Concise Report

Influenza vaccination as model for testing immune modulation induced by anti-TNF and methotrexate therapy in rheumatoid arthritis patients

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Objectives. To compare serological response to influenza vaccine in patients with long-standing rheumatoid arthritis (RA) treated with tumour necrosis factor (TNF) blockers and/or methotrexate (MTX) and controls.

Methods. Altogether, 149 patients with RA and 18 healthy subjects were vaccinated. Fifty patients were treated with TNF blockers (etanercept or infliximab) in combination with MTX (TNF blockers + MTX), while 62 patients received TNF blockers alone or with other disease-modifying anti-rheumatic drugs (DMARDs) (TNF blockers without MTX). Thirty-seven patients were treated with MTX without TNF blockers (MTX). Vaccination was performed with trivalent vaccine (Influvax® or Vaxigrip®) both containing 15 μg haemagglutination inhibition (HI) of each of two A strains (H1N1 and H3N2) and one of B strains (B1 or B2). Serum samples were collected prior to and 4–6 weeks after vaccination and titrated against all four strains using HI assay. A positive immune response was defined as ≥4-fold increase compared with pre-vaccination titre levels. A titre ≥40 was considered protective. Pre- and post-vaccination geometric mean titres (GMT) were compared.

Results. Post-vaccination titre levels increased significantly in all groups, also reflected by high frequencies of positive immune responders. A positive immune response to combinations of all strains was significantly better for the MTX group. Individuals with protective levels before vaccination responded less well as a group.

Conclusions. RA patients treated with MTX without TNF blockers had significantly better serological response to influenza vaccination compared with those receiving TNF blockers alone or in combination with MTX and/or other DMARDs. However, the immune response is sufficiently large to warrant influenza vaccination to all RA patients regardless of treatment.

KEY WORDS: Influenza vaccination, Antibody response, TNF blockers, Methotrexate, Rheumatoid arthritis, Prednisolone.

Introduction

Influenza vaccination is considered to be the most effective measure for preventing influenza-related morbidity and mortality. The Swedish National Board of Health and Welfare [1], as well as the CDC Advisory Committee on Immunisation Practice [2], recommend annual influenza vaccination for subjects ≥65 yrs of age and patients suffering from chronic illness at high risk for influenza-related complications. Several studies have demonstrated that vaccination against influenza is effective in reducing hospital admissions and deaths due to pneumonia and influenza in elderly people with chronic lung disease and diabetes [3–9].

Rheumatoid arthritis (RA) patients have increased incidence of infections including those affecting the respiratory tract compared with age-matched subjects without RA [10, 11]. An aspect of immunomodulating treatments is their influence on antibody responses to vaccinations. An adequate immune response to influenza vaccine has been previously demonstrated in some groups of patients with RA [12, 13].

In this study, we aimed to use influenza vaccination as a model for testing immune modulation of anti-tumour necrosis factor (anti-TNF) and methotrexate (MTX) therapy in patients with long-lasting RA and compare the antibody responses with those of healthy subjects. We also wanted to compare this response with that of polysaccharide antigen challenge (pneumococcal vaccination) in the same patients [14].

Material and methods

All RA patients treated with TNF blockers at the Department of Rheumatology were offered pneumococcal and influenza vaccination. RA patients taking MTX without TNF blockers attending the Department of Rheumatology as well as healthy individuals among the medical staff served as control groups. We have previously reported on the effects of simultaneous pneumococcal vaccination in these individuals [14]. Patients were offered pneumococcal vaccination according to the guidelines of the Swedish National Health Board. Ethical approval from local Ethical Review Board at Lund University (LU 513-01) was obtained for vaccination of the medical staff.

Etanercept was given in a dosage of 25 mg/ml subcutaneously twice a week, and infliximab as an intravenous infusion of 3 mg/kg body weight at start after 2 and 6 weeks and thereafter as a rule, every 8 weeks.
For comparison, anti-TNF-treated patients were stratified into two groups according to concomitant use of MTX. One group of 62 patients was treated with TNF blockers as mono-therapy or combined with disease-modifying anti-rheumatic drugs (DMARDs) other than MTX (TNF blockers without MTX). Fifty patients received anti-TNF treatment combined with MTX (TNF blockers + MTX group). MTX treatment without TNF blockers was given to 37 patients (MTX group). Controls consisted of 18 hospital staff persons. For anti-TNF-treated patients, detailed information of disease characteristics and disease activity according to the South Swedish Arthritis Treatment Group protocol (SSATG) [14] was available prior to vaccination and before anti-TNF therapy initiation.

