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Infliximab and/or immunomodulators inhibit immune responses to trivalent influenza vaccination in adults with inflammatory bowel disease

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KEYWORDS
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Abstract

Background and aims: Appropriate influenza vaccination is important for patients with inflammatory bowel disease under immunosuppressive therapy. The purpose of this study was to evaluate the influence of immunosuppressive therapy on the immune response to the trivalent influenza vaccine in adult patients with inflammatory bowel disease.
Methods: In this cohort study, 91 participants received a single dose of influenza vaccine for the 2010/2011 season. Serum samples were collected at 3 different times (pre-vaccination, 3 weeks post-vaccination, and after flu season) to measure hemagglutination inhibition antibody titers. Immune responses were compared based on immunosuppressive therapy.
Results: Among the 88 subjects who completed the study, the influenza vaccine induced a more than 4-fold increase in the mean antibody level for all flu strains. The overall seroprotection proportion (post-vaccination titer $\geq$ 1:40) was 81% for H1N1, 61% for H3N2, and 86% for B. Treatment with an immunomodulator reduced the immune response to the H1N1 strain (OR = 0.20, p = 0.01), and treatment with infliximab reduced the immune response to the

Abbreviations: AZA, azathioprine; CAI, clinical activity index; CD, Crohn's disease; CDAI, Crohn's disease activity index; CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition; IFX, infliximab; IS, group immunosuppressive group; 6MP, 6-mercaptopurine; NIS, group non-immunosuppressive group; OR, odds ratio; SD, standard deviation; UC, ulcerative colitis; WHO, World Health Organization.

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other strains (H3N2 strain: OR = 0.37, $p = 0.02$; B strain: OR = 0.18, $p = 0.03$). Combination therapy with azathioprine/6-mercaptopurine and infliximab significantly inhibited the immune response to H1N1 (OR = 0.056, $p = 0.02$).
Conclusions: Infliximab and/or immunomodulators inhibit immune responses to some strains of trivalent influenza vaccination in adults with inflammatory bowel disease. For optimization of the trivalent influenza vaccination for patients with adult inflammatory bowel disease treated with immunosuppressive agents, establishing an effective vaccination method is crucial.

1. Introduction

Inflammatory bowel disease (IBD), ulcerative colitis (UC), and Crohn's disease (CD) are accompanied by chronic inflammation of the gastrointestinal tract due to a complex interplay between environmental factors, dysregulated immune systems, and genetic susceptibility.[1] Immunosuppressive (IS) therapeutics such as immunomodulators or anti-tumor necrosis factor-α (TNF-α) agents are frequently used as aggressive therapies for IBD. However, immunosuppressive agents such as systemic corticosteroids, azathioprines (AZA)/6-mecaptopurine (6-MP), tacrolimus, methotrexate, and anti-TNF-α agents (e.g., infliximab [IFX]) increase the risk for more frequent and severe infections in IBD patients.[2–4] Combination therapies using more than one IS agent are especially associated with increased risk for opportunistic infections,[5] including bacterial and many severe and fatal viral infections.[6–8] Recent publications recommend more appropriate vaccination strategies for IBD patients as infection prophylaxis prior to IS therapy.[9,10]

Influenza, caused by type A or type B viruses, is a prevalent respiratory illness that can lead to other associated complications and hospitalization. Influenza patients often seek medical attention in hospital emergency rooms, and absence rates for workers and students increase dramatically during the influenza season.[11] In the US, approximately 226,000 patients are hospitalized annually for influenza, and approximately 36,000 cases of influenza-related deaths are reported each year.[12,13] In 2009, the World Health Organization (WHO) reported of the human infection with influenza A(H1N1). HIN1 spread rapidly throughout the world during the 2009/2010 influenza season, leading WHO to declare a phase 6 pandemic alert.[14] Epidemiologic studies for the pandemic outbreak in 2009 revealed that the risk of influenza-associated complications for adults infected with influenza A(H1N1)pdm09 was higher than usual for seasonal influenza.[15]

Several recent studies that examined the immunogenicity of the influenza A(H1N1)pdm09 vaccine in IBD patients[16–23] have cautioned that combination therapy with anti-TNF-α agents and immunomodulators (AZA/6MP) may reduce the immune response to vaccines.[17,18] Similar findings have been reported for the trivalent influenza vaccine, which is routinely distributed as a seasonal influenza vaccine.[16,22,24] These reports also showed that children undergoing IS therapy for IBD exhibited reduced immune response to the vaccine. To the best of our knowledge, however, no studies have reported the effect of IS therapy on the specific immune response to the individual strains covered by the trivalent influenza vaccine in adults with IBD. Although adults are generally considered to generate a better immune response to the vaccine than children do, it is important to examine the effect of IS therapy in adult IBD patients. Therefore, the aim of the present study was to investigate the immune response to the trivalent influenza vaccine in adult IBD patients undergoing IS treatments.

