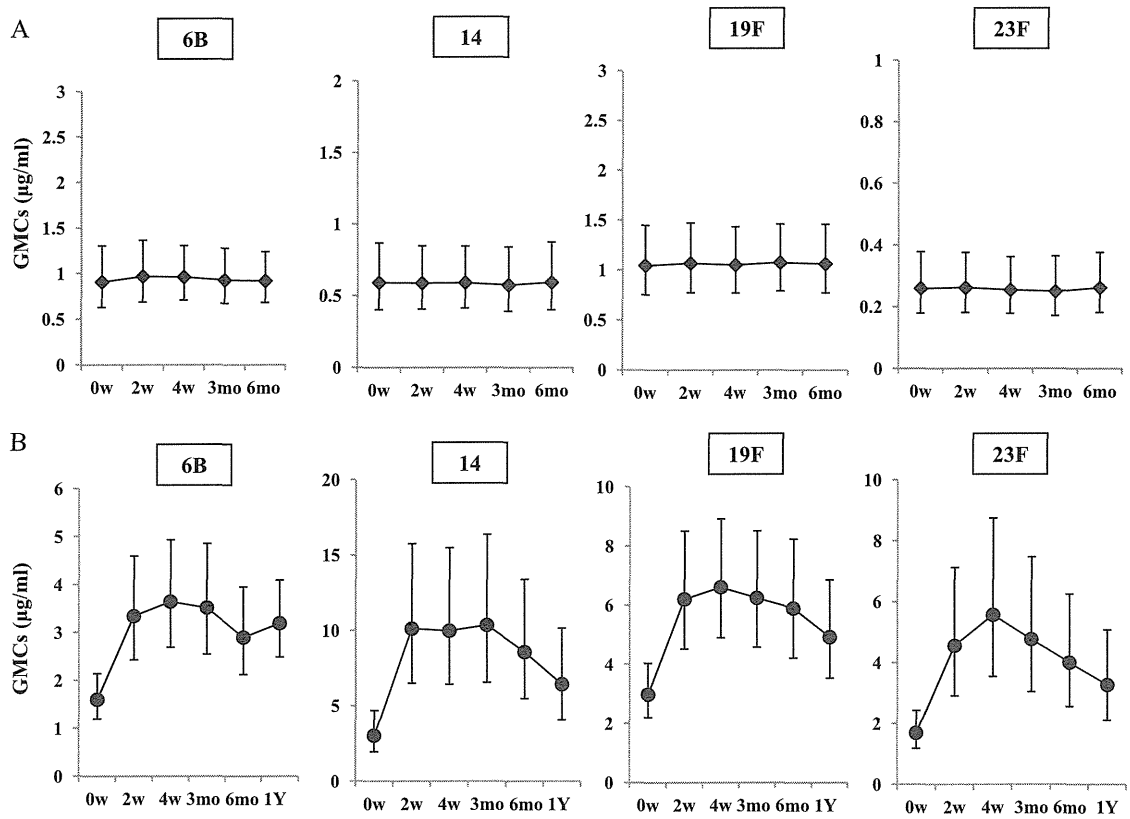

Fig. 1. Serum levels of anti-pneumococcal polysaccharide Abs after PPV injection. Concentrations of IgM (A: $n = 15$) and IgG (B: $n = 55$) Abs against each serotype of pneumococcal capsular polysaccharide in sera were measured at indicated time points after PPV administration. Data are shown as the geometric mean concentrations and 95% confidence intervals. GMCs, geometric mean concentrations; 0w, pre-vaccination; 2w, 2 weeks; 4w, 4 weeks; 3 mo, 3 months; 6 mo, 6 months; 1y, 1 year post-vaccination.

vaccination in the responder group, whereas no such significant increase in IgG concentration was observed in the low responder group, except for serotype 6B [pre-vaccination: 1.33 (95% CI was within 1.10–1.60) *vs.* peak: 2.02 (95% CI was within 1.57–2.59) ($n = 9$, $p < 0.05$)].

3.3. Alteration in the number of NKT cells in the peripheral blood after pneumococcal vaccination

We analyzed the number of NKT cells in the peripheral blood before vaccination and 2 weeks, 4 weeks, 3 months and 6 months after vaccination in 24 individuals, in whom the surface antigens on lymphocytes could be tested. NKT cells were identified as the lymphocytes positively stained with α-GalCer-CD1d tetramer or expressing both CD3 and CD56, and α-GalCer-CD1d tetramer$^+$ lymphocytes were further divided into CD4$^+$CD8$^-$ (CD4$^+$ iNKT), CD4$^-$CD8$^+$ (CD8$^+$ iNKT) and CD4$^-$CD8$^-$ (double negative: DN iNKT) subsets. As shown in Fig. 2, iNKT cell subsets did not show significant elevation in their cell count at any time point after vaccination, although increased iNKT cell counts were observed during the first two weeks in 11 or 12 individuals (data not shown).

3.4. NKT cell counts and serum levels of anti-pneumococcal Ab

In order to address the possible role of NKT cells in the humoral response to the pneumococcal vaccine, we analyzed the relationship between the degree of change in NKT cell counts during the first 2 weeks post-vaccination and the degree of change in serum anti-pneumococcal IgG levels from pre-vaccination to their peak. As shown in Fig. 3, a significant positive correlation was detected between increases in DN iNKT cells and increases in anti-serotype 14 IgG, and there were tendencies toward positive correlations between changes in CD8$^+$ iNKT and DN iNKT cell counts and increases in anti-serotype 19F IgG levels ($p = 0.069$ and 0.067, respectively), and between changes in DN iNKT cell counts and increases in anti-serotype 6B and 23F IgG levels ($p = 0.062$ and 0.082, respectively). By contrast, CD4$^+$ iNKT, CD8$^+$ iNKT and CD3$^+$CD56$^+$ cells showed neither a positive nor a negative correlation with changes in the serum levels of anti-pneumococcal IgG in all of the serotypes except for 19F in CD8$^+$ iNKT and CD3$^+$CD56$^+$ cells.

Finally, we compared changes in DN iNKT cell counts between responders and low responders, because these cells showed a tendency toward a positive correlation with Ab responses to PPV. As shown in Fig. 4, in serotype 19F, the increase in DN iNKT cells was significantly more marked in responders than in low responders. This tendency was also observed in serotypes 6B, 14 and 23F, although it was not statistically significant.

4. Discussion

In the present study, serum levels of anti-pneumococcal IgG increased after pneumococcal vaccination, peaking in the fourth week for serotypes 6B, 19F and 23F and in the third month for serotype 14; in 45–65% of vaccinated subjects, these levels increased more than two-fold. There were also low responders, however, producing smaller quantities of anti-pneumococcal Ab; these constituted 16%, 13%, 13% and 16% of our 55 subjects for serotypes 6B, 14, 19F and 23F, respectively. Of the low responders, 15 showed a low response to one of the four serotypes examined, nine showed a low response to two serotypes, and one showed a low response to three serotypes, indicating that 45% of our 55 subjects were low responders for at least one serotype. Although there is no standardized definite on of a low responder, our results appear

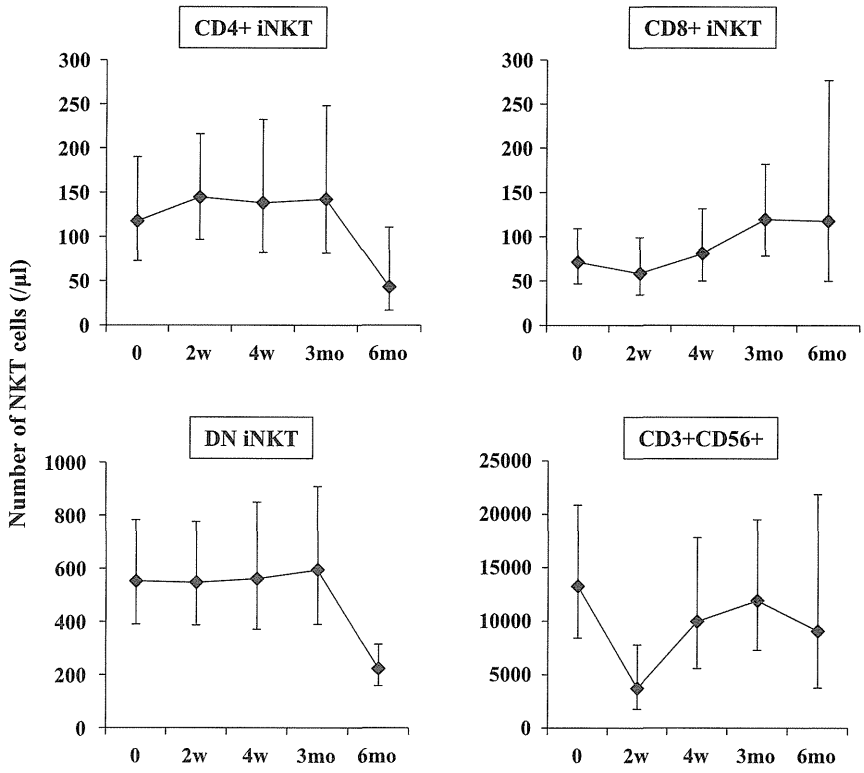

Fig. 2. NKT cells in the peripheral blood after PPV injection. Number of NKT cells in the peripheral blood was examined before PPV administration and 2 weeks, 4 weeks, 3 months and 6 months after PPV administration in 24 individuals. NKT cells were identified as the lymphocytes positively stained with α-GalCer-CD1d tetramer or expressing both CD3 and CD56, and α-GalCer-CD1d tetramer$^+$ lymphocytes were further divided into $CD4^+CD8^-$ ($CD4^+$ iNKT), $CD4^-CD8^+$ ($CD8^+$ iNKT) and $CD4^-CD8^-$ (double negative: DN iNKT) subsets. Data are shown as the geometric means and 95% confidence intervals in each NKT cell subset.

to be in accordance with those of previous investigations, which indicate that 16–31% of vaccinated subjects are low responders, whose anti-pneumococcal Ab levels increase less than two-fold for two among four to seven analyzed serotypes [27–29].

