccination in a large cohort of apparently healthy adults.

Methods: Autoantibodies including antinuclear antibodies (ANA), anticardiolipin antibodies (aCL), anti-β_2-glycoprotein I antibodies (anti-β_2-GPI), lupus anticoagulant (LA) and anti-extractable nuclear antigen antibodies (anti-ENA) were determined in 92 healthy adult subjects, staff at the University Children's Hospital Ljubljana. Blood samples were taken from each participant before the vaccination, 1 month and 6 months after the annual influenza vaccination.

Results: Before the influenza vaccination, 26% of participants were positive for ANA, 16% for aCL, 7% for anti-β_2-GPI, 2% for LA and 1% for anti-ENA. There were no statistically significant differences in the percentage of positive ANA, aCL, anti-β_2-GPI, LA and anti-ENA before, 1 month and 6 months after the vaccination. One month after the vaccination 24% of participants demonstrated changes in the levels of autoantibodies including 15% of participants with increased level of autoantibodies or appearance of new autoantibodies. Six months after the vaccination 26% of participants demonstrated changes in the levels of autoantibodies including 13% of participants with increased level of autoantibodies or appearance of new autoantibodies. Persistently elevated levels of autoantibodies were observed in 7 (8%) participants and 2 showed progressively increased levels of IgM aCL or IgA anti-β_2-GPI, respectively. Eleven participants had a transient increase in autoantibodies.

Discussion: Influenza vaccination in general did not alter the percentage of healthy adults with positive autoantibodies. Transiently or persistently increased levels of autoantibodies or appearance of new autoantibodies was demonstrated in up to 15% of apparently healthy adults after the influenza vaccination.

Contents

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1. Introduction

Vaccinations are one of the major achievements in medicine in the last century and the most effective method for preventing infections. Concern about safety of vaccinations has been heightened by several reports of possible vaccine-induced autoimmune phenomena following various vaccinations, including influenza vaccination [1,2]. According to the Vaccine Adverse Event Reporting System (VAERS), Guillain–Barre syndrome represents the most frequently reported autoimmune neurological adverse event following influenza vaccination [3]. A few cases of Henoch–Schoenlein purpura with induction of anti-phospholipid antibodies (aPL) have been described in children following influenza vaccination [4,5]. Moreover, isolated cases of microscopic polyangiitis and rheumatoid vasculitis were reported in adults following influenza vaccination [6,7]. The majority of published epidemiological studies did not confirm the connection between vaccinations and autoimmune diseases and the autoimmune reaction was usually transient [8].

To our knowledge, no study assessed the induction of autoimmune response after influenza vaccination in apparently healthy persons. This study was undertaken to evaluate the possibility of autoimmune responses following annual influenza vaccination in apparently healthy adult population.

2. Patients and methods

The study design was a prospective cohort study with 6 months follow-up after the annual influenza vaccination. The study population consisted of 92 apparently healthy adults (77 female and 15 male; mean age 41.2 ± 10.9 years) who were vaccinated with annual influenza vaccine (Influvac 2006/2007, Solvay Pharmaceuticals B.V., Netherlands). All participants were staff at the University Children's Hospital Ljubljana and received a routine annual influenza vaccination in November or December 2006. Each 0.5 ml dose of the vaccine contained 15 μg of inactivated purified surface fragments from the three different strains of the influenza virus (A/New Caledonia/20/99(H1N1)-like virus, A/Wisconsin/67/2005 (H3N2)-like virus or A/Hiroshima/52/2005, B/Malaysia/2506/2004-like virus) according to the latest recommendations of the World Health Organization (WHO).

All participants were surveyed and questioned at the time of vaccination about intercurrent infections, past and present medical history and family history of autoimmune diseases. No participant had clinical infection or any documented serious medical problems at the time of sample collection and none was allergic to egg proteins. Each participant was informed about the aim of the study and asked for a written informed consent for drawing blood before and after the vaccination. The study was approved by the Ethics' Committee of the Slovenian Ministry of Health and was conducted according to the principles of the Declaration of Helsinki.