2. Materials and methods

2.1. Subjects

We conducted this prospective, open label, cohort study from September 2010 to July 2011 in the Department of Gastroenterology at Osaka City University Hospital. Between 29 September 2010 and 14 October 2010, IBD outpatients (minimum age, 20 years) were recruited for participation in the study.

The exclusion criteria were as follows: patient had already received 2010 trivalent inactivated influenza vaccine; patient had history of influenza infection within the last 6 months; patient had history of anaphylactic reaction to previous influenza vaccine or vaccine components or of acute febrile illness or signs of severe acute illness at the time of vaccination. All participants provided written, informed consent following a detailed explanation of the nature and possible consequences of the study. All participants in the study signed informed consent forms. We estimated the appropriate sample size was 100 participants for the present study based on the reference of the guidance of the European Committee for Proprietary Medical Products.[25] The study protocol was approved by the Ethics Review Board of the Osaka City University Graduate School of Medicine.

2.2. Data acquisition

At the time of recruitment, we obtained the following patient information from the medical records: defined disease (ulcerative colitis [UC] or Crohn's disease [CD]); disease duration; current IS therapy (corticosteroids, tacrolimus, AZA, 6-MP and IFX), which has been continued for more than 3 months; and data from blood tests (white blood cell count, differential leukocyte count, serum albumin, hematocrit, C-reactive protein). All medications were required to be stable prior to vaccination and for at least 3 weeks after vaccination. Validated clinical activity scores, clinical activity index (CAI) of Rachmilewitz index,[26] and Crohn's disease activity index (CDAI),[27,28] were used to assess disease activity in patients with UC and CD, respectively. A CAI score of ≥ 5 for UC and a CDAI score of >150 for CD were defined as active

stage, and a CAI of ≤ 4 for UC and a CDAI of ≤ 150 for CD were defined as remission stage. Participants receiving IS therapy at the time of vaccination were classified as the immunosuppressive (IS) group, and the remaining participants were considered the non-immunosuppressive (NIS) group, which included participants treated with other medications (e.g., 5-aminosalicylates).

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

2.3. Vaccination with trivalent vaccine

All participants received a single subcutaneous dose of the 2010 trivalent inactivated influenza vaccine (Lot. HA101E, BIKEN, Osaka, Japan). This vaccine included the following antigen strains: A/California/7/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008. A standard 0.5-mL dose of the vaccine contained 15 μg of the hemagglutinin antigen of each strain.

2.4. Determination of hemagglutination inhibition antibody titers

Serum samples were collected at 3 time points: before vaccination (S0), 3 weeks post-vaccination (S1) according to our previous investigation[29], and after the influenza season (S2; approximately 7 months after vaccination). All serum specimens were stored at −80 °C until used for testing for hemagglutination inhibition (HAI) antibody titers against all 3 strains. HAI antibody titers were determined using the standard microtiter HAI method with the same antigens as in the vaccine.[29,30] All samples were assayed at the laboratory of the Research Foundation for Microbial Diseases of Osaka University between July and September 2011.

2.5. Assessment of side effects

Participants were surveyed regarding the presence of related symptoms for the following side effects: ocular and respiratory symptoms within 24 h after vaccination (red eyes, facial edema, and any respiratory symptoms—coughing, wheezing, chest tightness, difficulty breathing, difficulty swallowing, and sore throat), systemic symptoms within 48 h (fever, general malaise, myalgia, headache, and rash), and local symptoms within 48 h (redness, swelling, induration, itching, and pain).

2.6. Statistical analyses

The following outcomes were calculated to assess the immunogenicity of the influenza vaccine: geometric mean titer (GMT), mean fold-rise, seroresponse proportion ($\geq$4-fold rise), and seroprotection proportion (post-vaccination titer $\geq$1:40). For data processing, titers less than 1:10 were regarded as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results are presented in the original scale by calculating the antilogarithm. We also performed a stratified analysis to investigate the effect of potential confounders: age at vaccination (tertile), sex, defined disease (UC or CD), disease activity (remission or active), immunosuppressive treatment, and pre-vaccination titer (<1:10, 1:10–1:20, and $\geq$1:40). The significance of fold-rise within a category was assessed by the Wilcoxon signed-rank test, and intercategory comparisons were made using either the Wilcoxon rank-sum test or the Kruskal–Wallis test. The χ^2 test or Mantel-extension method for the trend test was also used where appropriate.

Furthermore, to consider the independent effect of individual immunosuppressive therapy for immune response, multivariate analyses were conducted using logistic regression

Table 1 Baseline characteristics of participants with inflammatory bowel disease.

