EXTENDED REPORT

Vaccination against influenza in rheumatoid arthritis: the effect of disease modifying drugs, including TNFα blockers

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Objective: To assess the efficacy and safety of vaccination against influenza virus in patients with rheumatoid arthritis, with special emphasis on the effect of disease modifying antirheumatic drugs (DMARDs), including tumour necrosis factor α (TNFα) blockers.
Methods: 82 rheumatoid patients and 30 healthy controls were vaccinated with a split-virion inactivated vaccine containing 15 μg haemagglutinin (HA) per dose of each of B/Hong Kong/330/2001 (HK), A/Panama/2007/99 (PAN), and A/New Caledonian/20/99 (NC). Disease activity was assessed by tender and swollen joint count, morning stiffness, evaluation of pain, Health Assessment Questionnaire, ESR, and C reactive protein on the day of vaccination and six weeks later. Haemagglutination inhibiting (HI) antibodies were tested by a standard WHO procedure. Response was defined as a fourfold or more rise in HI antibodies six weeks after vaccination, or seroconversion in patients with a non-protective baseline level of antibodies (<1/40). Geometric mean titres (GMT) were calculated to assess the immunity of the whole group.
Results: Six weeks after vaccination, a significant increase in GMT for each antigen was observed in both groups, this being higher in the healthy group for HK ($p = 0.004$). The percentage of responders was lower in rheumatoid patients than healthy controls (significant for HK). The percentage of responders was not affected by prednisone or any DMARD, including methotrexate, infliximab, and etanercept. Indices of disease activity remained unchanged.
Conclusions: Influenza virus vaccine generated a good humoral response in rheumatoid patients, although lower than in healthy controls. The response was not affected by the use of prednisone or DMARDs.

Infection is one of the leading causes of morbidity and mortality in patients with rheumatoid arthritis.[1 2] This may be because of inherently altered activity of the immune system or because of the deleterious effect of immunosuppressive drugs such as corticosteroids, methotrexate, tumour necrosis factor α (TNFα) blockers, and others.[3] Despite this well established propensity to infection, there is some reluctance to vaccinate patients with rheumatoid arthritis. This reluctance is based on sporadic case reports on the onset or exacerbation of the disease following vaccination with influenza, tetanus, hepatitis, and other vaccines.[4 5] However, the eventual capacity of influenza vaccination to induce a significant clinical flare of rheumatoid arthritis is still debated. One study reported post vaccination flare in six of 17 patients[6] while no flares were reported in other studies.[7–9]

In addition to these concerns over the safety of the influenza vaccine in rheumatoid arthritis, there is uncertainty about the immunogenicity of vaccines in immunocompromised patients such as rheumatoid patients. Likewise, the effect of commonly used drugs in rheumatoid arthritis, such as methotrexate and TNFα blockers, on the immunogenicity of the vaccine has not been well established.

We report a study aiming to assess the safety and immunogenicity of vaccination against influenza in rheumatoid arthritis, and investigating the effect of disease modifying antirheumatic drugs (DMARDs), including TNFα blockers, on the immunogenicity of the vaccine.

METHODS

Subjects

Eighty two consecutive outpatients routinely treated at departments of rheumatology who fulfilled the American College of Rheumatology criteria for rheumatoid arthritis[10] and 30 healthy personnel matched for age, sex, and institution participated in the study. The patients were required to be on stable drug treatment for the three months preceding the vaccination. All subjects were vaccinated with 0.5 ml of split virion inactivated vaccine (Vaxigrip, Promedico) containing a 15 μg haemagglutinin (HA) dose of B/Hong Kong/330/2001 (HK), A/Panama/2007/99 (PAN), and A/New Caledonian/20/ 99 (NC).

Exclusion criteria were pregnancy, a history of past vaccination allergy, a known allergy to egg products, hyposplenism, and active rheumatoid arthritis necessitating a recent change in the drug regimen.

Clinical assessment

Before vaccination, a complete history was obtained, a physical examination was done, and the subject's therapeutic drug use was recorded. Clinical assessment at the day of vaccination and six weeks later included the following: duration of morning stiffness (in minutes); evaluation of daytime and nocturnal pain using a visual analogue scale (VAS) in which 10 represented an extreme pain and 0 no pain; Health Assessment Questionnaire score (HAQ); and count of the number of tender and swollen joints (28 joint count).

