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Long-term Observation of the Serum Level of Anti-influenza Antibody Titers in Patients with Rheumatoid Arthritis on Receiving Vaccination with Identical Strains in the 2010 to 2012 Season

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Authors' contributions

This work was carried out in collaboration between all authors. Author TK designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript and managed the analyses of the study. Authors KY, NH and TT managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Influenza vaccination is effective in patients with rheumatoid arthritis (RA), although the humoral immune response to influenza vaccine is weak with some kinds of strain in RA patients having biologics. This study's purpose is to investigate the immune response in RA patients who are weak at a humoral response to the vaccination, because we assume CD4+ T cells already recognize the epitope of influenza viral antigen presented by antigen-presenting cells, while the humoral response is not yet sufficient.
Study Design: Observational study.
Place and Duration of Study: Department of Japanese Oriental Medicine, Gunma Central & General Hospital (GCH), between October 2010 and February 2012.

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Methodology: The strains of influenza vaccine in 2011-2012 were the same as those in 2010-2011 in Japan. Therefore, we investigated whether booster effects exist in the 2nd season compared with the response in the 1st season. In 38 RA patients (m/f: 2/36; age:60.4+/-13.7 mean+/-SD), we monitored the change in the serum level of anti-influenza antibody titers for 2 years.
Results: Booster effects were observed in the A/H3N2 strain; however, those effects were not observed in the A/H1N1 and B strains. There was no difference in the B strain at the baseline and at 4 weeks later, respectively, between 2010 and 2011. Titer's fold in 2011 was not higher than that in 2011 in the 5 RA patients treated with biologics.
Conclusion: The interaction between CD4+ T cells and B cells may be variable with each strain among influenza vaccine. The clinical efficacy of influenza vaccination with the B strain against RA patients having biologics may be not clear until further observational studies are developed concerning incidence rate of each strain.

Keywords: Influenza vaccine; clinical efficacy; rheumatoid arthritis; humoral response; booster effects; anti-rheumatic drugs.

1. INTRODUCTION

Previous clinical research suggested that patients with rheumatoid arthritis (RA) had an increased susceptibility to infection, even before the use of glucocorticoids became widespread [1]. RA is also associated with an increased incidence of seasonal influenza, and Blumentals et al. reported that there was a 2.75-fold increase in the incidence of influenza complications in the presence of RA [2]. Currently, annual influenza vaccination is recommended for patients with RA (Center for Disease Control and Prevention, 2002) [3].

Previously, we investigated the humoral immune response to influenza vaccination, and observed a significant response to the vaccination with each strain of influenza antigen [4,5]. There have been many reports investigating the association between responses to vaccination and anti-rheumatic drugs containing anti-tumor necrosis factor (TNF) drugs [6-9]. The research so far has demonstrated that the treatment with methotrexate (MTX) or prednisolone (PSL) does not influence the humoral immune response to influenza vaccine in patients with RA; however, anti-TNF drugs may be associated with the low response to vaccination in RA. In RA patients treated with Rituximab, an anti-CD20 monoclonal antibody, significantly lower post-vaccination titers were found [10]. We have also confirmed a lower immune response to the A/H3N2 strain in RA patients with biologics [5]. On the other hand, a large practice-based cohort, with the endpoint of the onset of influenza, revealed that influenza vaccination was effective in RA patients regardless of the disease activity or treatment (MTX or corticosteroid) in Japan [11]. Based on these phenomena, we consider that CD4-positive T cells may recognize the influenza antigens presented by antigen-presenting cells, even though the humoral response is low; therefore, influenza vaccination may lead to the development of antigen-specific memory T cells for influenza antigens. It is possible that patients previously inoculated with influenza vaccine show a higher response to influenza virus infection, even though their serum level of antibody titers after the vaccination is low.

To evaluate this hypothesis, we investigated the changes in serum levels of anti-influenza antibody titers for two years, from the season of 2010 to 2011, because the strain of influenza vaccine in the season of 2011-2012 was the same as that in 2010-2011. In this study, the booster effects in the 2nd season were examined in comparison with those in the 1st season.

