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ORIGINAL ARTICLE—ALIMENTARY TRACT

Booster influenza vaccination does not improve immune response in adult inflammatory bowel disease patients treated with immunosuppressives: a randomized controlled trial

Hiroko Matsumoto · Satoko Ohfuji · Kenji Watanabe · Hirokazu Yamagami · Wakaba Fukushima · Kazuhiro Maeda · Noriko Kamata · Mitsue Sogawa · Masatsugu Shiba · Tetsuya Tanigawa · Kazunari Tominaga · Toshio Watanabe · Yasuhiro Fujiwara · Yoshio Hirota · Tetsuo Arakawa

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Abstract
Background This research was conducted is to assess the effect of booster doses of the trivalent influenza vaccine in adult inflammatory bowel disease (IBD) patients treated with anti-tumor necrosis factor (TNF)-α agents and/or immunomodulators.
Methods Adult IBD patients and healthy individuals were subcutaneously administered the trivalent influenza vaccine. They were randomized into two groups: the single vaccination group and the two vaccination booster group. Blood samples were collected, and the antibody titers against each influenza strain were determined by hemagglutination inhibition at 3 different time points (pre-vaccination, 3 weeks post-vaccination, and after the flu season) in the single vaccination group and at 4 time points (pre-vaccination, 3 weeks post-first vaccination, 3 weeks post-second vaccination, and after the flu season) in the booster vaccination group.
Results Seventy-eight IBD patients and 11 healthy controls were randomized into the single vaccination group and the booster vaccination group. Twenty-nine patients received immunomodulators; 21 received anti-TNF-α agents; and 28 received a combination of both. No significant differences were observed in the evaluated immune response parameters between 3 weeks post-vaccination in the single vaccination group and 3 weeks post-second vaccination in the booster vaccination group (geometric mean titers: H1N1, $p = 0.09$; H3N2: $p = 0.99$; B: $p = 0.94$). A higher pre-vaccination titer was significantly associated with sufficient seroprotection rate after vaccination for the H1N1 strain (odds ratio 11.93, $p = 0.03$).
Conclusions The second booster of trivalent influenza vaccination did not improve the immune response in adult IBD patients who were treated with immunomodulators and/or anti-TNF-α agents.

Keywords Inflammatory bowel disease · Immunomodulator · Anti-tumor necrosis factor-α agent · Booster vaccination · Influenza vaccine

Abbreviations

ADA	Adalimumab
AZA	Azathioprines
CD	Crohn's disease
GMT	The geometric mean titer
HAI	Hemagglutination inhibition
HBI	Harvey–Bradshaw index
HBV	Hepatitis B virus
IBD	Inflammatory bowel disease
IFX	Infliximab
OR	Odds ratio
TNF-α	Anti-tumor necrosis factor-α
UC	Ulcerative colitis

H. Matsumoto · K. Watanabe (✉) · H. Yamagami · N. Kamata · M. Sogawa · M. Shiba · T. Tanigawa · K. Tominaga · T. Watanabe · Y. Fujiwara · T. Arakawa
Department of Gastroenterology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
e-mail: m1158756@med.osaka-cu.ac.jp

S. Ohfuji · W. Fukushima · Y. Hirota
Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan

K. Maeda
Research Foundation for Microbial Disease of Osaka University, Osaka, Japan

Y. Hirota
Clinical Epidemiology Research Center, Medical Co. LTA, Fukuoka, Japan

UMIN-CTR University Hospital Medical Information Network Clinical Trial Registry
6MP 6-Mercaptopurine

Introduction

Patients with inflammatory bowel diseases (IBDs) such as ulcerative colitis (UC), Crohn's disease (CD), and intestinal Behçet's disease have chronic intestinal inflammation from various causes of environmental factors, dysregulated immune systems, and genetic susceptibility [1]. Immunosuppressive therapy, immunomodulators, or anti-tumor necrosis factor (TNF)-α agents are currently used in IBD patients to improve clinical outcomes with remission induction and maintenance; however, these treatments can increase adverse events, including infections [2–4]. In particular, elderly IBD patients may be at increased risk for opportunistic infections [5, 6].

Influenza is an annual respiratory infection that can cause serious complications. In the United States, influenza causes about 226,000 hospitalizations and about 36,000 related deaths every year [7, 8]. Patients who are compromised, elderly, or treated with immunosuppressive agents are at a higher risk of having complications if they are infected with the influenza virus [9]. Therefore, it is recommended for IBD patients who are treated with immunosuppressive agents to get the annual influenza vaccination [9].

We previously reported that immune responses to the trivalent influenza vaccination were inhibited for some strains in adult IBD patients who were treated with infliximab (IFX) and/or immunomodulators [10]. This has also been reported in pediatric IBD patients [11].