Appropriate informed consent was obtained from all patients, and the clinical research was conducted in

Abbreviations: DMARD, disease modifying antirheumatic drug; GMT, geometric mean titre; HA, haemagglutinin; HAQ, Health Assessment Questionnaire; HIT, haemagglutination inhibition test; NA, neuraminidase; RF, rheumatoid factor; TNFα, tumour necrosis factor α

Table 1 Clinical and demographic characteristics of rheumatoid arthritis patients and control subjects

	RA (n = 82)	Controls (n = 30)
Age (years) (mean (SD))	59 (12)	53 (7)
Sex (F;M, (n (%))	63 (77%); 19 (23%)	21 (70%); 9 (30%)
Disease duration (years) (mean)	14	0

F, female; M, male; RA, rheumatoid arthritis.

Table 2 Drugs used by rheumatoid arthritis patients at the time of vaccination

Drug (mean (SD) dose)	RA patients (n = 82)
Methotrexate (12 (4.6) mg/week)	56
Prednisone (8.0 (3.7) mg/day)	48
Hydroxychloroquine	25
Infliximab	22
Etanercept	5
NSAIDs	33
Sulfasalazine	7
Gold	4
Minocycline	8
Leflunomide	2
Combination therapy*	47

*At least two DMARDs.
NSAID, non-steroidal anti-inflammatory drug; RA, rheumatoid arthritis.

accordance with guidelines for human experimentation specified by the Tel Aviv Sourasky Medical Centre. The study was approved by the ethics committee of the Medical Centre.

Laboratory assessment of disease activity was made using erythrocyte sedimentation rate (ESR), C reactive protein, and IgM rheumatoid factor (RF) by enzyme linked immunosorbent assay before and after vaccination.

Haemagglutination inhibition test

Influenza virus has two important surface glycoproteins—the haemagglutinin (HA) and the neuraminidase (NA). Antigenic classification and subtyping of influenza viruses is based on these two glycoproteins. HA plays a key role in virus cell entry by binding to cell surface receptors, which are found also on red blood cells of certain species. Binding to red cells results in haemagglutination, which can be observed as a carpet of agglutinated red cells at the bottom of a tube or microtitre well. In the haemagglutination inhibition test (HIT), antibody directed against the viral haemagglutinins block the virus from binding to the blood cells and thus inhibits the haemagglutination reaction.

The pre- and postimmunisation HI antibodies were tested at the Central Virology Laboratory of the Israeli Ministry of Health using the HIT according to a standard WHO procedure.[11] Sera were separated, code labelled, and stored at −20°C until tested. Sera were treated with receptor destroying enzyme cholera filtrate to remove non-specific inhibitors, and with turkey red blood cells to remove non-specific agglutinins. The treated sera were tested by HIT against the following antigens: B/Hong Kong/330/2001 (HK), A/Panama/2007/99 (PAN), and A/New Caledonian/20/99 (NC). The working dilution (test dose) of each antigen contained four haemagglutinin units in 25 μl of antigen. Test doses were diluted in phosphate buffered saline (PBS) and added to serial dilution of antiserum. The haemagglutinin inhibition titre was determined as the highest dilution of serum that completely inhibits haemagglutination of red blood cells.

The titre of an antiserum not showing any inhibition was recorded as <10. Response was defined as either a fourfold or more rise in titre, or a rise from a non-protective baseline level of $<1/40$ to $\geqslant 1/40$ in HI antibodies six weeks after vaccination.[12 13] Geometric mean titres of antibody were calculated to assess the immunity of the whole group.

Outcomes of the study

The primary outcome was the percentage of patients in rheumatoid arthritis and control groups showing a humoral response to each of the three serotypes. Secondary outcomes included the effect of current DMARDs on the humoral response and the safety of the vaccine.

Statistical methods

Associations between the response to vaccination and patient group and drug use were examined using the χ^2 test.

Patients with positive reactions to vaccination were compared with those who did not react with respect to the number of drugs at baseline, change in the number of drugs, and change in disease indices such as the number of tender and swollen joints, morning stiffness, pain intensity, HAQ, ESR, and C reactive protein using the Mann–Whitney U test. Multiple regression analysis was used to assess the importance of the different variables relative to the humoral response. Continuous variables were compared between groups using *t* tests.