	All (n = 88)
Characteristics	*n* (%)
Age at vaccination (years)	
Mean (±SD)	44.4 (±14.4)
Gender	
Male	51 (58)
Female	37 (42)
Disease	
UC	45 (51)
CD	43 (49)
Disease activity	
Remission stage	74 (84)
Active stage	14 (16)
Immunosuppressive therapy	
Not receiving(NIS group)	30 (34)
Receiving(IS group)	58 (66)
Corticosteroids	6 (7)
Tacrolimus	2 (2)
AZA/6MP	31 (35)
AZA/6MP monotherapy	21 (24)
AZA/6MP + IFX	10 (11)
IFX	33 (38)
IFX monotherapy	23 (26)
IFX + AZA/6MP	10 (11)
Pre-vaccination titer	
H1N1 < 1:10	51 (58)
1:10–1:20	20 (23)
$\geq$1:40	17 (19)
H3N2 < 1:10	53 (60)
1:10–1:20	25 (28)
$\geq$1:40	10 (11)
B < 1:10	26 (30)
1:10–1:20	29 (33)
$\geq$1:40	33 (38)

SD, standard deviation; UC, ulcerative colitis; CD, Crohn's disease. NIS group, non-immunosuppressive group; IS group, immunosuppressive group.
AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Data are expressed as *n* (%) of patients, unless otherwise indicated.

Table 2 Changes in the geometric mean titer for each strain of trivalent influenza vaccine during the study period.

Category	Influenza A(H1N1)			Fold rise [a]	Influenza A(H3N2)			Fold rise [a]	Influenza B			Fold rise [a]
	Geometric mean titer [a]				Geometric mean titer [a]				Geometric mean titer [a]			
	Before vaccination	After vaccination	After season	S1/S0	Before vaccination	After vaccination	After season	S1/S0	Before vaccination	After vaccination	After season	S1/S0
	(S0)	(S1)	(S2) [b]		(S0)	(S1)	(S2) [b]		(S0)	(S1)	(S2) [b]	
Total patients	11	83	30	7.7***	8.5	55	26	6.4***	21	95	44	4.6***
Tertile age at vaccination (years)												
<38	16	94	39	5.9***	8	61	30	7.4***	20	118	57	5.8***
38–48	10	108	37	11***	6.6	40	17	6.1***	26	113	53	4.3***
≥49	8	54	18	7.1***	12	69	34	5.8***	16	62	28	3.9***
	$p = .08$	$p = .11$	$p = .03$	$p = .43$	$p = .12$	$p = .30$	$p = .09$	$p = .71$	$p = .29$	$p = .16$	$p = .07$	$p = .44$
Gender												
Male	11	87	31	7.8***	8	45	21	5.9***	21	90	41	4.3***
Female	10	77	28	7.6***	10	71	36	7.3***	20	102	50	5.1***
	$p = .82$	$p = .27$	$p = .14$	$p = .99$	$p = .43$	$p = .14$	$p = .43$	$p = .71$	$p = .88$	$p = .80$	$p = .68$	$p = .73$
Disease												
UC	9	69	24	7.5***	9	75	35	8.5***	21	93	45	4.5***
CD	21	97	37	7.9***	8	39	19	4.8***	21	97	44	4.7***
	$p = .49$	$p = .17$	$p = .11$	$p = .74$	$p = .51$	$p = .06$	$p = .02$	$p = .15$	$p = .98$	$p = .60$	$p = .86$	$p = .84$
Disease activity												
Remission stage	10	76	27	7.6***	8	50	24	6.2***	19	90	$p = .38$	4.6***
Active stage	16	131	47	8.0**	11	88	38	8.0**	30	131	49	4.4**
	$p = .30$	$p = .13$	$p = .17$	$p = .89$	$p = .49$	$p = .04$	$p = .23$	$p = .53$	$p = .30$	$p = .38$	$p = .90$	$p = .74$
Immunosuppressive therapy												
−(NIS group)	10	88	33	8.4***	13	73	35	5.7***	27	90	45	3.3***
+(IS group)	11	80	28	7.4***	7	47	22	6.8***	18	98	44	5.5***
	$p = .76$	$p = .58$	$p = .61$	$p = .99$	$p = .001$	$p = .12$	$p = .045$	$p = .28$	$p = .18$	$p = .56$	$p = .99$	$p = .10$