Children generally receive two trivalent influenza vaccinations in one season because of their immunogenicity [12–14]. A second booster influenza vaccination is effective in children for improving immune responses after insufficient immune responses following the first vaccination [15]. However, it has not been clarified whether a first and a second booster influenza vaccination might be less effective for adult IBD patients treated with IFX and/or IM in comparison with healthy controls.

Pediatric IBD patients treated with IFX are highly susceptible to hepatitis B virus (HBV) reactivation; thus, clinicians need to screen for HBV immunity when they are diagnosed with IBD. The HBV vaccine, the same inactive vaccine, showed an anamnestic immune response after the second booster vaccination [16]. In patients with rheumatoid arthritis who are receiving treatment with immunosuppressive drugs, optimization with a booster dose of the trivalent influenza vaccine is also considered [17, 18]. We conducted the first prospective randomized controlled study to evaluate the efficacy of booster doses of the trivalent influenza vaccination in adult IBD patients who were treated with anti-TNF-α agents and/or immunomodulators.

Methods

Subjects

We conducted a prospective, open label, randomized, controlled, parallel-group comparison study from November 2012 to July 2013 in the Department of Gastroenterology at the Osaka City University Hospital. The study protocol was approved by the Ethics Review Board of the Osaka City University Graduate School of Medicine, and it was registered at the University Hospital Medical Information Network Clinical Trial Registry in advance (UMIN000009259).

Study subjects consisted of IBD patients receiving immunosuppressive therapy, immunomodulators and/or anti-TNF-α agents, and healthy volunteers were the controls (≥20 years). The exclusion criteria were as follows: (1) subjects who had already received the 2012 trivalent inactivated influenza vaccine; (2) subjects with a history of influenza infection within the last 6 months; and (3) subjects with a history of anaphylactic reaction to a previous influenza vaccine or vaccine components, or an acute febrile illness or signs of severe acute illness at the time of vaccination. All subjects provided written informed consent after the study design and possible risks were explained. We estimated that the appropriate sample size for the primary objective was 108 IBD patients and 20 controls. This was based on the assumption of a 2.5 odds ratio (OR) for an appropriate immune response in the booster two vaccination group compared to the single vaccination group, according to the data of our preliminary study, and a power of 80 % and an alpha of 0.05. The IBD patients were randomized into a single or booster vaccination group with a 1:1 ratio, allocation for age (<49 and ≥49 years), and the type of immunosuppressive therapy (i.e., immunomodulator monotherapy, anti-TNF-α agent monotherapy, or a combination of both). The controls were randomized into the single vaccination group or booster vaccination group.

Data collection

At the time of recruitment, we collected the following clinical information from the IBD patients' medical records: age, sex, diagnosed disease (UC, CD, or intestinal

Behçet's disease), duration of disease, current therapy [azathioprines (AZA), 6-mercaptopurine (6MP), IFX, and adalimumab (ADA)] that has been continued for >3 -months, disease activity [UC: partial Mayo score; CD: Harvey–Bradshaw index (HBI)]. A partial Mayo score of ≤2 for UC and HBI of ≤4 for CD are defined as remission stage.

Before vaccination, the subjects were asked to complete a self-administered questionnaire, which collected the following information: age at vaccination, body height and weight, underlying illnesses, past medical history, and allergic history (including allergy to eggs).

Vaccination with the trivalent vaccine

Each subject received a single dose or two doses as a booster of the 2012–2013 seasonal trivalent inactivated influenza vaccine (Lot HA119E; Biken, Osaka, Japan) subcutaneously. In Japan, subcutaneous administration is the routine for influenza vaccinations. The vaccine strains were A/California/7/2009 (H1N1) pdm09, A/Victoria/361/2011 (H3N2), and B/Wisconsin/01/2010 (B). A standard 0.5 mL dose of the vaccine contained 15 μg of the hemagglutinin antigen of each strain. For the booster vaccination group, the subjects received a second vaccination after 3 weeks from the first vaccination.

Measurement of hemagglutination inhibition antibody titers

Figure 1 presents an outline of the present study design. Serum samples were collected at 4 time points in the booster vaccination group: before vaccination (S0), 3 weeks after the first dose (S1), 3 weeks after the second dose (S2), and after the influenza season (after April 2013; S3). For the single vaccination group, the serum samples were collected at the following 3 time points: before vaccination (S0), 3 weeks after the first dose (S1), and after the influenza season (after April 2013; S3). All serum specimens were stored at −80 °C until they were tested for hemagglutination inhibition (HAI) antibody titers against all strains simultaneously. The HAI antibodies were measured using the standard microtiter HAI method with the same antigens as in the vaccine [19]. All samples were measured at the laboratory of the Research Foundation for Microbial Disease of Osaka University between July 2013 and September 2013.