Change in drug use was evaluated by the McNemar test, in number of drugs by the Wilcoxon test, and in drug dosage by paired *t* tests.

Statistical analysis was carried out using the SAS system for Windows, release 8.02.

RESULTS

Characteristics of patients and control subjects

The two groups were statistically similar in terms of age and sex. All groups were predominantly female (table 1), with a mean age of 59 years for rheumatoid arthritis and 53 years for controls. At the time of vaccination, all rheumatoid patients were being treated with at least one DMARD. Fifty six of 82 patients were being treated with methotrexate at a

Table 3 Effects of vaccination on disease activity in rheumatoid arthritis patients

	Before vaccination	After vaccination	p Value
Tender joints (n)	10.3 (12.3)	9.1 (12.1)	0.12
Swollen joints (n)	2.2 (3.7)	1.5 (5.1)	0.1
Morning stiffness (min)	34.7 (55.9)	29.3 (49.3)	0.08
Day and night pain (VAS)	4.8 (3.1)	4.8 (3.4)	0.41
Daytime activity (HAQ)	1.28 (0.85)	1.26 (0.88)	0.49
ESR (mm/h)	28.7 (17.9)	31.8 (22.2)	0.13
C reactive protein (mg/l)	16.05 (17.60)	18.23 (26.60)	0.15
Rheumatoid factor	236.9 (531.2)	487.5 (281.7)	0.10

Values are mean (SD).
HAQ, Health Assessment Questionnaire; VAS, visual analogue score.

Table 4 Geometric mean titres of HI antibodies (μg/ml) against influenza antigens in rheumatoid patients and controls before and six weeks after vaccination

	RA		Control	
Vaccine	Week 0	Week 6	Week 0	Week 6
B/Hong Kong/330/2001 (HK)	4 (1.4)	5.3 (1.2)	3.83 (1.5)	6.1 (0.9)*
A/Panama/2007/99 (PAN)	4.03 (1.4)	5.33 (1.5)	4.4 (1.4)	5.8 (1.2)
A/New Caledonia/20/99 (NC)	3.4 (1.4)	4.7 (1.4)	2.9 (1.0)	5.1 (1.3)

*$p<0.05$.
HI, haemagglutination inhibition; RA, rheumatoid arthritis.

mean dose of 12 mg/week, 48 were on prednisone at a mean dose of 8 mg/d, 22 were receiving infliximab, and five were on etanercept. These drugs had been given for at least three months by the time of vaccination (table 2).

Effect of vaccination against influenza on disease activity

Vaccination against influenza was not associated with a significant worsening of any clinical or laboratory index of disease activity (table 3).

A small number of subjects reported mild adverse events following vaccination: six rheumatoid patients and four controls developed symptoms of mild upper respiratory tract infection within two weeks following vaccination, one rheumatoid patient had transient pain in the atlanto-occipital joint, and one control complained of mild soreness at the site of injection. Thus vaccination against influenza appeared to be safe and well tolerated in patients with rheumatoid arthritis.

Immunogenicity of influenza vaccine

Prevaccination HI antibody levels, as a result of previous infection or vaccination, did not differ significantly in rheumatoid patients and controls. Six weeks after vaccination, both rheumatoid patients and controls had significant increases in their geometric mean titres of HI antibody against each of the antigens tested (Hong Kong, Panama, and New Caledonian), suggesting a good humoral response of the whole group. For the Hong Kong antigen, the rise was significantly greater in the healthy group ($p = 0.004$) (table 4).

Table 5 Number (%) of responders: rheumatoid arthritis patients and Controls

Vaccine	RA	Controls
B/Hong Kong/330/2001 (HK)	51(67)	26(87)*
A/Panama/2007/99(PAN)	43(53)	16(54)
A/New Caledonia/20/99 (NC)	43(53)	20(68)

*$p<0.05$.
RA, rheumatoid arthritis.

Individual responses of rheumatoid patients and controls to vaccination against influenza

Although as a group, both the rheumatoid patients and the controls responded to vaccination, the vaccine did not appear to be uniformly immunogenic in all patients. Levels of HI antibodies <1/40 are considered to be non-protective. A satisfactory humoral response was defined as a fourfold rise or more in HI antibodies six weeks after vaccination in patients with baseline HI antibody levels above 1/40, or if a rise to HI levels of ⩾1/40 was observed in patients with non-protective baseline levels of <1/40. Using this definition, for HK, the proportion of responders in the rheumatoid arthritis group was significantly lower than in controls (67% *v* 87%; $p = 0.05$). The proportion of responders was similar for PAN (53% *v* 54%) and NC (53% *v* 68%) (table 5). Response to more than one antigen was obtained in 70% of rheumatoid patients *v* 82% of controls.