Previous studies have shown NKT cells to be involved in immune responses to TI-2 antigens, as a possible source of the secondary stimulatory signal for B cell activation [25] as well as in protection against pneumococcal infection [24]. These earlier observations suggest that NKT cells may play a certain role in the clinical effects of anti-pneumococcal vaccination. In agreement with this possibility, in the present study, a significant positive correlation was detected between changes in the number of DN iNKT cells, though not of $CD4^+$ iNKT cells, and increases in Ab levels against serotype 14 antigen. Moreover, the increase in DN iNKT cells was more marked in responders than in low responders, and this difference was statistically significant for serotype 19F. However, the positive correlation between DN iNKT cells and Ab levels and the difference in DN iNKT cells between responders and low responders were not significantly detected in other serotypes, although there were such tendencies with lower *p* values. The increase of study subjects would help in making these differences statistically significant. In addition, there is a possibility that the increase of DN iNKT cell number in responders may be due to overall immune activation of these individuals in response to vaccine, rather than selective effect on NKT cells. This may not apply to our case, because there was no tendency of difference between low responders and responders in other NKT cell subsets (data not shown).

$CD4^+$ and DN iNKT cells are major subsets in humans, both of which secrete large amounts of IFN-γ upon stimulation [21]. Yet these subsets differ in their secretion of such Th2 cytokines as IL-4, IL-5 and IL-13, and in their expression of chemokine receptors, integrins and NK receptors [21,30–32]. Galli and co-workers have demonstrated that iNKT cells promote immunoglobulin production by B cells, an activity that is more potent in $CD4^+$ iNKT cells than in DN iNKT cells [33]. The same group has also reported that activated human iNKT cells directly support the proliferation of and immunoglobulin production by naive and memory B cells. All these experiments were conducted *in vitro*, however, and frequent stimulation of iNKT cells during culture has been reported to cause a shift in their cytokine profile toward a Th2-dominant condition [34], raising the possibility that cultured NKT cells are not always equivalent to those in circulation *in vivo*. In the present clinical study of individuals receiving PPV, the relationship between iNKT cells and Ab production does not seem to be identical between $CD4^+$ and DN iNKT cells. Taken together, the data suggest that these subsets play distinct roles in Ab production by B cells after PPV administration. Further investigation is necessary to define the precise mechanism by which this occurs.

On the other hand, only a limited subset of NKT cells expressing NK cell markers, such as CD56 or CD161, is reactive to α-GalCer-loaded CD1d tetramer [31]. Therefore, $CD3^+CD56^+$ NKT cells, described as NKT-like cells, are distinguished from iNKT cells by certain characteristics, including the differences in their cytokine production profiles and their TCR αβ chains [18]. Our results suggest that iNKT cells rather than NKT-like cells may be particularly involved in IgG production caused by pneumococcal capsular polysaccharides, because no correlation was observed between $CD3^+CD56^+$ NKT cell count and Ab response.

To the best of our knowledge, the current study is the first report presenting clinical data that suggests a possible relationship between the activation of iNKT cells and Ab responses after PPV administration. The increase in DN iNKT cell count seems to be particularly correlated with serotype-specific IgG production, suggesting a higher contribution from DN iNKT cells than from other subsets. The population size in this study was limited, and the

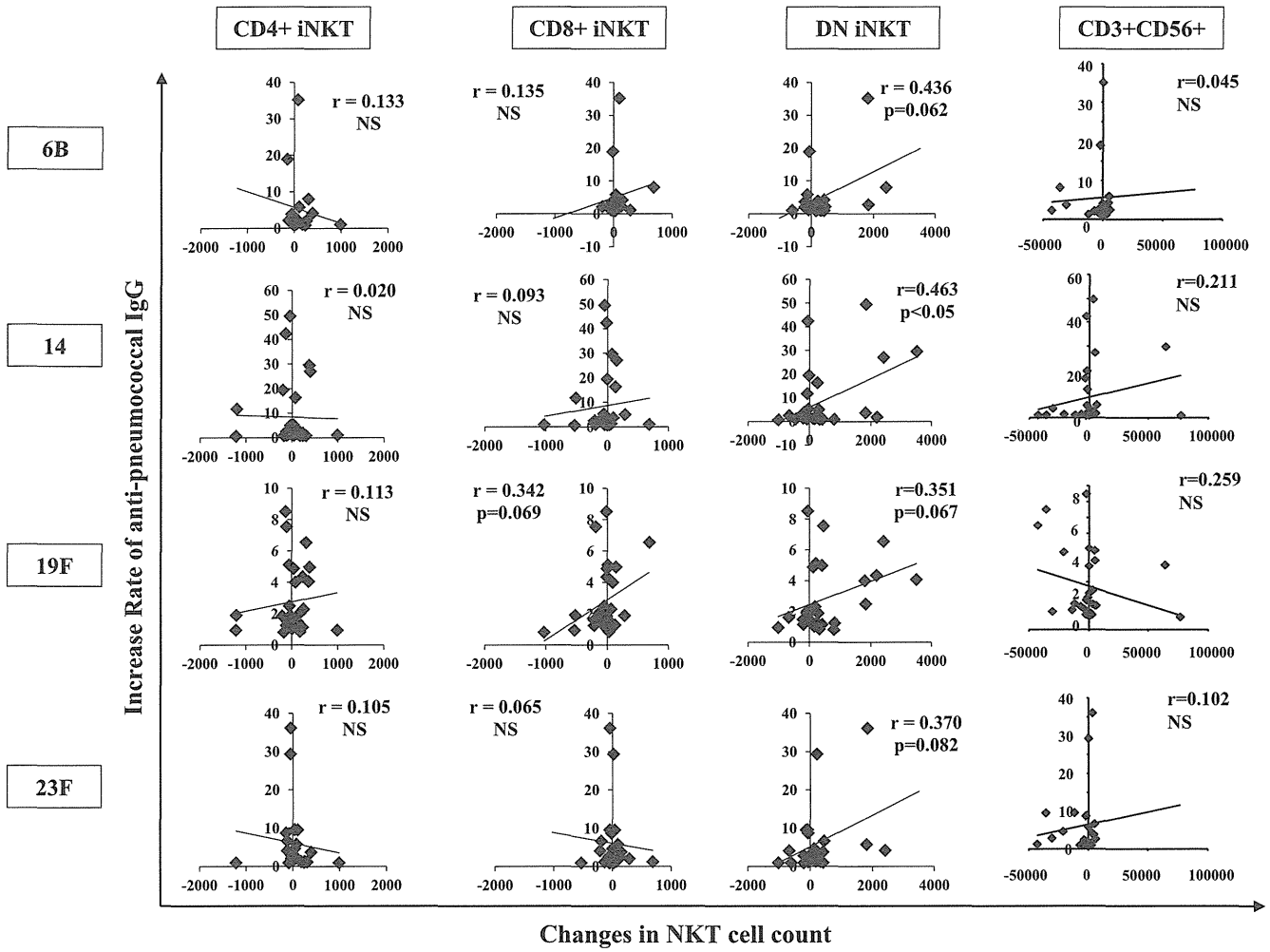

Fig. 3. Relationship between NKT cell counts and anti-pneumococcal IgG. Relationship between changes in NKT cell counts during the first 2 weeks post-vaccination and degree of change in serum anti-pneumococcal IgG levels from pre-vaccination to peak. Each symbol indicates the relationship for one subject. *R* and *P* values and number of subjects in each analysis are shown.