2. MATERIALS AND METHODS

2.1 Patient Profiles

Patients who visited our department between 2010 and 2012 had to fulfill the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA and were selected by a random sampling method, which was one of the most popular types of random or probability sampling. They also had to fulfill the 2010 ACR-EULAR (European league against rheumatism) classification criteria [12] for RA based on retrospective analysis. Patients with other systemic diseases, including malignancy, diabetes mellitus, and viral hepatitis, were excluded from this study.

In the season of 2010-2011, the 45 RA patients received influenza vaccination in our department,

and 38 of them were inoculated with influenza vaccine in the next season while under our observation. Thus, 38 patients were recruited for this study (Fig. 1).

2.2 Vaccine

The strain of influenza vaccine in the season of 2011-2012 was the same as in the 2010-2011 season, according to the National institute of infectious diseases in Japan. Thus, we used a trivalent influenza subunit vaccine (2010-2011 and 2011–2012; Daiichi-Sankyo Co., Ltd., Tokyo, Japan) containing purified hemagglutinin and neuramidase of the following strains: 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 strain).

2.3 Hemagglutination Inhibition Assay

The hemagglutination inhibition assay (HIA) was used for the detection of anti-influenza antibodies. HIAs were performed with guinea pig erythrocytes in accordance with standard procedures [13]. The following parameters to assess the efficacy of the vaccination based on the anti-influenza antibody response were evaluated: geometric mean titer (GMT), fold increase in titer, and titer rise to ≥40 (seroprotection). HIA titers of 40 are generally considered to be protective in healthy adults [14].

2.4 Study Design

An observational design was utilized in the present study. The protocol (time series) is demonstrated in Fig. 2. First, patients received the influenza vaccine intramuscularly from October to December in 2010. Blood was collected immediately before and 4 weeks after the vaccination to measure the C-reactive protein levels (CRP), erythrocyte sedimentation rate (ESR), matrix metalloproteinase-3 (MMP-3), class-IgM rheumatoid factor (IgM-RF), and anti-influenza antibodies. The disease activity score-28 (DAS28) [15] was recorded before and 4 weeks after the vaccination. In the next season, patients registered to receive this protocol were inoculated with the influenza vaccine (the same strain as last season) in a similar fashion. Furthermore, information on previous influenza vaccinations was obtained from all participants, and adverse effects occurring in the first 7 days post-vaccination were recorded.

This study was approved by the Ethics Committee of Gunma Central and General Hospital in Aug. 2010 and Aug. 2011, and all authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