Vaccination procedure and measurement of virus-specific serum antibody titres by haemagglutination inhibition (HI) assays

The study was conducted during the winter seasons 2000/01 and 2001/02. Each participant received a single intramuscular or deep subcutaneous dose of 0.5 ml of commercially available inactivated trivalent influenza vaccines (Influvac® or Variflu®) containing 15 μg HI of two different A strains: A/Moscow/10/99 (H3N2)–like virus and A/New Caledonia/20/99 (H1N1)–like virus. B virus strains differed during two seasons and the vaccine contained either 15 μg HI of B/Yamanashi/116/98 (2000/01) (B1) virus or B/Guangdong/120/00 (2001/02) (B2) virus according to the annual recommendations of the World Health Organisation [15].

Blood samples were collected prior to and 4–6 weeks after the vaccination. HI assays were performed at ViroClinics, Rotterdam, The Netherlands, according to World Health Organisation standard procedure using haemagglutinin antigens representing the strains of virus included in the vaccine [16]. HI was performed using 2-fold dilution of the serum and the results are given as titres, meaning the highest dilution of the serum which achieves complete inhibition of haemagglutination. All sera were titrated simultaneously in duplicate. The analysis was done blinded for clinical data.

Statistical analysis

In order to assess the immunity of the different groups, the geometric mean titres (GMT) of HI antibodies (from log-transformed values) were calculated, and differences between pre-vaccination and post-vaccination levels were compared using paired-samples t-test. Patients with a positive immunization response defined as having pre-vaccination titres <40 and a ≥4-fold titre increase to different strains were identified. Comparisons between groups were done with chi-square test for ordinal variables and Mann–Whitney U-test for nominal variables. Because of differences at baseline, a logistic regression model adjusting for age, gender, disease duration and prednisolone dosage was used. Because of low number, post hoc univariate analysis of variance using Tukey HSD test was performed on the subgroup of subjects with pre-vaccination titre levels <40 and ≥4-fold titre increase but not reaching post-vaccination protective levels of ≥40. This analysis was also used on subjects with protective titre levels prior to vaccination and ≥4-fold titre increase. The B1 and B2 HIA identified both B1 and B2 strains to a similar level (Spearman’s ρ = 0.93). Therefore, only B1 HIA results were used for the B strain analyses. Comparisons were performed for different treatment groups and each strain and also, all strains combined.

Results

Altogether, 149 patients with established RA and 18 healthy volunteers participated in this study. Demographic and clinical characteristics of patients prior to vaccination are previously described in detail [14]. Briefly, 112 patients with ongoing anti-TNF treatment with either etanercept (N = 48) or infliximab (N = 64) and 37 patients treated with MTX without anti-TNF drugs simultaneously received influenza and pneumococcal vaccine. MTX patients were older and disease duration was longer for TNF blockers without MTX patients. There were no differences in MTX dosage and duration of MTX treatment before vaccination. Anti-TNF treatment duration was similar between TNF blockers without MTX and TNF blockers + MTX. Disease activity at vaccination was similar in all patient groups.

Antibody titres

Post-vaccination GMT of HI antibodies increased significantly compared with pre-vaccination levels for all treatment groups and controls and also each strain (Table 1). Controls had high proportions of pre-vaccination protective levels to all strains making them unsuitable to be included in the statistical analyses.

Numbers of individuals with protective titre levels (≥40) before and after vaccination are shown in Table 1.

A positive immune response to combinations of all strains (H1N1 + H3N2 + B1) was significantly better for the MTX group also after adjustments in the regression model (Fig. 1).

RA patients with protective levels before vaccination responded less well to vaccination as a group.

The frequency of subjects with pre-vaccination titre levels <40 and ≥4-fold titre increase but not reaching protective levels (i.e. subjects with post-vaccination titre levels of <40) did not differ between the treatment groups. The number of patients with protective titre levels prior to vaccination and ≥4-fold titre increase did not reveal any significant differences for different treatment groups or strains. However, the number of these subjects was low in all subgroups.