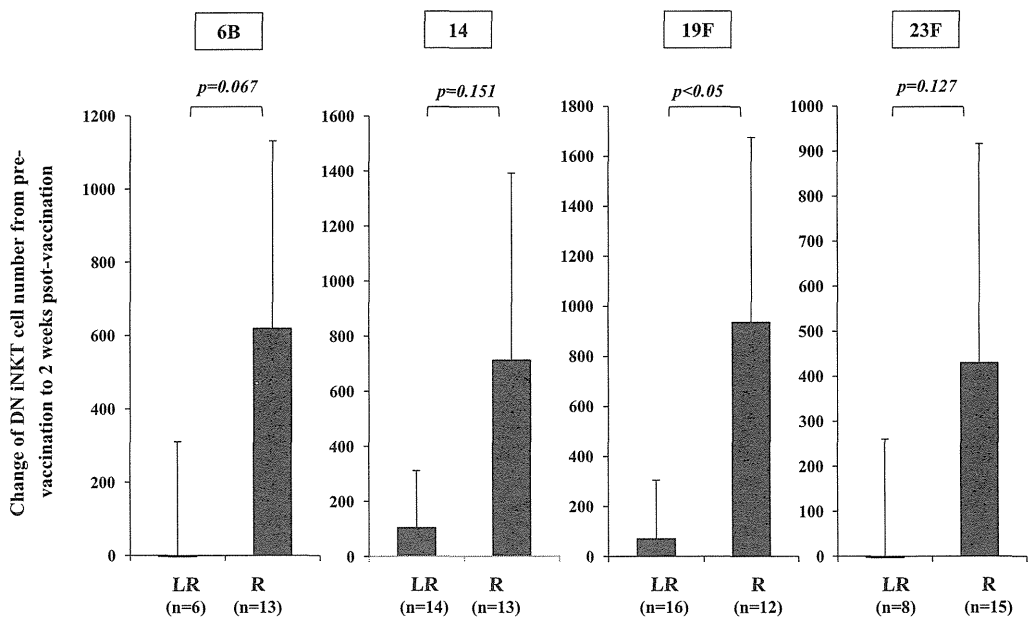

Fig. 4. Changes in DN iNKT cell counts in responders and low responders. Degree of change in DN iNKT cell count during the first 2 weeks after vaccination was compared between responders and low responders for each serotype. Data are expressed as the arithmetic means and 95% confidence intervals of indicated number of subjects. LR, low responders; R, responders.

enrolled subjects were aged (74.4 ± 6.6 years) and had underlying diseases that affected their immune condition. In these respects, there are some limitations in interpreting the results. At present, it remains to be elucidated how iNKT cells are involved in humoral immune responses to pneumococcal capsular polysaccharides in the clinical setting, but further investigations are already under way in our laboratory to define the precise mechanism underlying the relationship between iNKT cells and Ab responses.

Acknowledgments

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CONCISE REPORT

Impact of tocilizumab therapy on antibody response to influenza vaccine in patients with rheumatoid arthritis

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ABSTRACT

Objectives We assessed the influence of tocilizumab (TCZ), a humanised monoclonal anti-interleukin-6 receptor antibody, on antibody response following influenza vaccination in patients with rheumatoid arthritis (RA).

Methods A total of 194 RA patients received inactive trivalent influenza vaccination (A/H1N1, A/H3N2 and B/B1 strains). All patients were classified into the TCZ (n=62), TCZ+methotrexate (MTX) (n=49), MTX (n=65) and RA control (n=18) groups. Antibody titres were measured before and 4–6 weeks after vaccination using the haemagglutination inhibitory assay.

Results For the A/H1N1 and A/H3N2 strains, the TCZ and TCZ+MTX groups achieved fold increases of 9.9–14.5, postvaccination seroprotection rates greater than 70% and seroresponse rates greater than 40%. For the B/B1 strain, seroresponse rates were approximately 30%, but fold increases and seroprotection rates were 5.0–5.4 and greater than 70%, respectively, in these treatment groups. MTX had a negative impact on vaccination efficacy, but adequate responses for protection were nevertheless demonstrated in the MTX group. Neither severe adverse effects nor RA flares were observed.

Conclusions TCZ does not hamper antibody response to influenza vaccine in RA patients. Influenza vaccination is considered effective in protecting RA patients receiving TCZ therapy with or without MTX.

INTRODUCTION

Influenza vaccination is the most effective method for preventing influenza virus infection and its potentially severe complications. Patients with rheumatoid arthritis (RA) are at an increased risk for infectious diseases due to the nature of RA and its treatment with immunosuppressive agents;[1] therefore, this patient population is a potential candidate for influenza vaccination. Treatment with antitumour necrosis factor α (anti-TNFα) agents may impair antibody response to influenza vaccination in patients with RA and other rheumatic diseases, but the response is large enough to warrant influenza vaccination for such patients.[2–8]

Tocilizumab (TCZ), a humanised monoclonal interleukin-6 (IL-6) receptor antibody, is effective in the treatment of patients with moderate to severe RA who have shown inadequate responses to methotrexate (MTX) and one or more anti-TNFα agents.[9] Our concern is the impact of TCZ on protective antibody response to influenza vaccination because IL-6 was originally identified as a factor that plays an essential role in terminal differentiation of B cells into antibody producing plasma cells.[10] Data regarding the efficacy and safety of influenza vaccination are lacking in RA patients receiving TCZ. Only one attempt at evaluating the efficacy of influenza vaccine has so far been made in a small number of paediatric patients receiving TCZ therapy for systemic onset juvenile idiopathic arthritis.[11]

To address this issue, we determined antibody response to trivalent inactivated influenza vaccine in RA patients being treated with TCZ, MTX or both agents, and compared parameters for efficacy of vaccination among these groups.

METHODS

Patients

RA patients aged 18 or older who had been receiving TCZ (an intravenous infusion of 8 mg/kg every 4 weeks) for at least 4 weeks and/or MTX (6–18 mg per week) for 12 weeks or more at our rheumatology outpatient clinics were invited to participate in this open-label study. RA patients who had been receiving bucillamine or salazosulphapyridine were also included as RA controls. All participants fulfilled the 1987 American College of Rheumatology criteria for diagnosis of RA. Exclusion criteria were current use of 10 mg/day or more of prednisolone, current use of tacrolimus or leflunomide, a recent history (within 3 months) of influenza infection, and a recent history (within 6 months) of influenza vaccination.

Vaccine

We used commercially available inactivated trivalent influenza vaccine (Biken HA, Mitsubishi Tanabe Pharm Corporation, Osaka, Japan) containing 30 μg of purified haemagglutinin of each of the following: A/California/7/2009 (H1N1)-like strain (A/H1N1 strain), A/Victoria/210/2009 (H3N2)-like strain (A/H3N2 strain) and B/Brisbane/60/2008-like strain (B/B1 strain). Patients received a single dose of vaccine (0.5 ml) subcutaneously from October 2011 until January 2012. For RA patients receiving TCZ, the vaccination was done on the same day as TCZ infusion.

HI tests

Sera were collected immediately before and 4–6 weeks after vaccination. For the detection of

influenza antibodies, haemagglutination inhibition (HI) tests were performed in duplicate at SRL (Tachikawa, Tokyo, Japan), according to WHO standard procedure using haemagglutinin antigens representing all three strains that were included in the vaccine. Geometric mean titres (GMTs) of HI antibodies before and after vaccination, and fold increases relative to prevaccination titres (geometric means of postvaccination to prevaccination antibody titre ratios) were determined. GMTs were calculated from log-transformed values of HI antibody titres. For statistical analysis, a titre of 5 was arbitrarily assigned to sera with undetectable titres of <10. Seroprotection was defined as antibody titres of ≥40. Seroconversion was defined as postvaccination antibody titres of ≥40 in patients whose prevaccination titres were <10. Seroresponse was defined as seroconversion or fold increases in antibody titres of ≥4 in patients whose prevaccination titres were ≥10.

Monitoring adverse effects and disease activity

Systemic adverse events and worsening of RA occurring 4–6 weeks after vaccination were recorded. Systemic adverse effects included fever, tiredness, sweating, myalgia, chills, headache, arthralgia, diarrhoea and common cold-like symptoms. RA activity was monitored using a disease activity score for 28 joints and a clinical disease activity index.

Statistical analysis

In univariate analyses for categorical variables, differences between treatment groups were analysed using the χ^2 test or Fisher's exact probability test. Continuous variables were assessed by the Mann–Whitney U test for comparisons of non-parametric data between the two treatment groups, and analysis of variance with post hoc Tukey's honestly significant difference test for comparisons of parametric data between the four treatment groups. A paired-sample t test was used to compare differences in GMTs between prevaccination and postvaccination.

For all tests, probability values (p values) <0.05 were considered to indicate statistical significance. All calculations were performed using Excel Statistical Analysis 2008 (SSRI Co., Tokyo, Japan) or PASW Statistics V.18 (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Clinical and demographic characteristics of participants

A total of 194 RA patients were classified into four groups according to their ongoing anti-RA therapy. One group of 62 patients was treated with TCZ as a monotherapy (TCZ group); 65 patients were treated with MTX alone (MTX group); 49 patients received a combination therapy consisting of TCZ and MTX (TCZ+MTX group); and 18 patients received bucillamine or salazosulphapyridine monotherapy (RA control group). Clinical and demographic characteristics are shown in table 1.