The presence of autoantibodies was assessed at three time points: (1) before influenza vaccination; (2) 1 month after the vaccination; and (3) 6 months after the vaccination. All participants were screened for the presence of antinuclear antibodies (ANA), antibodies against extractable nuclear antigens (anti-ENA), IgG/IgM anticardiolipin antibodies (aCL), IgG/IgM/IgA anti-β_2 glycoprotein I antibodies (anti-β_2-GPI) and lupus anticoagulant (LA) before and after vaccination.

ANA were detected by a standard indirect immunofluorescence technique on HEp-2 cells (Immuno Concepts, Sacramento, CA, USA). ANA titer of 1:80 or higher was considered positive. Anti-ENA antibodies were detected by a standard counter-immunoelectrophoresis using rabbit thymus and human spleen extracts as the antigen substrates [9].

aCL and anti-β_2-GPI antibodies were determined by our in-house enzyme-linked immunosorbent assays (ELISAs) as described previously [10]. The cut-off values for IgG/IgM aCL were defined by statistical evaluation of data obtained from 147 blood donors as reported earlier [11]. The cut-off values for IgG/IgM/IgA anti-β_2-GPI were based on the control group comprised of 434 healthy blood donors. The level of anti-β_2-GPI antibodies in each sample was derived from the standard curve according to the defined dilutions of monoclonal antibodies [12].

The LA was detected by one-stage clotting assay, using simplified dilute Russell viper venom time test (dRVVT) and confirmation dRVVT test with a high phospholipids concentration (Dade Behring, Marburg, Germany) to neutralize the possible LA effect [13]. The normalized ratio of both tests was calculated and values 1.2 or higher indicated the presence of LA.

Antibody titers against the three vaccinal influenza viruses were measured before and after the vaccination using the haemagglutination inhibition assay with the use of turkey erythrocytes according to the WHO established procedures [14]. Antibody titer of 1:80 or higher was considered seropositive before the vaccination (convalescent titer) [15]. Seroconversion was defined as at least 4-fold increase in antibody titers in seronegative individuals. For individuals with seropositive titers before vaccination seroconversion was defined as 2-fold increase in antibody titers [16].

Statistical tests were performed using subroutines from the statistical analysis package by MS Excel version 11.0 and MegaStat version 9.0 for MS Office. The statistical significance of the difference between the inter group frequency rates before and after the vaccination was determined using the chi-square test. Student's *t*-test was used to compare the mean values of anti-β_2-GPI autoantibodies before and after the vaccination and antiviral antibodies before and after the vaccination.

Significance threshold of 95% was used, which means that differences were considered statistically significant if $p<0.05$.

3. Results

3.1. General characteristics

The study was conducted on 92 apparently healthy adults between November 2006 and August 2007. No clinical signs of moderate or serious adverse events after the influenza vaccination were observed during the 6 months follow-up period. No participant had clinical signs of systemic autoimmune disease; however, 12 (13%) participants had positive family history of autoimmune diseases among first-degree relatives.

All participants had three blood sample collections: at the time of vaccination, 1 month after the vaccination and 6 months (±14 days) after the vaccination. The percentage of participants with positive autoantibodies before and after the vaccination is presented in Table 1. Overall, there were no statistically significant differences in the percentage of positive ANA, aCL, anti-β_2-GPI, LA and anti-ENA before, 1 month and 6 months after the vaccination (Table 1).

Before the vaccination 35 (38%) participants were seronegative to all three strains of the influenza virus and 57 (62%) participants were seropositive for at least one strain. Among seropositive participants, 9 (16%) were seropositive for all three strains of the influenza virus.

No statistically significant correlation was found between seropositivity to three vaccinal strains of the influenza virus and positive autoantibodies before vaccination.