Statistical analyses

The following outcomes were calculated to assess the immunogenicity of the influenza vaccine: the geometric mean titer (GMT), mean fold-rise, seroresponse rate (≥4-fold rise), and seroprotection rate (HI titer ≥1:40). For data processing, titers <1:10 were regarded as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results were presented in the original scale by calculating the antilogarithm. A stratified analysis was also performed to investigate the effect of potential confounders: sex, age at vaccination, disease duration, immunosuppressive treatment, defined disease, disease activity (remission or active), and pre-vaccination titer (<1:10, 1:10–1:20, and ≥1:40). The significance of the fold-rise within a category was assessed using the Wilcoxon signed-rank test, and inter-category comparisons were made by using either the Wilcoxon rank-sum or Kruskal–Wallis tests. The Chi square test or Mantel-extension method for the trend test was also used when appropriate. Furthermore, to consider the independent effect of the booster dose on the immune response, multivariate analyses were conducted using logistic regression models with potential confounders. We chose to adjust the variables,

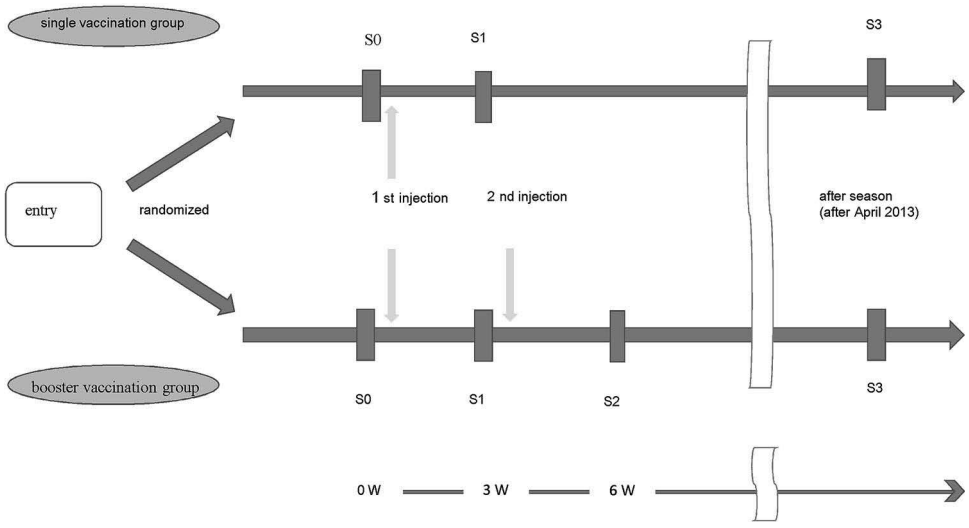

Fig. 1 An outline of the present study design

which revealed the differences in the stratified analysis, or we reported the effect on the immune response from previous studies, as potential confounders. All analyses were performed using SAS, version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Study participants

Seventy-eight IBD patients and 11 controls were enrolled. The baseline characteristics were well matched for sex, age at vaccination, disease, and disease duration between the two groups after randomization (Table 1). The immunosuppressive therapy, immunomodulator monotherapy, anti-TNF-α agent monotherapy, and combination therapy were also randomized well between the two groups ($p = 0.82$). Thirty-eight patients had CD, 33 had UC, and 7 had Behçet's disease. There were no statistically significant differences in disease activity between the two groups (HBI: $p = 0.66$; partial Mayo score: $p = 0.19$).

Forty-six participants received a single dose of the influenza vaccination and 43 participants received two doses between November 5, 2012 and December 28, 2012. All 89 subjects had follow up until July 2013.

Changes in the parameters of immunogenicity

The immune responses to the trivalent influenza vaccination for the 3 strains during each phase are shown in Table 2. Among 78 IBD patients, there were no significant differences in GMTs after vaccination between S1 in the single vaccination group and S2 in the booster vaccination group (H1N1: $p = 0.09$; H3N2: $p = 0.99$; B: $p = 0.94$). There were also no significant differences in GMTs after the flu season for each strain between the groups (H1N1: $p = 0.54$; H3N2: $p = 0.93$; B: $p = 0.90$). Although the seroprotection rate for the H1N1 strain after two vaccinations (S2) was lower in the booster vaccination group than that (S1) in the single vaccination group ($p = 0.04$), the seroprotection rates for the other strains were similarly observed in both groups. The seroprotection rates after the flu season were equally distributed for each strain between the groups (H1N1: $p = 0.35$; H3N2: $p = 0.80$; B: $p = 0.31$).