Antibody titres

After vaccination, GMTs for all strains were increased significantly. Regarding the A/H3N2 strain, a significantly higher post-GMT was obtained in the TCZ group compared with that in the MTX group (p=0.009) (table 2). The TCZ group also showed a higher post-GMT for the B/B1 strain than did the MTX group and the RA control group (p=0.044 and p=0.031,

Table 1 Clinical and demographic characteristics of RA patients prior to influenza vaccination

	MTX group (n=65)	TCZ+MTX group (n=49)	TCZ group (n=62)	RA control (n=18)	p Values between treatment groups
Male/female	11/54	5/44	11/51	3/15	NS
Age, years, mean (95% CI)	67 (65.0 to 68.9)	62.9 (59.8 to 65.9)	65.2 (61.6 to 68.8)	67.3 (62.3 to 72.4)	NS
Prior influenza vaccination, number of patients (%)	47 (72.3)	36 (73.5)	50 (80.6)	12 (66.7)	NS
RA duration, years, mean (95% CI)	9.8 (7.7 to 11.9)	7.5 (5.8 to 9.2)	14.6 (11.5 to 17.7)	11.1 (4.8 to 17.4)	0.029 (M vs T) 0.001 (T/M vs T)
MTX dose, mg/week, median (25th, 75th percentiles)	8 (6, 8)	8 (6, 8)	–	–	NS
MTX duration, months, median (25th, 75th percentiles)	58 (17, 78)	54 (29, 89)	–	–	NS
TCZ duration, weeks, median (25th, 75th percentiles)	–	68 (24, 104)	64 (21, 107)	–	NS
Use of prednisolone, number of patients (%)	13 (20)	12 (24.5)	22 (35.5)	1 (5.6)	0.016 (T vs C)
Prednisolone dose, mg/day, mean (95% CI)	0.87 (0.4 to 1.34)	0.90 (0.33 to 1.47)	1.02 (0.54 to 1.49)	–	NS
Positive RF, number of patients (%)	38 (58.5)	42 (85.7)	46 (74.2)	7 (38.9)	0.002 (M vs T/M) 0.0001 (T/M vs C) 0.005 (T vs C)
Positive anti-CCP Abs, number of patients (%)	46 (70.8)	43 (87.8)	56 (90.3)	6 (33.3)	0.030 (M vs T/M) 0.006 (M vs T) 0.004 (M vs C) <0.0001 (T/M vs C) <0.0001 (T vs C)
CDAI (25th, 75th percentiles)	5.3 (3.7–7.8)	6.2 (4.5–7.8)	9.5 (7.9–11.1)	8.2 (4.8–11.5)	0.001 (M vs T) 0.027 (T/M vs T)
Lymphocytes, /μl, mean (95% CI)	1368 (1237 to 1500)	1395 (1255 to 1535)	1622 (1500 to 1744)	1478 (1098 to 1857)	0.038 (M vs T)

Data were obtained immediately before influenza vaccination. Prior influenza vaccination represents that administered last season (2010/2011). p Values between treatment groups were determined by the Mann–Whitney U test, post hoc ANOVA using Tukey's HSD test, the χ^2 test or Fisher's exact probability test.
ANOVA, analysis of variance; anti-CCP Abs, anti-cyclic citrullinated peptide antibodies; C, RA control group; CDAI, clinical disease activity index; HSD, honestly significant difference; M, MTX group; MTX, methotrexate; NS, not significant; RA, rheumatoid arthritis; RF, rheumatoid factor; T, TCZ group; T/M, TCZ+MTX group; TCZ, tocilizumab.

Table 2 GMTs and fold increases of HI antibodies for three influenza strains in the RA treatment groups prior to and after influenza vaccination

	MTX group (n=65)	TCZ+MTX group (n=49)	TCZ group (n=62)	RA control group (n=18)	p Values between treatment groups
GMTs					
A/H1N1					
Before	31.7 (16.1–47.2)	59.5 (19.9–99.1)	62.0 (25.4–125.4)	15.3 (8.3–22.3)	NS
After	120.5 (75.3–165.6)*	162.1 (86–238.2)**	211.7 (142–281.4)*	169.4 (11.5–327.4)*	NS
A/H3N2					
Before	37.9 (15.5–60.4)	42.6 (25.2–59.9)	55.2 (31.8–78.7)	36.9 (11.9–62.0)	NS
After	120.2 (80.2–160.2)*	140.7 (82–199.4)***	237.8 (169.1–306.5)*	93.9 (54.1–133.6)**	0.009 (M vs T)
B/B1					
Before	45.5 (30.2–60.7)	43.2 (29.8–56.5)	72.1 (53.3–90.9)	23.9 (12.2–35.6)	0.017 (T vs C)
After	103.1 (74.9–131.3)*	105.1 (69.4–140.8)*	161.8 (123.8–144)*	68.9 (45.7–92.1)*	0.044 (M vs T) 0.031 (T vs C)
Fold increase					
A/H1N1	12.6 (5.8–19.5)	14.5 (7.2–21.9)	12.0 (9.8–17.7)	11.2 (3.0–19.4)	NS
A/H3N2	9.6 (5–14.2)	9.9 (5.2–14.6)	12.0 (6.6–17.3)	5.3 (2.7–8.0)	NS
B/B1	3.5 (2.5–4.4)	5.4 (2.4–8.3)	5.0 (3.3–5.7)	5.8 (3.1–8.4)	NS

Data are expressed as the mean (95% CIs). Differences between prevaccination and postvaccination GMTs were assessed using the paired-sample t test. Comparisons between the four treatment groups were performed by post hoc ANOVA using Tukey's HSD test.
*p<0.0001, **p=0.009 and ***p=0.001 based on comparisons with prevaccination titres.
ANOVA, analysis of variance; C, RA control group; GMT, geometric mean titre; HI, haemagglutination inhibition; HSD, honestly significant difference; M, MTX group; MTX, methotrexate; NS, not significant; RA, rheumatoid arthritis; T, TCZ group; TCZ, tocilizumab.

respectively). Fold increases in GMTs for the three strains were ≥3.5-fold in all treatment groups. These groups achieved similar levels of fold increases for each strain and there were no statistically significant differences.

Seroprotection, seroresponse and seroconversion rates

After vaccination, seroprotection rates for the three influenza strains were increased significantly in all treatment groups (figure 1A). The TCZ and TCZ+MTX groups achieved postvaccination protection rates of >70% for all the influenza strains. Regarding the A/H3N2 and B/B1 strains, postvaccination seroprotection rates were significantly higher in the TCZ group compared with those in the other three treatment groups (for A/H3N2, p<0.0005 vs MTX, p=0.001 vs TCZ + MTX p=0.006 vs RA control; for B/B1, p=0.007 vs MTX, p=0.023 vs TCZ +MTX, p=0.007 vs RA control). Seroprotection rates for the A/H1N1 strain were similar among all the groups tested.

For the A/H1N1 and A/H3N2 strains, seroresponse rates were >40% in the MTX, TCZ and TCZ+MTX groups, while the rates for the B/B1 strain in these groups were approximately 30% (figure 1B). The seroresponse rate for the A/H3N2 strain was significantly higher in the TCZ group compared with that in the MTX group (p=0.04). Seroconversion rates for the three influenza strains were greater than 40% in all treatment groups (figure 1C). The TCZ group showed a significantly higher seroconversion rate for the A/H3N2 strain than did the MTX group (p=0.032).

Predictive factors for seroresponse to influenza vaccination

In multivariate logistic regression analysis, TCZ use was not identified as the predictive factor for seroresponse to influenza vaccination (see online supplementary table S1). For the A/H3N2 strain, the negative association of current MTX use with seroresponse was confirmed (p=0.04). Prior influenza vaccination was negatively associated with seroresponse for all the three strains (for A/H1N1, p=0.006; for A/H3N2, p=0.01; for B/B1, p<0.0001). This may have reflected ceiling effects; that is, higher prevaccination protection rates may, at least in part, have influenced the observed seroresponse rates.

Vaccination safety

Neither systemic adverse effects nor exacerbation of RA was experienced by any patients during a follow-up period of 4–6 weeks after vaccination.

DISCUSSION

Antibody response to the A/H1N1 and A/H3N2 strains in the TCZ and TCZ+MTX groups met all three requirements of the European Medicines Agency (EMA) guidance for assessment of influenza vaccines specified by the Committee for Proprietary Medical Products (CPMP).[12] For the B/B1 strain, these treatment groups met two of the EMA/CPMP criteria. The MTX group fulfilled two of the EMA/CPMP criteria for all strains. Multivariate logistic analysis confirmed that TCZ use is not a predictive factor for inadequate antibody response for any influenza strain.