Corticosteroids												
−	10	78	29	7.6***	8.7	47	22	5.3***	20	86	39	4.2***
+	20	180	45	9**	6.3	508	226	81**	28	403	202	14*
	$p = .20$	$p = .12$	$p = .39$	$p = .94$	$p = .28$	$p = .0004$	$p = .001$	$p = .0002$	$p = .63$	$p = .002$	$p = .002$	$p = .07$
Tacrolimus												
−	11	83	30	7.7***	8.6	56	27	6.4***	21	96	45	4.5***
+	10	80	20	8	5	28	10	5.7	7	80	20	11
	$p = .47$	$p = .97$	$p = .62$	$p = .79$	$p = .26$	$p = .52$	$p = .30$	$p = .85$	$p = .24$	$p = .69$	$p = .34$	$p = .29$
AZA/6MP												
−	12	97	32	7.8***	9.4	51	25	5.4***	21	91	43	4.3***
+	8.2	61	26	7.5***	7.2	63	28	8.7***	20	102	47	5.2***
	$p = .12$	$p = .04$	$p = .53$	$p = .94$	$p = .12$	$p = .56$	$p = .64$	$p = .099$	$p = .41$	$p = .41$	$p = .70$	$p = .76$
IFX												
−	10	82	31	8.3***	9	77	37	8.2***	25	112	55	4.4***
+	12	83	28	6.8***	7	31	15	4.2***	15	72	30	4.9***
	$p = .61$	$p = .85$	$p = .77$	$p = .67$	$p = .27$	$p = .004$	$p = .002$	$p = .09$	$p = .04$	$p = .19$	$p = .09$	$p = .73$
Pre-vaccination titer												
<1:10	5	73	21	15***	5	42	19	8.3***	5	58	23	12***
1:10–1:20	15	83	29	5.7***	12	70	30	5.7***	13	76	31	5.6***
≥1:40	74	120	77	1.6**	61	130	86	2.1**	91	170	102	1.9***
	$p < .0001$	$p = .02$	$p = .001$	$p < .0001$	$p < .0001$	$p = .002$	$p = .001$	$p < .0001$	$p < .0001$	$p = .004$	$p < .0001$	$p < .0001$

UC, ulcerative colitis; CD, Crohn's disease; NIS group, non-immunosuppressive group; IS group, immunosuppressive group; AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.

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

[b] Excluded influenza infected patients for 3 physician diagnosed influenza patients and patients(3 of H1N1 and 1 of B) with serologically diagnosed influenza.

* $p < .1$.

** $p < .05$.

*** $p < .0001$.

Table 3 Seroprotection proportion to trivalent influenza vaccine during the study period.