3.2. Autoantibody determinations 1 month after influenza vaccination

One month after the influenza vaccination, 70 (76%) participants showed no change in the level of ANA, aCL, anti-β_2-GPI, LA or anti-ENA. Changes in the levels of autoantibodies were observed in 22 (24%) participants: ANA titer in 1 (1%) participant, aCL levels in 12 (13%) participants, anti-β_2-GPI levels in 8 (9%) participants, changes in LA levels in 6 (7%) participants, while 1 (1%) participant became low positive for non-specific cytoplasmic immunofluorescence antibodies. Six participants developed changes in titers of two different autoantibodies.

Table 1
Percentage of positive autoantibodies in 92 healthy adults before, 1 and 6 months after the influenza vaccination

	Before vaccination	1 month after vaccination	6 months after vaccination
	N (%)	*N* (%)	*N* (%)
ANA	24 (26)	24 (26)	24 (26)
aCL	15 (16)	12 (13)	14 (15)
Anti-β2-GPI	6 (7)	8 (9)	8 (9)
LA	2 (2)	4 (4)	3 (3)
Anti-ENA	1 (1)	1 (1)	1 (1)

ANA—antinuclear antibodies, aCL—anticardiolipin antibodies, anti-β_2-GPI—anti-β_2-glycoprotein I antibodies, LA—lupus anticoagulant, anti-ENA—anti-extractable nuclear antigen antibodies.

Increased level of autoantibodies or appearance of new autoantibodies was observed in 14 (15%) participants. One participant who was initially positive for ANA showed an increased titer after the vaccination. Among participants with changes in the level of aCL, no statistically significant differences were detected between the number of participants with increased (4/92) or decreased (8/92) values. All four participants who showed increased levels of aCL (2 IgG aCL and 2 IgM aCL) were positive for aCL also before vaccination. Among participants with changes in the level of anti-β_2-GPI, no statistically significant differences were detected between the number of participants with increased (6/92) or decreased (2/92) values. Three participants who were initially negative for anti-β_2-GPI became positive (2 IgA and 1 IgM anti-β_2-GPI) and 3 who were initially low positive for anti-β_2-GPI (1 IgG, 1 IgM and one IgG/IgM/IgA anti-β_2-GPI) showed increased values. Two participants who were initially positive for aCL and anti-β_2-GPI showed an increase in the level of both aPL subtypes. Among participants with changes in the level of LA, no statistically significant differences were detected between the number of participants with increased (4/92) or decreased (2/92) values.

Only one of the 35 seronegative participants did not respond to the vaccine, 3 had an inadequate response, 19 (54%) responded with 4-fold increase of antibody titers against at least one strain of the influenza virus and 12 (35%) participants responded to all three strains of the influenza virus 1 month after the vaccination. Among 57 initially seropositive individuals, 4 (7%) showed no response, 38 (67%) responded with an increase of antibody titer against at least one strain of the influenza virus and 15 (26%) participants responded to all three strains of the influenza virus 1 month after the vaccination.

3.3. Autoantibody determinations 6 months after influenza vaccination

Six months after the influenza vaccination, 68 (74%) participants showed no change in the level of ANA, aCL, anti-β_2-GPI, LA or anti-ENA. Changes in levels of autoantibodies were observed in 24 (26%) participants: ANA titer in 3 (3%) participants, aCL levels in 11 (12%) participants, anti-β_2-GPI levels in 7 (8%) participants, LA levels in 3 (3%) participants and 2 (2%) were low positive for non-specific cytoplasmic immunofluorescence antibodies. Two participants developed changes in the level of two different autoantibodies. No participant had changes in the level of anti-ENA antibodies 6 months after the influenza vaccination.

Increased levels of autoantibodies or appearance of newly synthesized autoantibodies were observed in 12 (13%) participants. One participant initially low positive for ANA (1:80) became high positive (1:320). Five participants initially negative for autoantibodies developed *de novo* autoantibodies after 6 months, including one participant who developed ANA with high titer (1:640), one became low positive for aCL, one for anti-β_2-GPI, one for LA and one developed low non-specific cytoplasmic immunofluorescence.