IL-6 works as a B cell differentiation factor, which induces activated B cells to produce immunoglobulin.[10] The blockage of IL-6 activity following TCZ therapy, therefore, would be expected to reduce humoral immune response to influenza vaccination. Kopf *et al*[13] indicated that T cell-dependent antibody response against virus infection is impaired in IL-6-deficient mice. Unlike anti-infliximab or antiadalimumab antibodies, anti-TCZ antibodies rarely developed in RA patients receiving 8 mg/kg of TCZ, even as monotherapy.[14 15] Nevertheless, the present study has clearly indicated that RA patients receiving TCZ therapy can be effectively and safely immunised with influenza vaccine. One possible explanation may be that, unlike rituximab, TCZ is not a B cell-targeting antibody that can induce B cell depletion. Given that a variety of cytokines are released from activated helper T cells, antibody production may not depend simply on IL-6. Costelloe *et al*[16] showed that IL-6 is not required for antigen (influenza virus)-specific antibody responses by non-fractionated tonsillar mononuclear cells or by T cell-depleted B cells in the presence of IL-2. Another explanation may be that IL-6 signalling is not inhibited completely in lymphoid tissue, locations in which vaccination-mediated immune response is initiated, even when maximum saturation of soluble IL-6 receptors in the circulation is achieved with

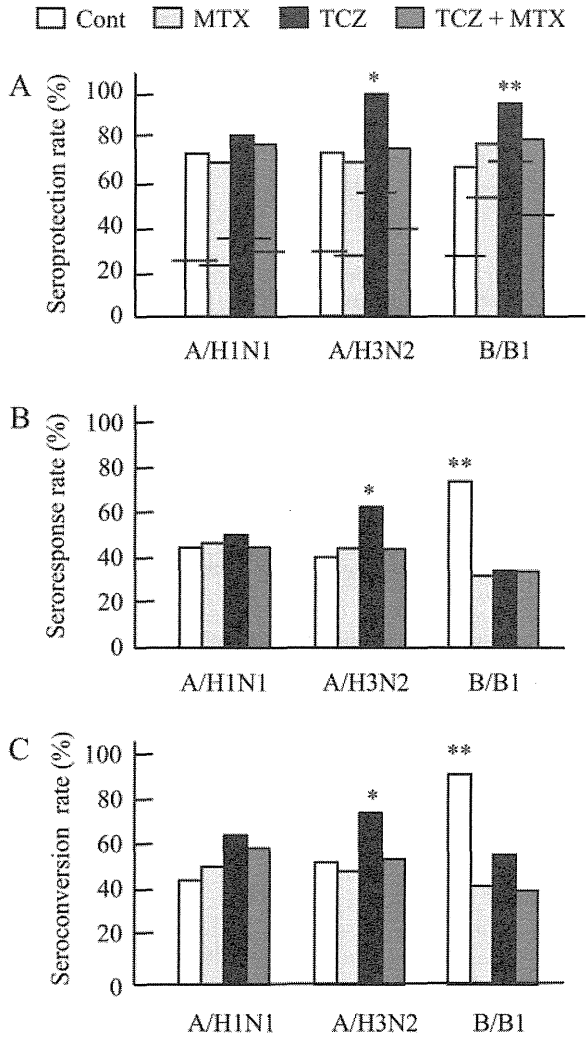

Figure 1 (A) Seroprotection rates for three influenza strains in the RA treatment groups prior to and after influenza vaccination. Horizontal bars represent levels of prevaccination protection rates for each influenza strain. *p=0.006 (TCZ vs Cont), p<0.0005 (TCZ vs MTX) and p=0.001 (TCZ vs TCZ+MTX). **p=0.007 (TCZ vs Cont, TCZ vs MTX) and p=0.023 (TCZ vs TCZ+MTX). (B) Seroresponse rates for three influenza strains in the RA treatment groups. *p=0.04 (TCZ vs MTX). **p=0.0009 (Cont vs MTX), p=0.002 (Cont vs TCZ) and p=0.022 (Cont vs TCZ+MTX). (C) Seroconversion rates for three influenza strains in the RA treatment groups. Seroconversion rates are expressed as percentages of patients with seroconversion out of seronegative patients before vaccination (antibody titres<10). *p=0.032 (TCZ vs MTX). **p=0.003 (Cont vs MTX) and p=0.002 (Cont vs TCZ+MTX). Data were compared using the χ^2 test or Fisher's exact probability test. Cont, RA control group; MTX, methotrexate group; RA, rheumatoid arthritis; TCZ, tocilizumab group; TCZ +MTX, combination therapy group.

TCZ. Uchiyama *et al*[17] reported that anti-TCZ antibodies are induced in monkeys receiving 30 mg/kg of TCZ weekly, suggesting that IL-6 does not play a crucial role in antibody production.

Most previous studies have shown that the use of MTX is unlikely to affect antibody response to influenza vaccine.[2–4 7 18] However, Gabay *et al*[19] have indicated that MTX significantly reduced responsiveness to AS03-adjuvanted pandemic H1N1 2009 (A/H1N1/2009) vaccine in patients with rheumatic diseases. The mechanism by which MTX impairs antibody response following vaccination is unknown, but several studies have proposed that MTX prevents proliferation of T cells and induces apoptosis in these cells.[20]

In conclusion, despite TCZ therapy, the immunogenicity of influenza vaccination appears to be conserved and sufficient in RA patients. MTX had a negative impact on vaccination efficacy, but adequate immune responses for protection were achieved by RA patients in the MTX and MTX+TCZ groups. Neither severe adverse effects nor RA flares were observed following vaccination. RA patients, even those receiving TCZ as monotherapy or in a combination therapy with MTX, should therefore be encouraged to receive influenza vaccination.

Contributors All authors contributed to study conception and design, acquisition of data, analysis and interpretation of data, and drafting of the manuscript with regard to important intellectual content.

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Impact of tocilizumab therapy on antibody response to influenza vaccine in patients with rheumatoid arthritis

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CONCISE REPORT

Pneumococcal polysaccharide vaccination in rheumatoid arthritis patients receiving tocilizumab therapy

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ABSTRACT

Objectives We assessed the impact of tocilizumab (TCZ), a humanised monoclonal anti-interleukin-6 receptor antibody, on antibody response following administration of the 23-valent pneumococcal polysaccharide vaccine (PPV23).

Methods A total of 190 patients with rheumatoid arthritis (RA) received PPV23. Patients were classified into TCZ (n=50), TCZ + methotrexate (MTX) (n=54), MTX (n=62) and RA control (n=24) groups. We measured serotype-specific IgG concentrations of pneumococcal serotypes 6B and 23F using ELISA and functional antibody activity using a multiplexed opsonophagocytic killing assay, reported as the opsonisation indices (OIs), before and 4–6 weeks after vaccination. Positive antibody response was defined as a 2-fold or more increase in the IgG concentration or as a ≥10-fold or more increase in the OI.

Results IgG concentrations and OIs were significantly increased in all treatment groups in response to vaccination. The TCZ group antibody response rates were comparable with those of the RA control group for each serotype. MTX had a negative impact on vaccine efficacy. Multivariate logistic analysis confirmed that TCZ is not associated with an inadequate antibody response to either serotype. No severe adverse effect was observed in any treatment group.

Conclusions TCZ does not impair PPV23 immunogenicity in RA patients, whereas antibody responses may be reduced when TCZ is used as a combination therapy with MTX.

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) infection is responsible for substantial mortality and morbidity among adults aged ≥65 years or those with underlying chronic or immunosuppressive conditions. The CDC Advisory Committee on Immunization Practice has recommended the use of the 23-valent pneumococcal polysaccharide vaccine (PPV23) for prevention of invasive pneumococcal disease in at-risk populations.[1] Patients with rheumatoid arthritis (RA) are at an increased risk of contracting infectious diseases because of immunological changes that are intrinsic to RA and that result from immunosuppressive agents, and thus it is likely that pneumococcal vaccination can benefit this patient population.

Tocilizumab (TCZ), a humanised monoclonal antibody against the interleukin-6 (IL-6) receptor, is effective and generally well tolerated when administered either as monotherapy or in combination with methotrexate (MTX) in patients with moderate to severe RA. IL-6 was originally identified as a factor essential for B cell differentiation into antibody-producing plasma cells,[2] and IL-6-deficient mice had reduced antigen-specific IgG following immunisation with a T-cell-dependent antigen.[3] PPV23 induces serotype-specific IgG in a T-cell-independent polysaccharide antigen pathway, which can enhance pneumococcal opsonisation, phagocytosis and killing by phagocytic cells.[4] PPV23 immunogenicity is often impaired in certain groups of immunocompromised patients,[1] but evidence of PPV23 efficacy and safety is lacking in RA patients receiving TCZ.

The objective of the present study was to evaluate the influence of TCZ therapy on antibody response to PPV23 in RA patients. We determined the serum concentrations of serotype-specific IgG using ELISAs and the functional antibody activity using multiplexed opsonophagocytic killing assays (OPAs) in RA patients being treated with TCZ, MTX or TCZ and MTX, and in control RA patients who received neither drug.

METHODS

Patients

RA patients who were receiving TCZ therapy (at least the first dose of an intravenous infusion of 8 mg/kg every 4 weeks) and/or MTX (4–18 mg per week) for ≥12 weeks at our rheumatology outpatient clinics were invited to participate in this open-label study. RA patients who had been treated with bucillamine or salazosulfapyridine were also included as RA controls. All participants fulfilled the 1987 American College of Rheumatology criteria for RA diagnosis. Exclusion criteria were current prednisolone use (≥10 mg/day), current use of immunosuppressive antirheumatic drugs other than MTX (such as tacrolimus, cyclosporine, leflunomide, cyclophosphamide and azathioprine), a recent history (within 6 months) of pneumococcal infection and a history of pneumococcal vaccination. Patients who had changed treatments during the follow-up period or those who had received biological agents other than TCZ were also excluded from this study.