In the study subjects, the pre-vaccination titer for the B strain was very high compared to that of the H1N1 or H3N2 strains. Therefore, the seroprotection rates of the B strain for all participants after vaccination (S1 or S2) were 100 %.

In the single vaccination group, the seroprotection rate after vaccination (S1) was >70 % for every strain (H1N1: 85 %, H3N2: 82 %, B: 100 %). This means that the trivalent 2012/2013 seasonal influenza vaccine used in this study provided sufficient immune responses from a single vaccination, even though the IBD patients were treated with immunosuppressive agents [14].

Among the control subjects, there were no significant differences between the single vaccination and booster vaccination groups with regard to the GMT, fold-rise, and seroprotection rate at each point. In the control group, a relatively low pre-vaccination titer (≤1:20) was noted for H1N1 in 5 participants, for H3N2 in 9 participants, and for B in 0 participants. However, only one participant was unexpectedly randomized into the booster vaccination group; the GMT of this patient after the second vaccination did not improve.

Stratified immunogenicity analysis

To focus on the influences of the type of immunosuppressive treatment or pre-vaccination titer for the immune responses of the 3 strains at each phase, we performed a stratified analysis of the single vaccination and booster vaccination groups (Table 3). However, there were no associations between the types of immunosuppressive treatment (AZA or 6MP, IFX or ADA, AZA/6MP, and IFX/ADA) or the immune responses between the groups after vaccination (S1 in the single vaccination group vs. S2 in the booster vaccination group). Figure 2a shows the changing process of GMTs for the 3 strains according to the types of immunosuppressive treatment at each phase in only the booster vaccination group. The second booster vaccination did not influence GMTs in relation to the type of immunosuppressive treatment in adult IBD patients.

Conversely, subjects with a higher pre-vaccination titer showed higher GMTs and seroprotection rates for H1N1 and H3N2 strains after vaccination (S1 or S2; Table 3), as described in our previous study [10]. Figure 2b shows the changing process of GMT for the 3 strains according to the pre-vaccination titer in the booster group. All participants had a higher pre-vaccination titer (≥1:40) for the B strain. The second booster vaccination did not improve the GMT of the lower pre-vaccination titer group (<1:40) in adult IBD patients who were treated with immunosuppressive agents. Additionally, Fig. 2c shows the changing process of GMT for the 3 strains according to the immune response at S1 after the first vaccination in the booster vaccination group. The second booster vaccination also did not improve the GMT of the lower immune response group (<1:40) in adult IBD patients who were treated with anti-TNF-α agents and/or immunomodulators.

After adjusting for immunosuppressive therapy and the pre-vaccination titer, the booster vaccination group had a lower OR for seroprotection compared to the single vaccination group. Particularly, the decrease in OR of the

Table 1 Baseline characteristics of the study subjects

Characteristics	Study subjects *n* (%) All ($N = 89$)	Single group 46	Booster group 43	*p*
Gender				
Male	51 (57 %)	25 (54 %)	26 (60 %)	0.56
Female	38 (43 %)	21 (46 %)	17 (40 %)	
Age at vaccination (years ± SD)	43.9	45.3 (26–73)	42.4 (21–72)	0.29
Immunosuppressive therapy				
Healthy control	11 (12 %)	7 (15 %)	4 (1 %)	
AZA or 6MP	29 (33 %)	14 (30 %)	15 (35 %)	0.82
IFX or ADA	21 (24 %)	10 (22 %)	11 (26 %)	
IFX/ADA and AZA/6MP	28 (31 %)	15 (33 %)	13 (30 %)	
Disease duration (years ± SD)	9.37	8.8 (1–30)	10.0 (1–27)	0.76
Disease				
Crohn's disease	38 (43 %)	20 (22 %)	18 (20 %)	0.77
Ulcerative colitis	33 (37 %)	15 (17 %)	18 (20 %)	
Intestinal Behçet disease	7 (7 %)	4 (4 %)	3 (3 %)	
Disease activity				
HBI (CD)	4.03	3.61 (1–7)	4.47 (1–12)	0.66
Partial Mayo score (UC)	3.12	2.44 (0–8)	3.72 (0–10)	0.19

Data are expressed as no. (%) of patients, unless otherwise indicated

CD Crohn's disease, *UC* ulcerative colitis, *IFX* infliximab, *ADA* adalimumab, *AZA* azathioprine, *6MP* 6-mercaptopurine, *HBI* Harvey–Bradshaw index

booster group was marginally significant for the H1N1 strain (OR 0.34, $p = 0.05$; Table 4). Combination therapy with AZA/6MP and IFX/ADA did not result in a lower OR compared to monotherapy with AZA, 6MP, IFX, or ADA. A higher pre-vaccination titer (≥1:40) was significantly associated with a sufficient seroprotection rate after vaccination for the H1N1 strain (OR 11.93, $p = 0.03$).