Category	Number with Seroprotection proportion (≥1:40), *n* (%)								
	Influenza A(H1N1)			Influenza A(H3N2)			Influenza B		
	S0	S1	S2	S0	S1	S2	S0	S1	S2
Total patients	17 (19%)	71 (81%)	41 (51%)	10 (11%)	54 (61%)	31 (37%)	33 (38%)	76 (86%)	55 (66%)
Tertile age at vaccination (years)									
<38	10 (33%)	26 (87%)	18 (69%)	3 (10%)	20 (67%)	12 (42%)	13 (43%)	29 (97%)	22 (81%)
38–48	4 (13%)	27 (90%)	16 (57%)	0 (0%)	15 (50%)	6 (21%)	13 (43%)	27 (90%)	20 (69%)
≥49	3 (11%)	18 (64%)	7 (26%)	7 (25%)	19 (68%)	13 (48%)	7 (25%)	20 (71%)	13 (48%)
		p = .03	*p* = .002	*p* = .06	*p* = .95	*p* = .70	*p* = .53	*p* = .006	*p* = .01
Gender									
Male	11 (21%)	40 (78%)	27 (55%)	5 (10%)	29 (57%)	14 (29%)	19 (37%)	42 (82%)	30 (63%)
Female	6 (16%)	31 (84%)	14 (44%)	5 (14%)	25 (68%)	17 (49%)	14 (38%)	34 (92%)	25 (71%)
	p = .65	*p* = .53	*p* = .325 (14%)	*p* = .56	*p* = .31	*p* = .06	*p* = .59	*p* = .20	*p* = .40
Disease									
UC	5 (11%)	34 (76%)	16 (37%)	5 (11%)	32 (71%)	19 (43%)	16 (36%)	40 (89%)	31 (70%)
CD	12 (28%)	37 (86%)	25 (66%)	5 (12%)	22 (51%)	12 (30%)	17 (40%)	36 (84%)	24 (62%)
	p = .048	*p* = .21	*p* = .01	*p* = .57	*p* = .06	*p* = .21	*p* = .61	*p* = .48	*p* = .39
Disease activity									
Remission stage	13 (18%)	58 (78%)	33 (49%)	8 (11%)	23 (33%)	47 (68%)	26 (35%)	63 (85%)	47 (68%)
Active stage	4 (29%)	13 (93%)	8 (62%)	2 (14%)	8 (57%)	8 (57%)	7 (50%)	13 (93%)	8 (57%)
	p = .63	*p* = .21	*p* = .39	*p* = .93	*p* = .04	*p* = .43	*p* = .50	*p* = .44	*p* = .43
Immunosuppressive therapy									
−(NIS group)	5 (17%)	25 (83%)	14 (50%)	7 (23%)	23 (77%)	13 (45%)	11 (37%)	26 (87%)	19 (66%)
+(IS group)	12 (21%)	46 (79%)	27 (51%)	3 (5%)	31 (53%)	18 (33%)	22 (38%)	50 (86%)	36 (67%)
	p = .50	*p* = .65	*p* = .94	*p* = .002	*p* = .06	*p* = .27	*p* = .02	*p* = .95	*p* = .92
Corticosteroids									
−	15 (18%)	65 (79%)	37 (49%)	10 (12%)	48 (59%)	26 (33%)	30 (37%)	70 (85%)	49 (64%)
+	2 (33%)	6 (100%)	4 (67%)	0 (0%)	6 (100%)	5 (83%)	3 (50%)	6 (100%)	6 (100%)
	p = .44	*p* = .21	*p* = .4110 (12%)	*p* = .45	*p* = .04	*p* = .01	*p* = .66	*p* = .31	*p* = .07
Tacrolimus									
−	17 (20%)	69 (80%)	41 (52%)	10 (12%)	53 (62%)	31 (38%)	33 (38%)	74 (86%)	54 (67%)
+	0 (0%)	2 (100%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	2 (100%)	1 (50%)
	p = .03	*p* = .48	*p* = .14	*p* = .51	*p* = .74	*p* = .27	*p* = .54	*p* = .57	*p* = .62
AZA/6MP									
−	13 (23%)	50 (88%)	28 (53%)	8 (14%)	35 (61%)	18 (33%)	20 (35%)	49 (86%)	35 (65%)
+	4 (13%)	21 (68%)	13 (46%)	2 (6%)	19 (61%)	13 (45%)	13 (42%)	27 (87%)	20 (69%)
	p = .36	*p* = .02	*p* = .58	*p* = .29	*p* = .99	*p* = .27	*p* = .12	*p* = .88	*p* = .70
IFX									
−	9 (16%)	44 (80%)	24 (48%)	8 (15%)	39 (71%)	24 (46%)	23 (42%)	50 (91%)	39 (75%)
+	8 (24%)	27 (82%)	17 (55%)	2 (6%)	15 (45%)	7 (22%)	10 (30%)	26 (79%)	16 (52%)
	p = .55	*p* = .83	*p* = .55	*p* = .50	*p* = .02	*p* = .03	*p* = .16	*p* = .11	*p* = .03
Pre-vaccination titer									
<1:10	0 (0%)	35 (69%)	18 (40%)	0 (0%)	28 (53%)	12 (24%)	0 (0%)	17 (65%)	11 (42%)
1:10–1:20	0 (0%)	19 (95%)	8 (40%)	0 (0%)	16 (64%)	9 (38%)	0 (0%)	26 (90%)	15 (57%)
≥1:40	17 (100%)	17 (100%)	15 (94%)	10 (100%)	10 (100%)	10 (100%)	33 (100%)c	33 (100%)	30 (94%)
	p < .0001	*p* = .003	*p* = .001	*p* < .0001	*p* = .02	*p* < .0001	*p* < .0001	*p* = .0005	*p* = .0001

UC, ulcerative colitis; CD, Crohn's disease; NIS group, non-immunosuppressive group; IS group, immunosuppressive group.
AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Data are expressed as *n* (%) of patients, unless otherwise indicated.
χ^2 test between 2 categories and the Mantel-extension method for trend test among 3 categories.

models with potential confounders. The models were constructed with seroprotection after vaccination as the dependent variable, and the following factors were selected as potential confounders (age, disease activity, and pre-vaccination titer) because these variables were suggested to be associated with seroprotection for at least 1 of 3 vaccine strains in the univariate analyses ($p < 0.05$). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

To assess side effects, we compared the proportion of patients with each symptom across the 2 groups (IS and NIS groups) using the χ^2 test or Fisher's exact test. All tests were 2-sided. All analyses were performed using SAS, version 9.1.3 (SAS Institute).

3. Results

3.1. Study participants

Ninety-one IBD patients received a single dose of the influenza vaccine between 29 September 2010 and 14 October 2010. The participants were followed-up until July 2011 (i.e., study period). Serum samples at S0 (before vaccination), S1 (3 weeks post-vaccination), and S2 (after influenza season) were collected from 91, 88, and 88 patients, respectively. Between S1 and S2, however, 3 subjects were diagnosed with influenza by the rapid test at a medical institution. Another 4 subjects were serologically diagnosed with influenza infection (3 with A(H1N1) and 1 with B; titer increased more than 4 times in S2 compared with S1). Thus, the data from these infected subjects were excluded from S2 analysis.