Persistently elevated levels of autoantibodies were observed in 7 (8%) participants and 2 showed progressively increased levels of autoantibodies during the 6 months' follow-up including one with IgM aCL and one with IgA anti-

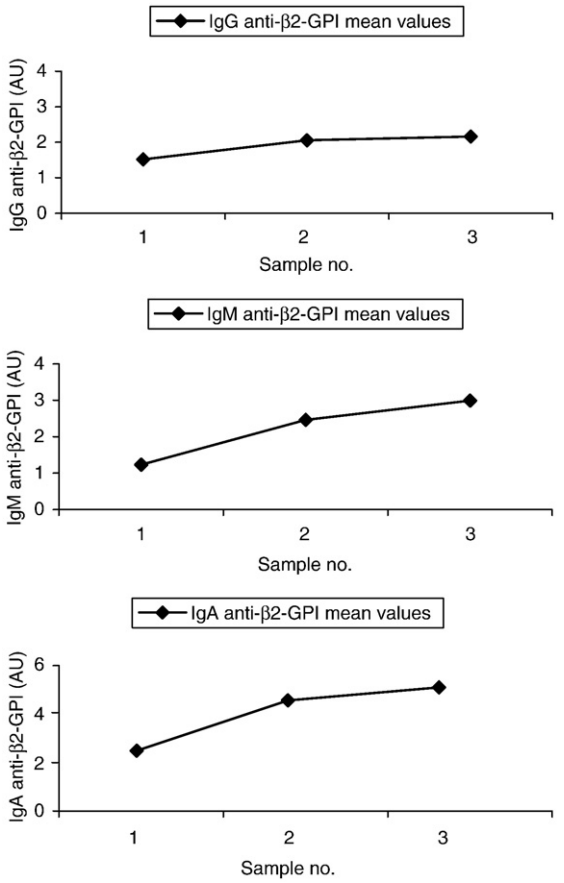

Fig. 1. Differences between the mean values of IgG/IgM/IgA anti-β_2-GPI before, 1 month and 6 months after influenza vaccination. Cut-off value for IgG anti-β_2-GPI was 5.9 arbitrary units (AU), for IgM anti-β_2-GPI 8.3 AU and for IgA anti-β_2-GPI 8.1 AU.

β_2-GPI, respectively. Eleven participants in our study developed a transient increase in autoantibodies (1 for ANA, 2 for aCL, 4 for anti-β_2-GPI and 4 for LA).

There were no statistically significant differences between the mean values of IgG/IgM/IgA anti-β_2-GPI before, 1 and 6 months after the vaccination (Fig. 1).

Six months after the vaccination, 74 (80%) participants showed increased titers of antibodies against at least one strain of the influenza virus ($1 \geq 80$) and 14 (19%) participants showed increased titers of antibodies against all three strains of the vaccinal influenza viruses.

4. Discussion

The present study assessed the induction of autoimmune response after annual influenza vaccination in apparently healthy adults. Our cohort consisted of 92 apparently healthy medical staff at the University Children's Hospital Ljubljana that exhibited a high percentage of positive autoantibodies even before the influenza vaccination. Sixty-two percent of participants had protective antibodies to at least one strain of the influenza virus before the vaccination; however, there were no correlation between the presence of autoantibodies and protective antibodies against the three different strains of the influenza virus before vaccination. No participant had clinical features of an acute infection or systemic autoimmune disease at the time of sample collection and changes in the level of autoantibodies in our cohort were most likely induced by the influenza vaccination.

Overall, our study demonstrated no statistically significant difference in the percentage of participants with positive autoantibodies before, 1 and 6 months after the influenza vaccination. Increased level of autoantibodies or appearance of new autoantibodies was observed 1 month after the vaccination in 15% and 6 months after the vaccination in 13% of participants, suggesting *de novo* induction of autoantibodies after the influenza vaccination in selected individuals. Changes in the level of autoantibodies were most frequently observed for aCL and anti-β_2-GPI antibodies, further supporting the evidence of infectious and/or vaccination-induced production of aPL [17]. Two participants demonstrated progressive increase in autoantibody levels including one with increasing levels of IgM aCL and one with increasing levels of IgA anti-β_2-GPI, respectively. No participant developed clinical signs of overt autoimmune disease and no participant demonstrated aPL-related thrombotic events during the 6 months follow-up period after the influenza vaccination.