Adverse events

There were no severe side effects such as fatalities or anaphylactic shock after vaccination. In the medical records, 4 patients in the single vaccination group and 4 patients in the booster vaccination group complained of pain with swelling at the site of the subcutaneous injection. The second booster vaccination did not result in additional adverse events.

Discussion

The influenza virus infection is an annual issue, and many people receive influenza vaccinations annually. The global H1N1 pandemic and its mortality in 2009 still remains in our memory, and the elderly and children are at high risk for the severe influenza infection [20]. Patients administered immunosuppressive agents are also at a high risk. Recent developments in immunomodulators or anti-TNF-α agents provide better prognoses for IBD patients, and several new immunomodulatory drugs, including biologics for individual target molecules, are under development in clinical trials [21]. Several guidelines recommend the annual influenza vaccination for IBD patients, especially those treated with immunosuppressive drugs, steroids, immunomodulators, or anti-TNF-α agents [26]. Educational intervention is effective for increasing the rate of vaccination [27], and the influenza vaccination is not associated with IBD flares [28].

However, some investigations reported an insufficient immune response to the influenza H1N1 vaccination in IBD patients treated with immunosuppressive drugs [29, 30]. For the first time, we also report an inhibited immune response to some strains of the trivalent influenza vaccination in adult IBD patients treated with IFX and/or immunomodulators [10]. Further investigations are needed to establish the appropriate influenza vaccination program for IBD patients taking immunosuppressive agents.

Lu et al. [11] reported that the trivalent influenza vaccination produces a high prevalence of seroprotection in pediatric IBD patients, particularly against A strains. IBD patients <8 years old received two booster vaccinations in that study. The proportion of seroprotected pediatric IBD patients and GMTs at post-vaccination was similar between the non-immunosuppressed therapy groups and the immunosuppressed therapy groups for all three strains. Regarding the other inactivated vaccine, the HBV vaccine, 76 % pediatric IBD patients who had an insufficient immune response after the first vaccination had an anamnestic response after the second booster vaccination [16]. Therefore, we conducted the present prospective

Table 2 Changes in the parameters of immunogenicity for the 3 strains of the trivalent influenza vaccine during the study period

IBD	Geometric mean titer[a]			Fold rise[a]	Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	S1/S0 for single S2/S0 for booster	Before vaccination (S0)	After vaccination[#]	After season (S3)
H1N1							
Single group	14	86	32	6.35***	8 (21)	33 (85)	27 (55)
Booster group	13	53	27	4.22***	8 (21)	25 (64)	20 (46)
p	0.98	0.09	0.54	0.41	1.00	0.04	0.35
H3N2							
Single group	16	81	57	4.95***	9 (23)	32 (82)	28 (72)
Booster group	20	77	50	3.86***	10 (26)	30 (77)	29 (74)
p	0.29	0.99	0.93	0.37	0.79	0.57	0.80
B							
Single group	68	169	116	2.48***	32 (82)	39 (100)	38 (97)
Booster group	96	169	120	1.77***	39 (100)	39 (100)	39 (100)
p	0.10	0.94	0.90	0.08	0.006	NA	0.31
Healthy control							
H1N1							
Single group	24	65	32	2.69*	4 (57)	6 (86)	5 (71)
Booster group	26	57	48	2.00*	3 (75)	4 (100)	3 (75)
p	0.77	0.92	0.68	0.77	0.55	0.43	0.90
H3N2							
Single group	16	40	24	2.44**	1 (14)	5 (71)	2 (29)
Booster group	28	57	28	2.00*	2 (50)	4 (100)	2 (50)
p	0.16	0.36	0.60	0.77	0.20	0.24	0.48
B							
Single group	98	160	108	1.64*	7 (100)	7 (100)	7 (100)
Booster group	95	160	80	1.68*	4 (100)	4 (100)	3 (75)
p	1.00	1.00	1.00	1.00	NA	NA	0.17

NA not applicable

[#] GMT after 1 vaccination (S1) for once group and GMT after 2 vaccinations (S2) for booster group

* $p < 0.1$, ** $p < 0.05$, *** $p < 0.0001$

[a] Wilcoxon signed-rank test for intracategory comparisons, and either the Wilcoxon rank-sum test or the Kruskal–Wallis test for intercategory comparisons

[b] Seroprotection rate (post-vaccination titer ≥1:40). $\chi 2$ test between 2 categories

randomized controlled study to evaluate the second booster of the trivalent influenza vaccination in adult IBD patients treated with immunosuppressive agents.