Vaccine

We used commercially available PPV23 (Pneumovax NP, Merck Sharp & Dohme Corp., Tokyo, Japan) containing 25 μg each of 23 capsular polysaccharide

types. From October 2011 to March 2012, each patient received a single dose of vaccine (0.5 ml) subcutaneously in the upper arm. For RA patients receiving TCZ, the vaccination was performed on the same day as the TCZ infusion.

ELISAs for serotype-specific IgG and multiplexed OPAs

Sera were collected immediately before and 4–6 weeks after vaccination and stored at −30°C until tested. To measure serotype-specific IgG concentrations and functional antibody activity against pneumococcus serotypes 6B and 23F, we performed ELISAs and multiplexed OPAs, respectively. For detailed protocols, see online supplementary text.

Antibody response

Fold increases relative to pre-vaccination values (post-vaccination value to pre-vaccination value ratios) were determined. Positive antibody response was defined as a 2-fold or more increase in IgG concentrations or as a 10-fold or more increase in opsonisation indices (OIs).[5]

Monitoring adverse effects

Adverse events that occurred during a follow-up period of 4–6 weeks after vaccination were recorded. Systemic adverse effects included fever, headache, myalgia, asthenia and fatigue. Local adverse events included pain/tenderness, swelling/induration and erythema at the injection sites.

Statistical analysis

To access the PPV23 immunogenicity in patients in each treatment group, IgG concentrations and OIs before and after vaccination were transformed into logarithmic values. IgG geometric mean concentrations (GMCs) and geometric mean OIs (GM-OIs) were calculated as the exponential of an arithmetic mean of log-transformed values. For details regarding statistical analysis, see online supplementary text.

RESULTS

Clinical and demographic characteristics

A total of 190 RA patients were divided into four groups according to their ongoing anti-RA therapy. There was one group of 50 patients treated with TCZ as monotherapy (TCZ group), 62 patients treated with MTX alone (MTX group), 54 patients who received a combination therapy consisting of TCZ and MTX (TCZ+MTX group) and 24 patients who did not receive either drug (RA control group). Prior to participating in this study, no patients had received a pneumococcal vaccination. Patients' clinical and demographic characteristics are shown in table 1.

Serotype-specific IgG concentrations

After vaccination, serotype-specific IgG GMCs to pneumococcal serotypes 6B and 23F in all four groups were increased significantly ($p<0.0005$; table 2). For serotype 6B, a significantly higher post-GMC was obtained in the TCZ group compared with that in the TCZ+MTX group ($p=0.004$). The TCZ group also showed a significantly greater fold increase than did the TCZ+MTX group ($p=0.036$). For serotype 23F, the TCZ group also showed a significantly higher post-GMC than did the MTX group ($p=0.027$). Increases were twofold or more in all treatment groups, and there were no statistically significant differences.

Opsonophagocytic killing assays

After vaccination, GM-OIs for the 6B and the 23F serotypes were increased significantly in all four groups ($p<0.0005$; table 2). For serotype 6B, the post-vaccination GM-OI was significantly higher in the TCZ group compared with that in the MTX group ($p=0.001$). The TCZ group also showed a significantly higher post-vaccination GM-OI for serotype 23F compared with the MTX group ($p=0.001$) or with the TCZ+MTX group ($p=0.042$). For either serotype, there were no significant differences in fold increases among the four treatment groups.

Table 1 Clinical and demographic characteristics of RA patients prior to pneumococcal vaccination

	MTX group (n=62)	TCZ+MTX group (n=54)	TCZ group (n=50)	RA control (n=24)	p Values between treatment groups
Male/female	11/51	4/50	7/43	5/19	NS
Age, mean (95% CI) (years)	68.3 (66.6 to 70.1)	65.1 (63.1 to 67.0)	68.3 (65.8 to 70.8)	69.2 (65.3 to 73.1)	NS
RA duration, mean (95% CI) (years)	10.0 (7.8 to 12.1)	9.1 (7.3 to 10.8)	12.5 (9.6 to 15.3)	11.3 (6.0 to 16.6)	NS
MTX dose, median (IQR) (mg/week)	8 (6 to 8)	8 (6 to 8)	–	–	NS
MTX duration, median (IQR) (months)	48 (14.3 to 86.3)	48.5 (26 to 81)	–	–	NS
TCZ duration, median (IQR) (weeks)	–	56 (16 to 95)	58 (15 to 98)	–	NS
Use of prednisolone, number of patients (%)	17 (27.4)	14 (25.9)	12 (24)	1 (4.2)	0.018 (M vs C) 0.029 (T/M vs C) 0.049 (T vs C)
Prednisolone dose, median (IQR) (mg/day)	0 (0 to 2)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	NS
Positive RF, number of patients (%)	35 (56.5)	39 (72.2)	31 (62)	8 (33.3)	0.001 (T/M vs C) 0.021 (T vs C)
Positive anti-CCP Abs, number of patients (%)	44 (71.0)	46 (85.2)	41 (82)	11 (45.8)	0.029 (M vs C) 0.0003 (T/M vs C) 0.001 (T vs C)
Lymphocytes, mean (95% CI) (/μl)	1374 (1230 to 1517)	1651 (1420 to 1881)	1717 (1545 to 1890)	1600 (1358 to 1842)	NS
Serum IgG, mean (95% CI) (mg/dl)	1286 (1194 to 1377)	1172 (1075 to 1269)	1196 (1121 to 1271)	1394 (1258 to 1530)	NS

Data were obtained immediately before pneumococcal vaccination. p Values between treatment groups were determined using the Mann–Whitney U test, ANOVA (analysis of variance) with a Tukey's HSD (honesty significant difference) post hoc test, the Kruskal–Wallis test with a Scheffe post hoc test, the χ^2 test or Fisher's exact probability test.
anti-CCP Abs, anti-cyclic citrullinated peptide antibodies; M, MTX group; MTX, methotrexate; NS, not significant; RA, rheumatoid arthritis; RF, rheumatoid factor; T, TCZ group; T/M, TCZ +MTX group; C, RA control; TCZ, tocilizumab.

Table 2 Concentrations of pneumococcal polysaccharide antigen serotype-specific IgG antibodies and opsonisation indices in the RA treatment groups before and after 23-valent pneumococcal polysaccharide vaccination

Serotype	MTX group (n=62)	TCZ+MTX group (n=54)	TCZ group (n=50)	RA control group (n=24)	p Values between treatment groups
IgG GMCs (μg/ml)					
6B					
Before	1.2 (1.0 to 1.5)	1.1 (0.9 to 1.3)	1.3 (1.0 to 1.7)	1.1 (0.8 to 1.6)	NS
After	2.2 (1.7 to 2.7)*	1.7 (1.3 to 2.3)*	6.1 (2.6 to 4.9)*	2.5 (1.5 to 4.4)*	0.004 (T/M vs T)
Fold increase	1.5 (1.1 to 3.0)	1.6 (1.2 to 1.9)	2.8 (1.4 to 4.4)	1.8 (1.3 to 3.7)	0.036 (T/M vs T)
23F					
Before	1.0 (0.8 to 1.2)	0.9 (0.7 to 1.2)	1.3 (1.0 to 1.7)	1.0 (0.6 to 1.5)	NS
After	2.4 (1.8 to 3.3)*	2.5 (1.8 to 3.5)*	4.6 (3.4 to 6.4)*	3.6 (1.8 to 5.7)*	0.027 (M vs T)
Fold increase	2.6 (1.4 to 4.1)	2.9 (1.0 to 6.9)	3.4 (1.5 to 6.8)	3.5 (1.7 to 5.6)	NS
GM-OIs					
6B					
Before	18.8 (18.7 to 32.1)	24.5 (14.7 to 42.1)	43.8 (22.4 to 85.6)	20.70 (7.0 to 61.0)	NS
After	115.6 (64.1 to 206.4)*	232.8 (124.0 to 437.0)*	692.3 (265.1 to 1366)*	262.4 (74.4 to 916.0)*	0.001 (M vs T)
Fold increase	4.5 (1 to 12.5)	6.8 (1.7 to 35.5)	12 (3.5 to 62.4)	8.5 (2.2 to 52.0)	NS
23F					
Before	10.1 (6.6 to 15.3)	15.5 (10.3 to 23.6)	27.9 (15.2 to 51.4)	17.6 (7.5 to 42.1)	0.018 (M vs T)
After	72.2 (39.3 to 133.0)*	124.0 (62.2 to 244.7)*	437.0 (221.4 to 862.6)*	219.2 (82.3 to 578.2)*	0.001 (M vs T) 0.042 (M/T vs T)
Fold increase	7 .0 (2.7 to 15.8)	5.0 (1 to 40)	18.8 (2.7 to 75.1)	11.0 (3.1 to 30.6)	NS

IgG GMCs and GM-OIs are expressed as the mean (95% CI). Fold increases are expressed as the median (IQR). Differences between pre- and post-vaccination GMCs of serotype-specific IgG and those between pre- and post-vaccination GM-OIs were assessed using a paired-sample t test. The four treatment groups were compared using ANOVA (analysis of variance) with a Tukey's HSD (honestly significant difference) post hoc test or the Kruskal–Wallis test with a Scheffe post hoc test.
*p<0.0005 compared with pre-vaccination IgG GMCs or GM-OIs.
GMC, geometric mean concentration; GM-OI, geometric mean opsonisation index; M, MTX group; MTX, methotrexate; NS, not significant; RA, rheumatoid arthritis; T, TCZ group; T/M, TCZ+MTX group; TCZ, tocilizumab.