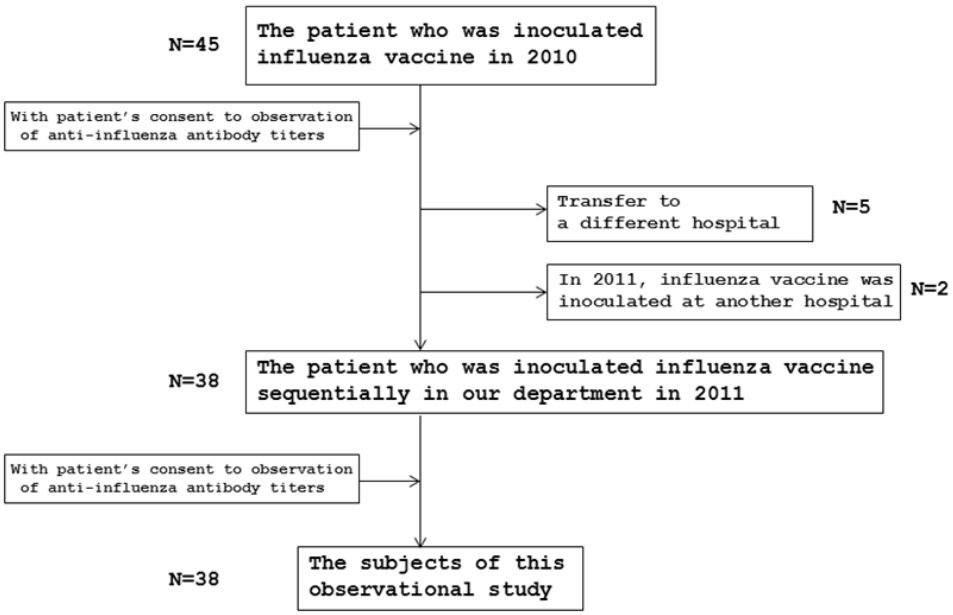

Fig. 1. Flow chart of subject (38 RA patients) recruitment and trial profiles

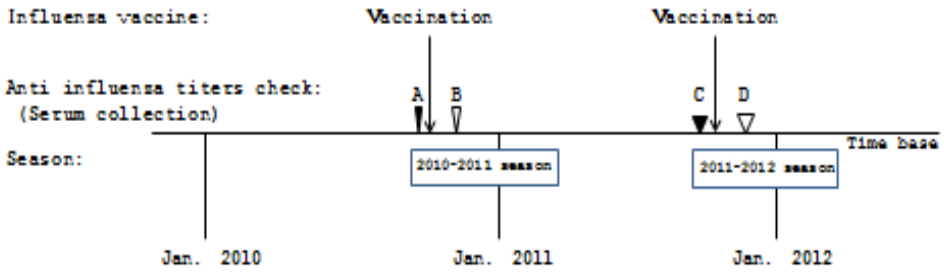

Fig. 2. The protocol of the observational study

The time courses for 2 years. A: Before vaccination (baseline) in 2010; B: Four weeks after vaccination in 2010 (1st season); C: Before vaccination (baseline) in 2011; D: Four weeks after vaccination in 2011 (2nd season)

2.5 Statistical Analysis

All data were inputted into a computer and analyzed using IBM SPSS Statistics Ver.19 (Released 2010. IBM SPSS Statistics for Windows, Version 19.0, Armonk, NY: IBM Corp). Whether or not each sample obeyed a normal distribution was confirmed by the Shapiro-Wilk test. Samples with a normal distribution were analyzed by the t-test, and those that did not obey a normal distribution were analyzed by the Mann-Whitney U-test. A p-value of less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Patient characteristics

The clinical characteristics of the 38 patients with RA are shown in Table 1. These data were monitored when we received the patient's consent for the observational study in October 2011. The contents of biologics were infliximab (one case), etanercept (three cases), and tocilizumab (one case). Abatacept and adalimumab were not administered.

3.1.2 The response to influenza vaccination in the season of 2011-2012

Each GMT 4 weeks after the vaccination was 87.1±204.6, 230.8±323.4, and 23.3±25.6 for the A/H1N1, A/H3N2, and B strain, respectively (Table 2). GMTs for the three strains were significantly increased after the vaccination. Furthermore, both the fold increase and the rate of seroprotection in each strain were markedly higher than before vaccination. Influenza vaccination of patients with RA was of clinical significance using A/H1N1 and A/H3N2. However, the rate of seroprotection with the B strain 4 weeks after vaccination was still below 40×(23.7%; see Table 2).

Data in the season of 2011-2012 were compared with those in the 2010-2011, to investigate the booster effects by vaccination. Because of that the strains in the season of 2010-2011 were same as those in the season of 2011-2012.

3.1.3 The change in serum levels of anti-influenza antibodies

GMTs in the three kinds of strain 4 weeks after influenza vaccination were significantly increased in the season of 2011-12, as described above (Table 2), and those in the season of 2010-2011 were also significantly increased.

The change of GMTs in each strain is shown in Fig. 3. In the GMTs of the A/H1N1 strain, there was a significant difference at the baseline between 2010-2011 and 2011-2012, but there was no significant difference at 4 weeks after vaccination between 2010-2011 and 2011-2012 (Fig. 3A). Although GMT in the A/H1N1 strain was maintained until the next season, the booster effect was unclear in the next season. In the GMTs of the A/H3N2 strain, there was a significant difference at the baseline between 2010-2011 and 2011-2012, and the GMT 4 weeks after vaccination in 2011-2012 was significantly higher than in the season of 2010-2011 (Fig. 3B). GMT in the A/H3N2 strain was maintained until the next season, and a booster effect in the next season was also observed. In the GMTs of the B strain, there was no significant difference at the baseline between 2010-2011 and 2011-2012, and the GMTs 4 weeks after vaccination in 2011-2012 did not significantly differ from those in 2010-2011 (Fig. 3C). GMT in the B strain was not maintained until the next season, and a booster effect in the next season was also not observed.