Patient characteristics are shown in Table 1. Forty-five patients had UC and 43 had CD. Five patients with UC and 9 with CD were in the active stage according to the respective disease activity index before vaccination. Fifty-eight patients were treated with immunosuppressive therapy (IS group) and the other 30 were placed into the NIS group.

3.2. Immune responses to the trivalent influenza vaccine

The immune responses (GMT, fold-rise, seroresponse proportion and, seroprotection proportion) to the trivalent influenza vaccine were calculated. Table 2 summarizes the change in the GMTs and fold-rise for each vaccine strain during the study period. In all participants, GMTs after vaccination (S1) increased to 83 (H1N1), (H3N2), and 95 (B), representing a mean fold-rise of 7.7 (H1N1), 6.4 (H3N2), and 4.6 (B), respectively. The corresponding seroresponse proportion ($\geq$4 fold-rise) was 73% (95% CI, 64–82%) for H1N1, 67% (57–77%) for H3N2, and 53% (43–63%) for B. These findings suggested that the trivalent 2010/11 seasonal influenza vaccine was immunogenic in adult IBD patients.[31] After the influenza season (S2), however, GMTs decreased to less than 50% for all 3 vaccine strains.

GMTs and fold-rise after vaccination (S1) did not differ significantly with respect to age, sex, disease, disease activity, or immunosuppressive therapy (NIS or IS group). On the other hand, patients with a higher pre-vaccination titer had higher GMTs and lower mean fold-rises (all 3 strains, $p < 0.0001$). For individual immunosuppressive therapy, participants treated with corticosteroids exhibited unexpectedly increased GMTs for H3N2 and B, whereas those treated with AZA/6-MP or with IFX had significantly decreased GMTs for H1N1 or H3N2, respectively.

Table 3 summarizes the changes in the seroprotection proportion for each vaccine strain during the study period. In all participants, vaccination increased the seroprotection proportion to 81% (73–89%) for H1N1, 61% (51–71%) for H3N2, and 86% (79–93%) for B; the proportion was slightly lower for H3N2 than for the other strains. After the influenza season (S2), these proportions decreased to 51% (40–62%) for H1N1, 37% (48–74%) for H3N2, and 66% (56–76%) for B, respectively.

In the stratified analyses, older participants exhibited significantly decreased seroprotection against H1N1 ($p = 0.03$) and B ($p = 0.006$) at S1. With respect to the clinical characteristics, participants in remission stage exhibited significantly reduced immune responses to H3N2 ($p = 0.04$). Participants treated with corticosteroids exhibited increased seroprotection especially against H3N2, whereas those treated with AZA/6-MP and those treated with IFX showed significantly decreased seroprotection against H1N1 and H3N2, respectively.

3.3. Effect of independent and combination immunosuppressive therapy on immune responses to the trivalent influenza vaccine

Data from the above-mentioned univariate assessments suggested that the immune response to the vaccine might be reduced in patients undergoing specific immunosuppressive therapy such as AZA/6MP and IFX. More significant differences were observed in the seroprotection rate than in the seroresponse rate to assess the effect of AZA/6MP or IFX. Thus, to investigate the independent effect of these treatments, multivariate analyses were carried out using the seroprotection rate (Table 4). The seroprotection proportion of all patients undergoing steroid treatment increased more than 40-fold ($\geq$1:40) at S1. Therefore, we could not add the category of steroids to the model used for the logistic regression analysis.

Even after considering the effect of potential confounders, however, participants treated with AZA/6MP exhibited significantly low ORs for seroprotection against H1N1 (OR = 0.20, $p = 0.01$). Participants treated with IFX exhibited significantly decreased ORs of seroprotection against H3N2 (OR = 0.37, $p = 0.02$) and B (OR = 0.18, $p = 0.03$).

We next performed multivariate analyses for the combination of these immunosuppressive therapies (Table 5). Participants undergoing IFX monotherapy showed significantly decreased ORs for seroprotection against H3N2 (OR = 0.13, $p = 0.01$). Combination therapy with AZA/6MP and IFX was associated with significantly decreased ORs for seroprotection against H1N1 (OR = 0.056, $p = 0.02$). Combination therapy with AZA/6MP and IFX also led to decreased ORs for the B strain; however, this finding was not statistically significant owing to the limited number of subjects analyzed. Thus, the multivariate analysis data showed that each individual drug or their combination therapy were likely to independently affect the immune response to at least 1 of the 3 influenza vaccine strains.