There was a moderate correlation between IgG concentrations and OIs for the 6B and the 23F serotypes (serotype 6B: r=0.623, p<0.0005; serotype 23F: r=0.601, p<0.0005).

Antibody response rates (percentages of patients with positive antibody response)

The TCZ group antibody response rates were comparable with those of the RA control group for serotypes 6B and 23F (figure 1).

For the IgG concentration specific to serotype 6B, the antibody response rate was significantly higher in the TCZ group (56%) compared with that in the MTX group (37%) and the TCZ+MTX group (24%, p=0.046 and p=0.0009, respectively; figure 1A). For serotype 23F, there was no significant difference in the antibody response rate among the four treatment groups (Control: 67%; MTX: 57%; TCZ+MTX: 56%; TCZ: 72%). The percentage of patients with positive antibody response for both strains were significantly greater in the TCZ group (46%) compared with the TCZ+MTX group (20%, p=0.005) and the RA control group (21%, p=0.044).

For OIs specific to serotype 6B, the TCZ group showed a significantly higher antibody response rate than did the MTX group (56% vs 34%, p=0.019; figure 1B). For serotype 23F, the antibody response rates were significantly higher in the TCZ group (58%) compared with those in the MTX group (37%, p=0.027) and the TCZ+MTX group (35%, p=0.020). For both strains, a higher proportion of patients in the TCZ group responded to pneumococcal vaccination compared with the patients being treated with MTX alone (34% vs 16%, p=0.028).

Predictive factors for antibody response to PPV23

In a multivariate logistic regression analysis, TCZ use was not identified as the predictive factor for antibody response to pneumococcal vaccination for either IgG concentrations or OIs. The negative association of current MTX use with antibody response was confirmed for IgG concentrations specific to serotypes 6B and 23F (for serotype 6B: OR 0.45, 95% CI 0.25 to 0.82, p=0.009; for serotype 23F: OR 0.56, 95% CI 0.31 to 1.04, p=0.007) and OIs for serotype 23F (OR 0.54, 95% CI 0.29 to 0.99, p=0.046).

Vaccination safety

Two patients in the TCZ+MTX group had a fever. Local adverse events were observed in 12 patients (2 in the MTX group, 7 in the TCZ+MTX group and 3 in the TCZ group). All adverse effects were mild.

DISCUSSION

Following immunisation with PPV23, IgG concentrations and OIs for the 6B and the 23F serotypes were significantly increased in all treatment groups. Antibody response rates in the TCZ group were comparable with those of the RA control group for each serotype. Ongoing use of MTX is likely to have affected the antibody response to PPV23.

Results of the present study indicate that TCZ does not diminish T-cell-independent antibody production after PPV23 immunisation. In addition, we recently reported that RA patients receiving TCZ can produce an adequate antibody response to influenza vaccine, which are T-cell-dependent protein antigens.[6] These findings suggest that both T-cell-dependent and T-cell-independent antibody response pathways are conserved in RA patients who are treated with TCZ. There is an increasing awareness of lethal synergism between influenza virus and pneumococcus; influenza virus contributes to secondary pneumococcal pneumonia and can subsequently increase mortality.[7 8] In addition, a large-scale trial suggested that a significant

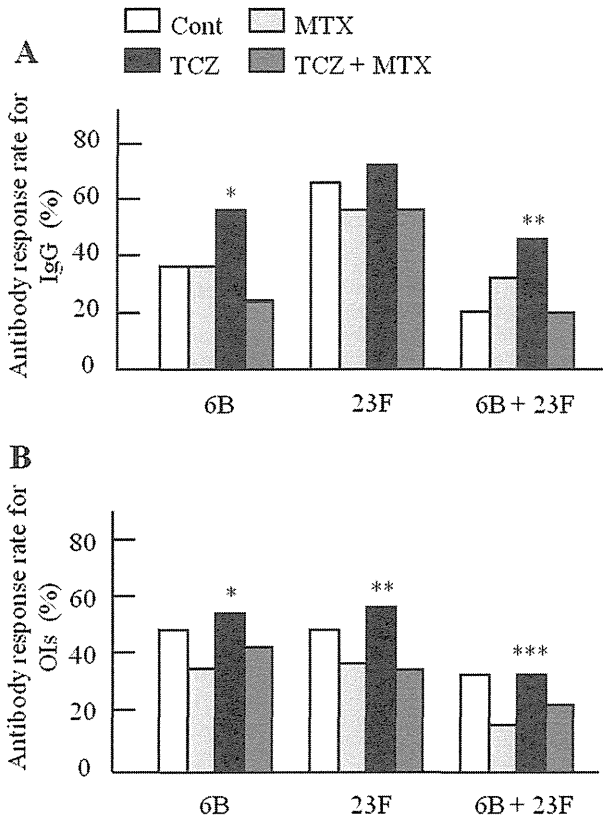

Figure 1 (A) Percentages of patients with twofold or more increases in serotype-specific IgG concentrations for serotypes 6B and 23F in the rheumatoid arthritis (RA) treatment groups. *p=0.046 (TCZ vs MTX) and p=0.0009 (TCZ vs TCZ+MTX). **p=0.005 (TCZ vs TCZ+MTX) and p=0.044 (TCZ vs Cont). (B) Percentages of patients with 10-fold or more increases in OIs for serotypes 6B and 23F in the RA treatment groups. *p=0.019 (TCZ vs MTX). **p=0.027 (TCZ vs MTX) and p=0.020 (TCZ vs TCZ+MTX). ***p=0.028 (TCZ vs MTX). Data were compared using the χ^2 test or Fisher's exact probability test. OIs, opsonisation indices; Cont, RA control group; MTX, methotrexate group; TCZ, tocilizumab group; TCZ+MTX, combination therapy group.

proportion of viral pneumonia, including influenza, is attributable to bacterial co-infection and that this co-infection may be preventable by bacterial vaccination.[9] Immunisation with both influenza and pneumococcal vaccines may, therefore, provide additive benefits for RA patients compared with a single vaccination, even if they are receiving TCZ therapy.

Previous studies have shown that MTX therapy reduced the antibody response to PPV23,[10–13] which is in agreement with the data obtained in the present study. Although T-cell-dependent protein antigens may be more immunogenic than polysaccharide antigens in immunocompromised patients,[14] MTX was also reported to be a strong predictive factor for an impaired antibody response to protein-conjugate pneumococcal vaccine.[15] Offering PPV23 vaccination before introduction of MTX therapy may be considered in RA patients.[11 16] In contrast, a study by Elkayam *et al*[17] did not demonstrate a detrimental effect of immunosuppressive drugs such as MTX on PPV23 immunogenicity in RA patients. Coulson *et al*[18] have also suggested that a single PPV23 administration offers up to 10 years of protection against the development of pneumococcal pneumonia in RA patients receiving MTX therapy. Determining serotype-specific IgG concentrations after PPV23 vaccination in patients receiving MTX therapy is recommended.[19]

In the present study, no patients were receiving high doses of prednisolone or antirheumatic agents with immunosuppressive effects other than MTX. In addition, there were no differences in the prednisolone dose among the four treatment groups, and the median dose of prednisolone was zero among all groups. The number of prednisolone users was significantly lower in the RA control group; however, there were no significant differences or trends in antibody response to each serotype compared with the other three groups. We can, therefore, say that the influence of such agents on PPV23-induced antibody response was minimal in the present study.

One limitation of this study is the relatively small number of patients in each group and the RA control group in particular. Since most RA patients had already received one or more immunosuppressive antirheumatic drugs, as recommended by the current therapeutic guidelines, it was difficult to recruit a sufficient number of patients who had never received such drugs. Another limitation is that we determined antibody response to only two pneumococcal serotypes. We chose serotypes 6B and 23F because these are the main causative serotypes of pneumococcal pneumonia in Japan and these are representative penicillin-resistant pneumococci.[20] However, the immune response to PPV23 may not be consistent among the 23 serotypes. Lastly, unlike influenza vaccines, antibody levels that are protective against invasive pneumococcal disease in adults have not been clearly defined. We used a 2-fold increase in the IgG concentration or a 10-fold increase in the OI as a measure of positive antibody response to PPV23 in this study, which was also used in previous studies;[5] however, how this threshold may best correlate with protection against invasive pneumococcal disease remains to be determined.

In conclusion, ongoing TCZ therapy does not preclude pneumococcal polysaccharide vaccination in RA patients; however, antibody responses may be reduced when TCZ is administered in combination with MTX.

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Competing interests TH has received lecture fees from Mitsubishi-Tanabe Pharmaceutical Co., Eisai Co. Ltd. and Abbott Japan Co. Ltd. The other authors have no financial relationships that could lead to a conflict of interest.

Patient consent Obtained.