Our findings indicate that the booster of the trivalent influenza vaccination does not improve the immune responses to the 3 strains in adult IBD patients who are treated with immunomodulators and/or anti-TNF-α agents. Of note, the second booster of the influenza vaccination did not result in an additional immune response in patients who had an insufficient immune response in the present study (Fig. 2c). Only the single vaccination responded enough to meet the international licensing criteria of the European Agency for the Evaluation of Medical Products. In this study, the seroprotection rate after the first vaccination was high (H1N1: 85 %, H3N2: 82 %, B: 100 %) compared to our previous study (H1N1: 81 %, H3N2: 61 %, B: 86 %) [10]. This good reaction may be the reason for the low increase in antibody titers after the second vaccination. Moreover, when comparing the pre-vaccination titers of each strain between the present study and our previous study, a lower pre-vaccination titer (≤1:10) was observed for H1N1 in 37 and 60 % of participants, for H3N2 in 8 and 60 % of participants, and for B in 0 and 30 % of participants in the present and previous studies, respectively. As a higher pre-vaccination titer will yield a better immunoresponse after a single vaccination, good results were obtained in the present study after single injection [31]. Comparison with healthy controls also helped to understand the immunological status in IBD patients that was not suppressed in this series. Furthermore, a history of

Table 3 Stratified immunogenicity analyses of the 3 strains of the trivalent influenza vaccine according to the type of immunosuppressive treatment or pre-vaccination titer during the study period

	Influenza A (H1N1)					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
Single group						
Treatment						
Healthy control	24	66	36	4 (57)	6 (86)	5 (71)
AZA or 6MP	14	76	46	2 (14)	10 (71)	10 (71)
IFX or ADA	9	98	20	2 (20)	10 (100)	3 (30)
AZA/6MP and IFX/ADA	17	88	32	4 (27)	13 (87)	8 (53)
p	0.21	0.81	0.30	0.19	0.29	0.19
Pre-vaccination titer						
<1:10	5	108	18	0	14 (86)	5 (31)
1:10–1:20	14	54	32	0	13 (72)	10 (56)
≥1:40	71	120	71	12 (100)	12 (100)	11 (92)
p	<0.0001	0.03	0.003	<0.0001	0.11	0.006
Booster group						
Treatment						
Healthy control	28	57	48	3 (75)	4 (100)	3 (75)
AZA or 6MP	11	61	24	2 (13)	10 (67)	6 (40)
IFX or ADA	13	45	24	2 (18)	8 (73)	5 (45)
AZA/6MP and IFX/ADA	14	52	34	4 (31)	7 (54)	7 (54)
p	0.51	0.98	0.62	0.08	0.37	0.68
Pre-vaccination titer						
<1:10	5	34	16	0	8 (47)	5 (29)
1:10–1:20	15	61	35	0	11 (73)	7 (47)
≥1:40	55	91	55	11 (100)	10 (91)	9 (82)
p	<0.0001	0.10	0.008	<0.0001	0.04	0.02
	Influenza A (H3N2)					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
Single group						
Treatment						
Healthy control	16	40	24	1 (14)	5 (71)	2 (26)
AZA or 6MP	19	93	84	4 (29)	11 (76)	12 (86)
IFX or ADA	20	92	49	2 (20)	9 (90)	8 (80)
AZA/6MP and IFX/ADA	13	66	44	3 (20)	12 (80)	8 (53)
p	0.33	0.52	0.08	0.88	0.81	0.03
Pre-vaccination titer						
<1:10	5	23	23	0	2 (40)	1 (20)
1:10–1:20	14	75	45	0	25 (81)	19 (61)
≥1:40	49	121	106	10 (100)	10 (100)	10 (100)
p	<0.0001	0.02	0.02	<0.0001	0.02	0.006
Booster group						
Treatment						
Healthy control	28	57	28	2 (50)	4 (100)	2 (50)