Table 4 Factors associated with seroprotection after trivalent influenza vaccination.

Category	Influenza A(H1N1	*P*	Influenza A(H3N2)	*P*	Influenza B	*P*
	OR (95% CI)		OR (95% CI)		OR (95% CI)	
Tertile age at vaccination (years)						
<38	1.00		1.00		1.00	
38–48	3.06 (0.45–21)	0.26	0.39 (0.11–1.31)	0.13	0.27 (0.02–3.25)	0.30
≥49	0.24 (0.24–1.41)	0.11	0.60 (0.16–2.21)	0.44	0.04 (0.003–0.56)	0.02
Disease activity						
Remission stage	1.00		1.00		1.00	
Active stage	4.44 (0.38–52)	0.24	4.01 (0.82–20)	0.09	1.96 (0.18–22)	0.59
AZA/6MP						
−	1.00		1.00		1.00	
+	0.20 (0.06–0.72)	0.01	1.64 (0.72–3.74)	0.24	1.42 (0.39–5.22)	0.60
IFX						
−	1.00		1.00		1.00	
+	1.19 (0.39–3.64)	0.76	0.37 (0.16–0.86)	0.02	0.18 (0.04–0.82)	0.03
Pre-vaccination titer						
<1:10	1.00		1.00		1.00	
1:10–1:20	8.25 (0.88–77)	0.06	1.62 (0.58–4.53)	0.36	8.92 (1.13–71)	0.04

AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Logistic regression model: CI, confidence interval; OR, odds ratio.
Pre-vaccination titer of ≥1:40 was excluded (Data of 71 for H1N1, 78 for H3N2, and 55 for B were analyzed.).
Model included age at vaccination (years), disease activity, AZA, IFX and pre-vaccination titer.

3.4. Side effects of the trivalent influenza vaccination

Severe side effects, including fatalities, did not occur in the present study. In addition, the disease activities of participants did not change significantly during the study period (data not shown). Table 6 summarizes the proportion of subjects who reported adverse reactions. Ocular and respiratory symptoms occurred in 11 subjects (13%) within 24 h, whereas systemic symptoms and local reactions occurred in 29 subjects (34%) and 58 subjects (67%), respectively, within 48 h. The most frequent systemic symptom (20 subjects) was general malaise (24%), and the most frequent local reaction (47 subjects) was redness (55%). Comparison of the IS group and NIS group revealed no significant difference in the frequency of the reported symptoms between the 2 groups.

4. Discussion

The recent development of immunomodulators or anti TNF-α agents has led to better clinical prognoses and outcomes for patients with IBD.[32] Efforts to control infectious diseases, including vaccination, however, have become an important issue for IBD patients undergoing these therapies.[33] Appropriate vaccinations against hepatitis B virus, pneumococcus, human papilloma virus, influenza virus, etc., prior to beginning immunosuppressive therapies, are recommended in several guidelines.[9,10,34,35] The pandemic of the influenza A(H1N1)pdm09 virus led to concerns regarding influenza vaccination in IBD patients.[36]

At least one previous study indicated that influenza vaccination did not influence IBD activity.[20] Thus, evaluation

Table 5 Inhibition for seroprotective efficacy of the trivalent influenza vaccination due to combination immunosuppressive therapy.

	Influenza A(H1N1)		*P*	Influenza A(H3N2)		*P*	Influenza B		*P*
	n (%)	OR (95% CI)		*n* (%)	OR (95% CI)		*n* (%)	OR (95% CI)	
Neither AZA/6MP nor IFX	23 (82)	1.00		19 (70)	1.00		17 (81)	1.00	
AZA/6MP monotherapy	12 (67)	0.19 (0.03–1.16)	0.07	12 (60)	0.66 (0.18–2.43)	0.53	10 (91)	6.83 (0.38–123)	0.19
IFX monotherapy	14 (88)	1.05 (0.13–8.30)	0.96	8 (36)	0.13 (0.03–0.58)	0.01	12 (75)	0.60 (0.05–6.89)	0.68
combination therapy of AZA/6MP + IFX	5 (56)	0.056 (0.005–0.62)	0.02	5 (56)	0.37 (0.07–2.14)	0.27	4 (57)	0.10 (0.01–2.15)	0.14

AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Logistic regression model: CI, confidence interval; OR, odds ratio.
Pre-vaccination titer of ≥1:40 was excluded (Data of 71 for H1N1, 78 for H3N2, and 55 for B were analyzed.)
Model included age at vaccination (years), disease activity, and pre-vaccination titer.

Table 6 Side effects of trivalent influenza vaccination in participants with inflammatory bowel disease.