Ethics approval The ethics committees of participating hospitals approved the protocol for this study.

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Pneumococcal polysaccharide vaccination in rheumatoid arthritis patients receiving tocilizumab therapy

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Notes

＜特集関連情報＞

7価肺炎球菌結合型ワクチン（PCV7）導入が侵襲性細菌感染症に及ぼす効果：2012

はじめに

肺炎球菌は，小児期における侵襲性感染症の起因菌として頻度が高い。細菌性髄膜炎，敗血症，肺炎はその代表的な疾患であり，治療が進歩した今日においても重篤な経過となることがあるため，ワクチンによる予防が重要である。これまでに小児結合型肺炎球菌ワクチンが定期接種となっている国々においては，侵襲性感染症の減少が報告されている[1)]。本邦では，2010年2月から結合型7価肺炎球菌ワクチン（PCV7）が市販され，2011年に入り多くの自治体では公費助成で接種可能になった。

われわれは，厚生労働科学研究事業研究班「ワクチンの有用性向上のためのエビデンス及び方策に関する研究」班（神谷班），「新しく開発されたHib，肺炎球菌，ロタウイルス，HPV等の各ワクチンの有効性，安全性ならびにその投与方法に関する基礎的・臨床的研究」班（2011年2月に神谷研究代表者が逝去したため庵原・神谷班に名称変更）として，小児侵襲性細菌感染症のアクティブサーベイランスを継続して実施している。今回は公費助成開始後2年間において，PCV7が侵襲性肺炎球菌感染症（IPD）に与えたインパクトについて報告する。

調査方法

本研究において報告対象とした患者は，生後0日～15歳未満で，肺炎球菌，インフルエンザ菌，B群レンサ球菌（GBS）による侵襲性細菌感染症（血液，髄液，関節液など，本来は無菌環境である身体内部から採取した検体から起因菌が分離された感染症）に罹患した全例とした。罹患率の算出には，総務省統計局発表の各年10月1日時点の5歳未満人口（ただし2012年は2013年2月14日時点でデータ未公表のため，2011年のものを使用）を用いた。2011年10月時点での10道県を合わせた5歳未満人口推計値は1,199,000人であり，全国の5歳未満人口の推計値（5,303,000人）の22.6%を占めていた。調査期間は，2008年1月～2012年12月までの5年間，前方視的に全数把握調査を実施した。

調査対象地域は，北海道，福島県，新潟県，千葉県，三重県，岡山県，高知県，福岡県，鹿児島県，沖縄県の10道県である。これらの地域で，人口ベースの患者発生状況調査を行った。菌の同定・血清型判定と薬剤感受性解析は，国立感染症研究所で実施した。なお，北海道は髄膜炎のみの調査であり，他の9県は侵襲性感染症すべての調査である。

表1．小児期侵襲性細菌感染症の報告患者数（2012年）

	北海道	福島	新潟	千葉	三重	岡山	高知	福岡	鹿児島	沖縄	全国
肺炎球菌髄膜炎	1	0	0	2	0	1	0	0	1	4	9
肺炎球菌非髄膜炎		0	7	27	4	1	5	33	4	25	106
Hib髄膜炎	0	0	0	3	0	0	0	3	1	0	7
Hib非髄膜炎		0	2	0	0	0	0	6	0	1	9
GBS髄膜炎	1	1	1	2	2	1	0	8	0	2	18
GBS非髄膜炎		0	2	5	0	0	0	3	2	0	12

＊各疾患の報告患者数は、すべて5歳未満の者のみ
＊北海道は髄膜炎のみが報告対象

表2．小児期侵襲性細菌感染症の罹患率
（5歳未満人口10万人当たり）

	2008-2010	2011	減少率（%）	2012	減少率（%）
肺炎球菌髄膜炎	2.8	2.1	25	0.8	71
肺炎球菌非髄膜炎	22.2	18.1	18	10.6	52
Hib髄膜炎	7.7	3.3	57	0.6	92
Hib非髄膜炎	5.1	3.0	41	0.9	82
GBS髄膜炎	1.3	1.3	0	1.5	-15
GBS非髄膜炎	1.2	1.1	8	1.2	0

結　果

1）IPD罹患率の変化

2008年1月～2012年12月に各県より報告された患者数を表1に示した。5歳未満の患者数は10道県合計で，肺炎球菌髄膜炎9例，髄膜炎以外のIPD（以下非髄膜炎）が106例であった。これらの報告数より，各疾患の5歳未満人口10万人当たりの罹患率を算出し，ワクチン公費助成前3年間（2008～2010年）と，2011年および2012年の罹患率比較を行った（表2）。2008～2010年のIPD平均罹患率は，髄膜炎2.8，非髄膜炎22.2であったが，2011年にはそれぞれ2.1，18.1に減少し，減少率は25%，18%であった。2012年も罹患率減

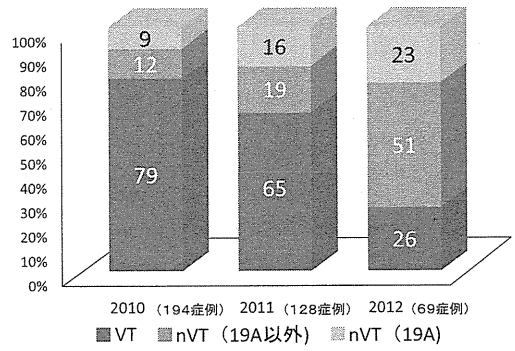

図1．IPD症例から分離された肺炎球菌の血清型、2010～2012年

少傾向は継続し，髄膜炎0.8（減少率71%），非髄膜炎10.6（減少率52%）にまで減少した。

2）侵襲性インフルエンザ菌b型（Hib），GBS感染症罹患率

侵襲性Hib感染症は，髄膜炎で92%，非髄膜炎感染症でも82%の罹患率減少を認めた。GBS感染症は減少傾向を示さなかった（前ページ表2）。

3）肺炎球菌血清型の変化

2010～2012年において，IPD症例から分離された菌の血清型について検討した（図1）。2010年はPCV7に含まれる血清型（vaccine serotypes, VT）が79%を占めていた。2011年には，VTは65%に減少し，PCV7に含まれない血清型（non-vaccine serotypes, nVT）は35%に増加し，特に血清型19Aの占める割合の増加が目立った（9%→16%）。2012年にはnVTの増加はさらに顕著となり（74%），VTは26%であった。nVTの増加として，19A以外の血清型の増加が主であった（19%→51%）。

4）ワクチン接種後罹患例

PCV7 1回以上の接種歴があるIPD症例について検討した。2010年は6例のみであったが，2011年は24例，2012年には62例に増加した。分離菌の血清型が判明した症例におけるVTが占める割合は，2010年は83.3%（5/6）であったが，2011年は15.8%（3/19），2012年は3.1%（1/32）と減少を認めた。

考　察

PCV7が導入された国々からはIPD発症数の大幅な減少が報告されている。米国ではPCV7導入後わずか1年で5歳未満のIPD罹患率が59%減少した[1]。その後Center for Disease Control（CDC）から5年後のデータが報告されており，5歳未満のPCV7血清型によるIPDは98%減少していた[2]。本研究班では，昨年の2011年調査において早くもIPDの罹患率減少が観察され始めたことを報告した[3]。今回は，ワクチン公費助成開始後2年目となる2012年の調査結果を加えて解析を行った。公費助成前期間と比較したIPD減少率は，髄膜炎で71%，非髄膜炎では52%であり，2011年に引き続き減少が観察された。欧米各国のデータと遜色の無い減少率であり，PCV7接種による発症抑制効果の現れと考えられる。しかしながら，Hib侵襲性感染症の減少率（髄膜炎92%，非髄膜炎82%）には及ばなかった。その要因として，1）本邦での市販開始時期の違い（Hibワクチンは2008年12月から，PCV7は2010年から市販開始），2）肺炎球菌の血清型の多様性，などが推察される。PCV7導入後のnVTの増加現象は，欧米ではすでにSerotype replacementとして報告されている[1,4]。米国では，血清型19Aを中心としたnVTによるIPDの増加があり，PCV7導入3年目以降のIPD罹患率がプラトーになった要因とされている[2]。本研究においても，VTの占める割合は2010年の79%から2012年は26%に減少し，19Aのみならず多様なnVTの増加が明らかであり，IPD罹患率に影響を及ぼしたと思われる。PCV7接種後罹患例においては，nVTの割合はさらに高くなり，VTによるBreakthrough infectionは1例のみであった。米国におけるABCs（Active Bacterial Core surveillance）による調査でも，ワクチン接種後罹患例は主としてnVTの感染によることが報告されており[5]，PCV7によるIPD発症抑制効果の高さを裏付けるデータと考える。

今回の調査により，本邦においてもPCV7導入が，5歳未満小児においてIPD罹患率の大幅な低下をもたらしたことが明らかとなった。さらに肺炎，中耳炎に対する効果や，PCV7非接種年齢層に対する間接効果も期待される。一方でSerotype replacementの発生，進行も確認されていることから，今後も分離菌の血清型解析に努め，全数把握アクティブサーベイランスを推進する必要があると考える。

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