Predictors of immunogenicity

We attempted to identify clinical or laboratory indices which might predict a poor response to the vaccine. We could not find any association between age, sex, disease duration, swollen and tender joint counts, duration of morning stiffness, level of pain, HAQ, ESR, or C reactive protein and the humoral response. Multivariate regression analysis did not identify any predictor of immunogenicity.

The use of drugs did not affect the humoral response. Within the rheumatoid group, the proportion of responders was similar for each of the antigens tested, independently of treatment with prednisone, methotrexate, infliximab, etanercept, or other DMARDs, as well as combination therapies (table 6).

DISCUSSION

We have shown that vaccination against influenza was safe and generated a good humoral response in rheumatoid patients, although lower than in healthy controls for one of the antigen tested (HK). The humoral response was not affected by different clinical and demographic characteristics of rheumatoid arthritis, or by the use of commonly administered DMARDs, including methotrexate and infliximab.

Vaccination against influenza is the primary strategy to reduce the mortality and morbidity associated with influenza.

Table 6 The effect of infliximab, methotrexate, hydroxychloroquine, and prednisone on the proportion (%) of responders to HK, PAN, and NC antigens

Vaccine	Infliximab treated/ not treated	Methotrexate treated/ not treated	Hydroxychloroquine treated/not treated	Prednisone treated/ not treated
B/HK	77/65	68/61	70/61	6/67
A/PAN	55/42	54/52	44/71*	50/53
A/NC	56/44	54/52	55/50	60/47

*$p = 0.04$.
A/NC, A/New Caledonian/20/99; A/PAN, A/Panama/2007/99; B/HK, B/Hong Kong/330/2001.

The vaccine is primarily recommended for persons at increased risk of severe influenza such as immunocompromised subjects (for example, patients with rheumatoid arthritis).[14] Despite this recommendation, the uptake of vaccination in rheumatoid patients is suboptimal, mostly because the patients have not been offered the vaccine.[15] Reduced compliance with vaccination against influenza is related to concerns about its safety and doubts over its immunogenicity. Our present results verify the safety of influenza in rheumatoid arthritis, confirming our own and other previous studies in rheumatoid arthritis and systemic lupus erythematosus, where patients did not experience significant clinical flares following vaccination.[7–9]

The immunogenicity of vaccines in rheumatoid patients has been a matter of controversy. Vaccination against *Streptococcus pneumoniae* has previously been shown by our group to induce an adequate humoral response in a group of rheumatoid patients, although this was lower than in controls.[16] Immunosuppressive drugs did not seem to affect the humoral response, with the exception of infliximab which was shown to reduce it.[17] Mease *et al* could not show a deleterious effect of etanercept on the humoral response to pneumococcal vaccine in a group of patients with psoriatic arthritis, while methotrexate caused a reduced response.[18] Similar results on the detrimental effect of methotrexate were suggested by O'Dell *et al* in a group of rheumatoid patients.[19]

Concerning influenza, Chalmers could not demonstrate any correlation between the use and dose of prednisone or gold on the immunogenicity of the influenza vaccine in a cohort of rheumatoid patients.[9] In children and adults with asthma, the immune response to inactivated influenza vaccine was not found to be adversely affected by corticosteroid therapy,[20] while ciclosporine has been related to a reduced immune response to influenza vaccination in lung transplant patients.[21] Our present study has shown that neither the use of methotrexate nor of infliximab affected the humoral response.

In conclusion, this study has shown that in a ''real life'' cohort of rheumatoid patients, immunisation against influenza did not modify the clinical picture of rheumatoid arthritis. In addition, our results indicate that long term immunosuppressive therapy at conventional doses, including TNFα blockers, did not adversely affect the humoral response to the vaccine. No differential effect of the DMARDs on the humoral response could be demonstrated. We are aware of the limitations of this study, which included a relatively small number of patients and controls, and this may have influenced the results. However, based on our present data, we feel that vaccination against influenza, which is strongly indicated in rheumatoid arthritis, can be recommended in patients with this disease.

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