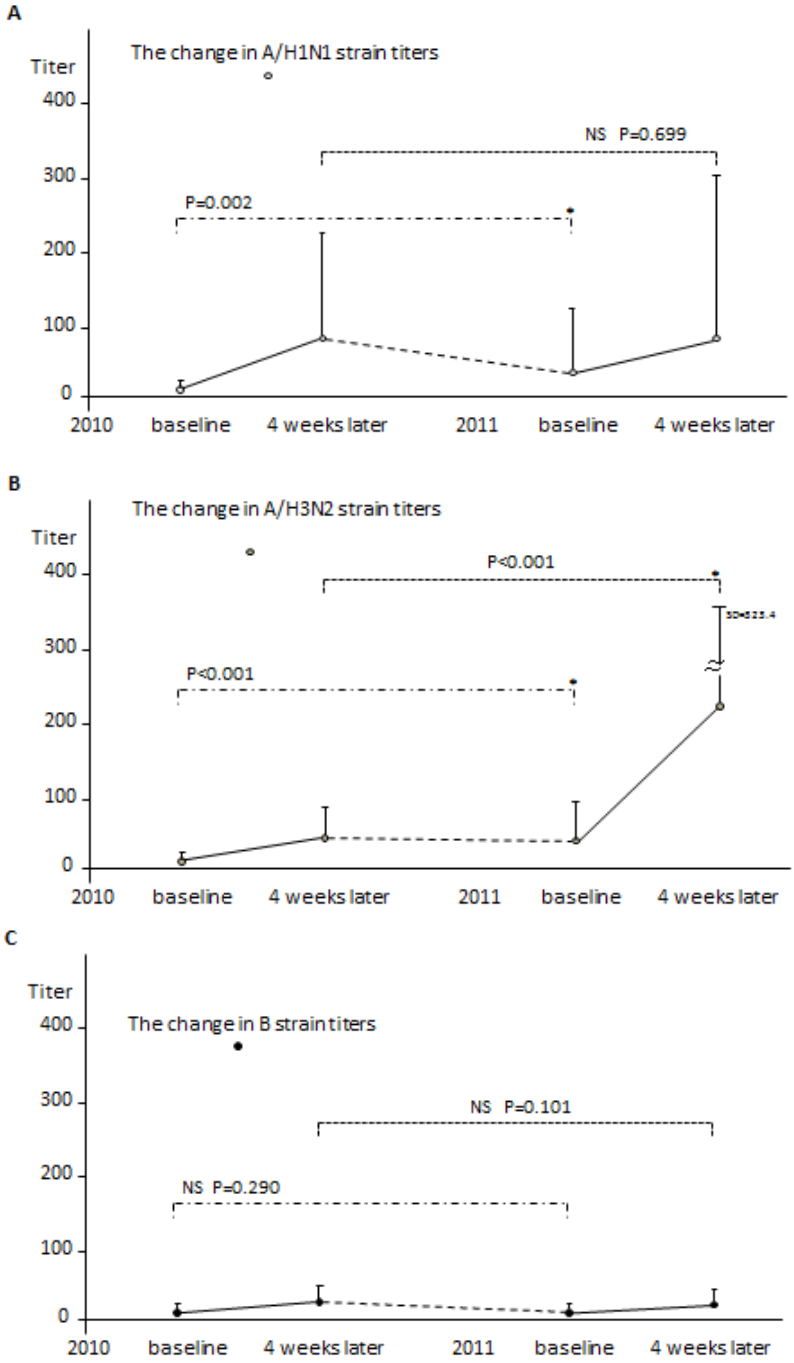

Fig. 3. The changes in the serum levels of GMTs for 2 years

A: A/H1N1 strain. The GMTs in 2011 were significantly higher than those in 2010 at the baseline. In contrast, there was no difference in the GMTs at 4 weeks after vaccination between 2010 and 2011.
B: A/H3N2 strain. The GMTs in 2011 were significantly higher than those in 2010 at both the baseline and 4 weeks after vaccination. The booster effects were particularly visible in the 2nd season.
C: B strain. There were no differences in the GMTs at both the baseline a
nd 4 weeks after vaccination between 2010 and 2011. The booster effects were not clear with this strain.
**GMT: geometric mean titer*
 # Mann-Whitney U-test: p-value less than 0.05 is considered significant