Discussion

The major finding of this study is that RA patients treated with MTX without TNF blockers exhibited the best serological responses to influenza vaccination. Patients treated with TNF blockers alone or in combination with MTX and/or other DMARDs had lower number of responders. The differences remained significant after adjusting for differences in age, gender, disease duration and prednisolone dosage using the regression model.

The impact of different therapeutic modalities was analysed for each virus strain and also for the combination of strains. Interestingly, the effect of different treatments on the immune response was strengthened when combination of the strains was analysed. This illustrates that a more comprehensive measuring probably increases the possibilities to find drug effects on immune mechanisms in biologically heterogeneous materials. We found a similar pattern of increased impact of treatment on the immune system after pneumococcal vaccination when looking at combined effects on different antibodies to pneumococcal antigens [14]. However, the impacts of treatments on the immune responses were totally different depending on the antigens studied. Antibody responses to polypeptide antigens were diminished by anti-TNF treatment, whereas the antibody responses to polysaccharide antigens were normal or increased by this treatment.

The mechanisms leading to differences in antibody response between treatment with TNF blockers and MTX are unknown. It is well known that polysaccharide antigens (included in pneumococcal vaccine) induce lower immune response compared with virus protein antigens [14], which is in line with the results in our study. Antibody production following influenza vaccination is T-cell mediated, while responses to polysaccharide antigen are considered to be T-cell independent. Our findings suggest that polysaccharide and polypeptide antigens are processed by
consistent evidence of lower ability in mounting protective immune response following influenza vaccination while on TNF blockers. On the contrary, normal or transiently elevated immunoglobulin levels [19] and development of autoantibodies during treatment with TNF blockers have been described.

Our findings of a positive immune response after influenza vaccination in RA patients while on MTX are consistent with previous reports [12, 17]. In an earlier study on immunogenicity of influenza vaccine it was shown that patients with RA receiving immunosuppressive medication including MTX and regardless of previous immunization status had similar immune responses compared with age-matched healthy controls [12]. In spite of differences in virus strains included in the vaccines, the rate of positive immune response among all three treatment groups, including anti-TNF-treated patient groups in our study, was about the same as that of the patient groups in the study above. One weakness of our study is that the healthy control group was unsuitable due to the presence of a large number of subjects with pre-vaccination antibody titres above the protective level. High pre-vaccination titres in this group probably reflected not only an exposure to influenza antigens due to former natural infections but also a previous influenza vaccination. Unfortunately, information concerning influenza vaccination status before the study was not available. Additionally, it has been shown that antibody titres decline more rapidly in immunosuppressed patients with rheumatological disease [20], which might be the reason why fewer patients in our study had protective pre-vaccination titre levels compared with those of controls.

Patients included in this study simultaneously received pneumococcal vaccination. We previously reported the results regarding immune response following pneumococcal vaccination [14], but HI assays were performed later for logistical reasons. Although the same patients were studied, the results are different in terms of the MTX group. The underlying mechanisms of these differences are unknown. The consequences of simultaneous exposure to antigens involving different immunological pathways are not known, but it may be speculated that such exposure could lead to boosting effects or otherwise changed response in vivo. Since these vaccinations often are administrated simultaneously in clinical practice, further studies are needed to clarify this issue.

The immune responses to vaccination are surrogate markers for protection against the disease and may identify persons at risk of unsatisfactory effect of the vaccine and possibly in need of revaccination. Checking of immunity may be most appropriate in patients treated with TNF blockers. Provided that good immune response reflects the effectiveness of the vaccine, immune response
in this study was sufficiently large as to warrant influenza vaccination to all RA patients regardless of treatment.

Acknowledgements

The authors thank ViroClinics BV, Erasmus MC, Rotterdam, The Netherlands, for performing the HI assays. This study was supported by grants from the Swedish Rheumatism Association, the Swedish Research Council, The Medical Faculty of the University of Lund, Alfred Österlund’s Foundation, The Crafoord Foundation, Greta and Johan Kock’s Foundation, The King Gustaf V Foundation and Lund University Hospital.

The authors have declared no conflicts of interest.

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