Table 3 continued

	Influenza A (H3N2)					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
AZA or 6MP	21	101	55	5 (33)	12 (80)	11 (73)
IFX or ADA	26	71	58	3 (27)	9 (82)	10 (91)
AZA/6MP and IFX/ADA	15	61	40	2 (15)	9 (69)	8 (62)
p	0.18	0.51	0.31	0.53	0.60	0.30
Pre-vaccination titer						
<1:10	5	20	28	0	1 (50)	1 (50)
1:10–1:20	15	63	37	0	21 (72)	18 (62)
≥1:40	57	143	95	12 (100)	12 (100)	12 (100)
p	<0.0001	0.01	0.004	<0.0001	0.08	0.04
	Influenza B					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
Single group						
Treatment						
Healthy control	98	160	108	7 (100)	7 (100)	7 (100)
AZA or 6MP	57	152	125	10 (71)	14 (100)	14 (100)
IFX or ADA	86	139	98	10 (100)	10 (100)	10 (100)
AZA/6MP and IFX/ADA	70	211	121	12 (80)	15 (100)	14 (93)
p	0.47	0.37	0.69	0.15	NA	0.55
Pre-vaccination titer						
<1:10						
1:10–1:20	18	98	88	0	7 (100)	9 (100)
≥1:40	92	184	120	39 (100)	39 (100)	3 (97)
p	<0.0001	0.02	0.17	<0.0001	NA	0.67
Booster group						
Treatment						
Healthy control	95	160	80	4 (100)	4 (100)	3 (75)
AZA or 6MP	96	175	121	15 (100)	15 (100)	15 (100)
IFX or ADA	117	132	132	11 (100)	11 (100)	11 (100)
AZA/6MP and IFX/ADA	80	198	110	13 (100)	13 (100)	13 (100)
p	0.78	0.64	0.98	NA	NA	0.02
Pre-vaccination titer						
<1:10						
1:10–1:20						
≥1:40	96	168	116	43 (100)	43 (100)	42 (98)

NA not applicable, *IFX* infliximab, *ADA* adalimumab, *AZA* azathioprine, *6MP* 6-mercaptopurine

[#] GMT after 1 vaccination (S1) for one group and GMT after second vaccination (S2) for booster group

[a] Wilcoxon signed-rank test for intracategory comparisons, and either the Wilcoxon rank-sum test or the Kruskal–Wallis test for intercategory comparisons

[b] Seroprotection rate (post-vaccination titer ≥1:40). The Mantel-extension method for trend test among 3 categories