3.1.4 The change of GMTs in 5 RA patients treated with biologics

A booster effect was observed in the GMTs of the A/H3N2 strain, and the GMTs of both the A/H1N1 and A/H3N2 strains were maintained until the next season, whereas these phenomena were not observed in the B strain. With these results, and we further analyzed the change in GMTs of the 5 RA patients treated with biologics. The fold of each strain is shown in Table 3. The response to influenza vaccination in 2011-2012 was very similar to that in the season of 2010-2011, although there was no significance in statistics due to the limited patient numbers.

3.2 Discussion

Patients with RA are more susceptible to influenza virus infectious diseases (IVID) than healthy subjects, and their yearly vaccination is recommended [3]. The clinical efficacy of influenza vaccination has been established through several clinical studies. Thus far, there are two kinds of method to demonstrate the efficacy of vaccination. One is the analytical epidemiological procedure, and the other is serological analysis of the immune response. The former is superior to the latter from the viewpoint of clinical evidence, and there are a large-scale cohort study, self-control method, and test-negative design as the analytical epidemiological procedures [11,16-18]. The results based on these designs generally demonstrated that influenza vaccination was effective for RA patients regardless of the disease activity or treatment. On the other hand, it is also not uncommon to investigate the immune response to influenza vaccine using serological analysis [6-9]. This kind of study has also demonstrated both a significant increase in anti-influenza antibody titers and seroprotective effects in RA patients. However, several studies demonstrated that immunosuppressant drugs such as anti-TNF inhibitor may influence the production of anti-influenza antibody. We have also reported that “receiving biologic therapy” is a predictive factor for a low humoral response to influenza vaccine, especially the A/H3N2 strain. The clinical efficacy of seasonal vaccination was shown by epidemiological analysis, while the humoral response was low in the population treated with biologics. The recognition of influenza vaccine by antigen-presenting cells and CD4-T cells may have led to this observation, although the humoral reaction did not become clear. Thus, we monitored the changes in the serum levels of anti-influenza antibody titers for two seasons between 2010 and 2011 to assess whether a booster effect exists in the 2nd season as the preliminary study, since the strains of influenza vaccine in the 2011 season were the same as those in 2010. This is the first report demonstrating the changes in serum levels of anti-influenza virus titers for 2 years using the same strains.

The GMTs after vaccination in both the 1st season and 2nd season were significantly increased in each strain. In A/H1N1 and B strains, there was no significant difference in GMTs after vaccination between the 1st and 2nd seasons. In contrast, GMTs of the A/H2N3 strain were significantly higher in the 2nd than 1st season (Fig. 3 B). We previously reported that GMTs of RA patients receiving biologics therapy were significantly lower than in RA patients not receiving biologics with the A/H2N3 strain [5]. Therefore, we consider that a booster effect exists in the A/H2N3 strain, and antigen-presenting cells and CD4-T cells certainly recognized the epitope of the A/H2N3 strain, although the humoral response was low in patients receiving biologics. These ideas partially explain why influenza vaccination resulted in prevention of the onset of IVID regardless of the disease activity or treatment. On the other hand, GMT of the B strain, in which seroprotection was not observed in RA patients receiving biologics [5], did not show significant differences between the 1st and 2nd seasons before and after vaccination in this study (Fig. 3C). Thus, a booster effect was not observed in the B strain, in other words, memory CD4-T cells recognizing the epitope of the B strain did not develop sufficiently. These findings suggest that the interaction between CD4+ T cells and B cells is probably variable with each strain among the strains of influenza vaccine [18]. However, there is a problem regarding this speculation. While we investigated the booster effects after a year, the immune response 2-3 months after vaccination is generally important in clinical practice. To solve this problem, it may be necessary to monitor the immune response in patients with IVID.