Possible mechanisms for this autoimmune phenomenon include molecular mimicry [18,19]. Experimental data which support the infective etiology of anti-phospholipid syndrome (APS) has been provided by Blank et al. in experimental APS. They identified a hexapeptide (TLRVYK) that was specifically recognized by pathogenic anti-β_2-GPI monoclonal antibodies and had high homology with peptide domain of various bacteria and viruses [19]. It has been suggested that several other mechanisms, such as bystander activation and exposure of normally hidden epitopes due to tissue destruction, may be involved in the induction of aPL by viral infections and vaccinations [20]. Newly synthesized autoantibodies detected in our study had no apparent clinical significance.

To our knowledge, this is the first cohort study investigating an autoimmune response following influenza vaccination in apparently healthy adults. Two studies evaluated the safety and immunogenicity of influenza vaccine in patients with systemic lupus erythematosus (SLE). Tarjan et al. found that repeated influenza vaccination in clinically stable SLE patients with low disease activity may result in increased production of anti-β_2-GPI antibodies and, therefore, may increase the risk of thrombotic manifestations [21]. Abu-Shakra et al. observed that influenza vaccination did not affect the clinical expression of SLE, but may trigger a transient production of various autoantibodies, including aCL, with no clinical significance [22].

Scarce accurate information exists regarding the induction of the synthesis of autoantibodies following other routine immunizations in humans. Our group investigated the synthesis of aPL following vaccination with recombinant DNA hepatitis B vaccine in 85 healthy volunteers. A transient increase of aCL titers in two participants and a transient increase of anti-β_2-GPI titers in one participant were observed. One participant who initially had low positive IgG anti-β_2-GPI showed a progressive increase of the antibody level during 6 months of follow-up. Although there was no statistically significant production of aPL antibodies after

vaccination with hepatitis B vaccine in healthy adults, the risk of developing a continuous long-term aPL response in genetically predisposed individuals could not be excluded [23].

The effects of vaccination on autoantibodies production were also studied in dogs following mandatory vaccination against rabies, canine distemper virus and canine parvovirus-9. Significant increase of the levels of autoantibodies to laminin and fibronectin were detected, without any evidence of autoimmune disease [24].

A possible limitation of our study is recruitment of the study cohort from apparently healthy medical workers, which represent a subgroup of healthy population with an increased risk of encountering infectious diseases. In particular, we cannot exclude a concomitant exposure to an infectious agent with subclinical presentation that might trigger a transient production of specific autoantibodies.

In conclusion, our study demonstrated that influenza vaccination in general did not alter the percentage of healthy adults with positive autoantibodies. However, an increased level of autoantibodies or the appearance of new autoantibodies was observed in 15% of apparently healthy adults 1 month after the influenza vaccination and in 13% of apparently healthy adults 6 months after the influenza vaccination. Changes in the level of autoantibodies were most frequently detected for aCL and anti-β_2-GPI antibodies, further supporting the evidence of possible infectious and/or vaccination-induced production of aPL with no clear clinical significance.

Take-home messages

- Influenza vaccination did not increase the percentage of positive autoantibodies in the general healthy adult population.
- Increased level of autoantibodies or appearance of new autoantibodies was observed in up to 15% of apparently healthy adults after the influenza vaccination.
- Out of 92 healthy adults included in our study, 11 participants developed transient and 7 persistently had elevated levels of autoantibodies after the vaccination.
- Newly synthesized autoantibodies after the influenza vaccination had no apparent clinical significance.
- Prolonged autoimmune response following influenza vaccination cannot be excluded.

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