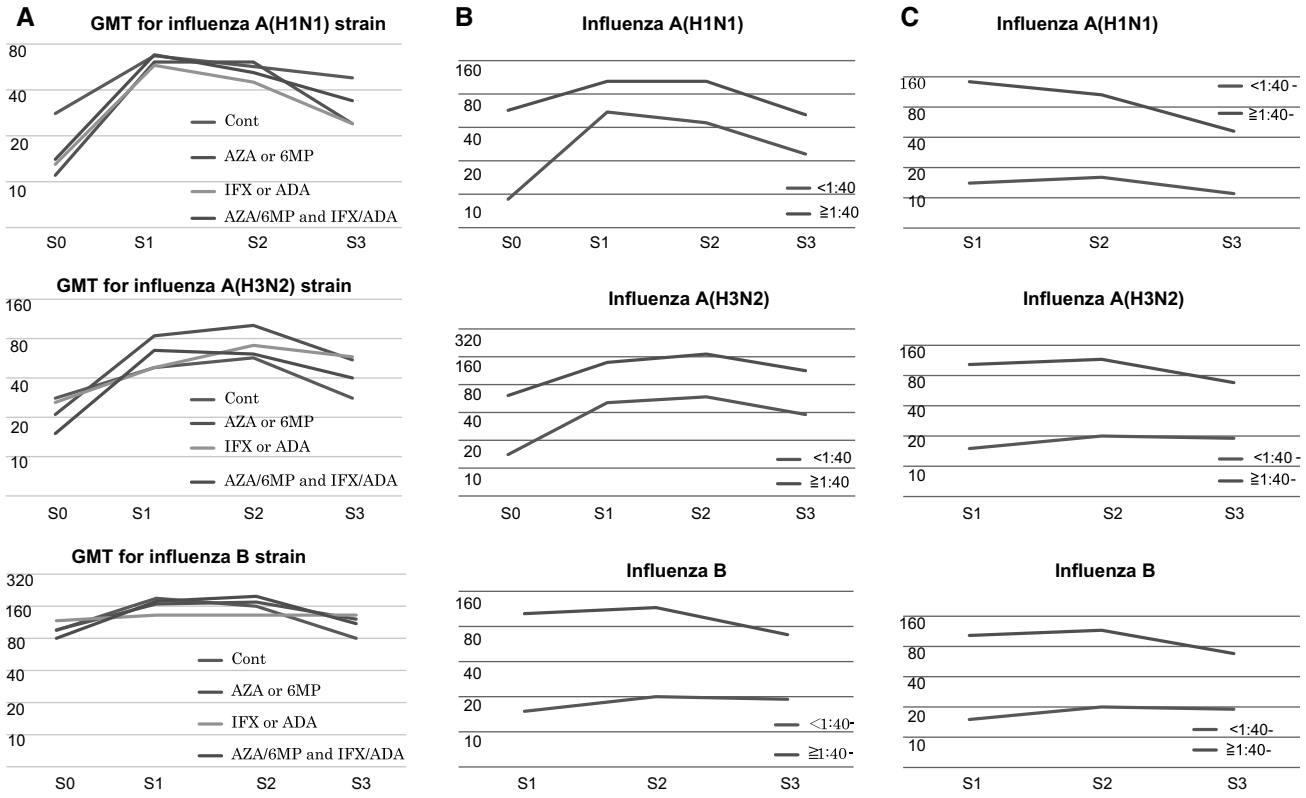

Fig. 2 **a** The change in the geometric mean titers for the 3 strains according to the immunosuppressive treatment in the booster vaccination group involving IBD patients and controls. **b** The changing process of geometric mean titers for the 3 strains according to the pre-vaccination titer in the booster vaccination group. **c** The changing process of geometric mean titer for the 3 strains according to the immune response at 3 weeks after the first dose (S1) after the first vaccination in the booster vaccination group

influenza infection or vaccination influences the immunoresponse for each strain. In Japan, vaccination for influenza has been performed for the same H1N1 strain annually after 2011. However, a difference was noted for strain B between 2010 and 2011 (Victoria lineage), and 2012 (Yamagata lineage) based on the estimation of the influenza epidemic.

A randomized study in children showed that the second booster of the influenza vaccination was effective for improving the seroprotection rate [32]. In contrast, the booster vaccination did not provide an anamnestic immune response in the elderly [32–34]. We reported that the second booster vaccination was not as effective in adults with severe motor and intellectual disabilities [35]. Thus, we expected that the most important factor of the immune response for the second booster vaccination was age. Humoral immune responses develop with increasing age, supporting the notion of broadening of immune responses and affinity maturation of the antibodies that are produced [33, 34].

There were limitations to the present study. The number of participants was small. When participant recruitment was initiated (November 2012), the influenza vaccination period had already begun in Japan. Hence, many IBD patients in our hospital may have been administered the vaccination in other hospitals or clinics. Therefore, we could not meet our target for the number of patients recruited. However, if the number of participants were increased, the results would not be so different from our current results according to the statistical analysis. The immune response to influenza strain B is usually less seroprotected compared to strain A [11]. Yet, the pre-vaccination titer of strain B was extremely high in our study. Pre-existing antibody titers provide a substantial effect on immune response [35]. Our study also showed that higher pre-vaccination titers to the H1N1 strain were associated with a sufficient immune response for the influenza vaccination (Fig. 2a; Table 4).

Immune response is different from the incidence rate of influenza. The approaches based on $CD4^+$ or $CD8^+$ T cells specific to the conserved viral core protein epitopes correlated with the cross-reactive cellular immune responses, not the strain-matched B cell, which may make the development of a novel influenza vaccine possible [35, 36].

In conclusion, this is the first prospective randomized controlled study to investigate the efficacy of a second booster vaccination on the immune response in adult IBD

Table 4 Multivariate analysis of the associated factors for a sufficient seroprotection rate after vaccination

	Influenza A (H1N1) OR (95 % CI)	*p*	Influenza A (H3N2) OR (95 % CI)	*p*
One S1	1		1	
Booster S2	0.34 (0.11–1.01)	0.05	0.68 (0.22–2.17)	0.52
Immunosuppressive therapy				
Healthy control	1		1	
AZA or 6MP	0.58 (0.05–6.41)	0.65	1.11 (0.16–7.74)	0.92
IFX or ADA	1.64 (0.12–22.05)	0.71	1.60 (0.20–12.58)	0.66
AZA/6MP and IFX/ADA	0.49 (0.04–5.38)	0.56	1.12 (0.17–7.60)	0.91
Pre-vaccination titer				
<1:10	1		1	
1:10–1:20	1.34 (0.44–4.09)	0.61	4.44 (0.80–24.77)	0.09
≥1:40	11.93 (1.30–109.19)	0.03	Not applicable	

Model included all variables in this table

IFX infliximab, *ADA* adalimumab, *AZA* azathioprine, *6MP* 6-mercaptopurine

Logistic regression model: *CI* confidence interval, *OR* odds ratio

patients taking immunosuppressive agents. The second booster of the trivalent influenza vaccination did not improve the immune response of adult IBD patients treated with immunomodulators and/or anti-TNF-α agents. The booster influenza vaccination does not appear to be necessary in adult IBD patients and healthy adults. With regard to the trends of immunosuppressive therapy for IBD patients, further investigations are essential for establishing an appropriate influenza vaccination strategy in high-risk IBD patients.

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