Furthermore, we investigated the changes in the GMTs of the RA patients treated with biologics, but significance could not be assessed due to the limited number of cases (N=5). As the results, no booster effects in any strains were observed. This suggests that the interaction between CD4+ T cells and B cells may be weak in RA patients treated with biologics, although booster effects

exist in RA patients. In addition, it is also possible for the immune response to be different in every strain of vaccine. So far, it has not been observed whether the incidence rate of IVID is different in each strain, such as the A or B strain, in epidemiologic analysis concerning RA patients. Therefore, to confirm whether these phenomena influence the onset of IVID, the incidence of IVID should be monitored with each strain based on a large-scale clinical study, especially controlled trial, and clinical significance had better be limited.

Table 1. Clinical characteristics of the patients with RA in this observational study (n=38)

Sex (number)	male 2	female 36	
Age	63 (median)	60.4+/-13.7 (mean+/-SD)	
Disease activity			
CRP* (mg/dl)	0.31 (median)	1.25+/-2.11 (mean+/-SD)	
ESR** (mm/hour)	24.5	32.1+/-25.1	
RF[#] (IU/L)	36.5	122.7+/-210.7	
MMP-3[$] (mg/dl)	89.1	169.6+/-176.3	
DAS28[##](CRP)	3.08	3.10+/-0.79	
DAS28(ESR)	3.89	3.77+/-0.95	
Treatments			
Biologics	5 (Number of treatment cases)		
Methotrexate (mg/w)	26	0-10 (min-max)	5.1+/-3.9 (mean+/-SD)
Tacrolimus (mg/day)	8	0-2	0.28+/-0.58
Prednisolone (mg/day)	8	0-7	0.7+/-1.6
SASP*** (mg/day)	10	0-1000	236.8+/-414.9

**These data were monitored before the vaccination in the season of 2011-2012.*
See Fig. 1, before C▼in Time Base.
**CRP: C-reactive protein; **ESR: erythrocyte sedimentation rate; [#]RF: class IgM-rheumatoid factor;*
[$]MMP-3: matrix metalloproteinase-3; [##]DAS28: disease activity score-28;
****SASP: salazosulfapyridine*

Table 2. The serum GMTs of anti-influenza virus antibodies before and after the influenza vaccination in the season of 2011-2012

	H1N1 strain		***H3N2 strain***		***B strain***	
	Baseline	**4 weeks later**	**Baseline**	**4 weeks later**	**Baseline**	**4 weeks later**
GMT[#]						
Min-max	5.0-320.0	5.0-1280.0	5.0-160.0	10.0-1280	5.0-40.0	5.0-160.0
Mean+/-SD	44.4+/-76.9	87.1+/-204.6*	39.9+/-43.0	230.8+/-323.4*	15.1+/-11.5	23.3+/-25.6*
Fold (mean)	3.0		9.4		1.8	
Min. – Max.	0.5 – 16.0		1.0 – 64.0		1.0-8.0	
Seroprotection						
≧40.0 x (%)	35.3	63.2	44.1	81.6	14.7	23.7

*[#]: geometric mean titer, *: P<0.01 vs. baseline titers by Mann-Whitney U-test*

Table 3. The change (fold) in the titers of anti-influenza antibodies in the RA patients with biologics (N=5)

	H1N1 strain	***H3N2 strain***	***B strain***
Season			
2010-2011 Mean+/-SD	4.2+/-6.6	2.4+/-1.5	1.4+/-0.5
Median	1.0	2.0	1.0
Season			
2010-2011 Mean+/-SD	2.9+/-2.9	1.8+/-1.3	1.2+/-0.4
Median	2.0	1.0	1.0

4. CONCLUSION

We monitored the changes in the serum levels of anti-influenza antibody titers for 2 seasons between 2010 and 2011 using the same strains. Booster effects were observed in the A/H2N3 strain in the 2nd season, but were not observed in the B strain. This observation suggests that the clinical efficacy of the vaccine of the B strain cannot be explained by the hypothesis that CD4+ T cells recognize the epitope of influenza vaccine antigen despite the humoral response being weak, unlike the A/H2N3 strain. The humoral response against the influenza vaccine may be variable with each strain among its strains. Additional vaccination each year may be necessary for the B strain from the viewpoint of humoral responses in RA patients having biologics treatment. Further observational studies are required to confirm the clinical efficacy in the RA patients of each strain of influenza vaccination.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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