	Total patients (n = 86)	IS group (n = 56)	NIS group (n = 30)	P
Ocular and respiratory symptoms within 24 h	11 (13%)	8 (14%)	3 (10%)	0.74
Red eyes	2 (2%)	2 (4%)	0	0.54
Facial edema	1 (1%)	1 (2%)	0	1
Any respiratory symptoms	7 (8%)	5 (9%)	2 (7%)	1
Coughing	7 (8%)	5 (9%)	2 (7%)	1
Wheezing	0	0	0	
Chest tightness	0	0	0	
Difficulty breathing	0	0	0	
Difficulty swallowing	0	0	0	
Hoarseness	2 (2%)	2 (4%)	0	0.54
Sore throat	5 (6%)	3 (5%)	2 (7%)	1
	29 (34%)	17 (31%)	12 (40%)	0.48
Systemic symptoms within 48 h	0	0	0	
Fever ($\geq$37.5 °C)	20 (24%)	12 (22%)	8 (27%)	0.61
General malaise	10 (12%)	5 (9%)	5 (17%)	0.44
Myalgia	8 (9%)	3 (5%)	5 (17%)	0.12
Headache	2 (2%)	1 (2%)	1 (3%)	1
Rash	58 (67%)	38 (68%)	20 (67%)	1
Local reaction within 48 h	47 (55%)	38 (68%)	20 (67%)	0.5
Redness	40 (47%)	29 (52%)	18 (60%)	0.37
Swelling	31 (36%)	20 (36%)	11 (37%)	1
Induration				
Itching	29 (34%)	19 (34%)	10 (33%)	1
Pain	25 (29%)	17 (30%)	8 (27%)	0.81
Medical office visit due to above symptoms	2 (2%)	1 (2%)	1 (3%)	1

NIS group, non-immunosuppressive group; IS group, immunosuppressive group.
Date are expressed as n (%) of patients, unless otherwise indicated.
χ^2 test or Fisher's exact test between 2 categories.

of immunogenicity by the trivalent influenza vaccine and optimization of the vaccination, especially in IBD patients treated with immunosuppressants, is an important next step. To date, no study has elevated the immune responses to the trivalent influenza vaccine in adult IBD patients. The findings of the present study revealed that HAI antibody titers reduced for all 3 vaccine strains after the influenza season. Influenza A and B virus variants result from frequent antigenic change (i.e., antigenic drift), which renders an individual susceptible to new strains despite previous exposure to influenza. For these reasons, the trivalent influenza vaccine must be modified according to the strain antigens, and patients must be immunized annually. Different efficacies of the trivalent influenza vaccine in IBD patients, in particular, those treated with immunosuppressive drugs, must be evaluated carefully and precisely, and the process of trivalent influenza vaccination should be optimized for these patients.

To prevent and control influenza infection and the associated complications, seroprotective levels of the antibody must be acquired before the influenza season. Evaluation of the characteristics associated with the immune response after vaccination in the stratified analyses revealed that GMT and the seroprotection proportion were significantly higher in participants treated with corticosteroids than in those not treated with corticosteroids. This finding was unexpected, because steroid treatment is known to inhibit the immune response to influenza vaccine.[37] Our results do not clarify why patients undergoing corticosteroid treatment exhibited higher antibody titers. One possibility, however, is that some patients under corticosteroid treatment might develop an asymptomatic infection between S0 and S1, as evidenced by the extremely high individual antibody titer (more than 640) in some of the study participants. Thus, this positive relationship must be cautiously interpreted.

Conversely, seroprotection proportion against H1N1 was significantly lower in participants treated with AZA/6MP (p = 0.01). In addition, treatment with IFX significantly reduced the immune response to H3N2 (p = 0.02) and B (p = 0.03), whereas combination therapy with AZA/6MP and IFX showed significant inhibitory effects on H1N1 (p = 0.02). The results of multivariate analyses showed that each drug alone or in combination therapy independently reduced the immune response to the influenza vaccine for at least 1 of the 3 influenza vaccine strains. Some studies have also reported that combination therapies with anti-TNF-α agents and immunomodulators reduces the immunogenicity of the influenza A(H1N1)pdm09 vaccine more than monotherapy with anti TNF-α agents does in adult IBD patients.[17,18] In addition, Mamula et al.[22] reported that combination therapy reduced the immunogenicity of the seasonal influenza vaccine more than monotherapy did in pediatric IBD patients. Furthermore, studies in pediatric IBD patients treated with whole immunosuppressive therapy or anti-TNF-α agents indicated that the immunogenicity of influenza B is inhibited more than that of influenza A(H1N1, H3N2).[16,24] However, thiopurines do not affect the immunogenicity of influenza B in adult IBD patients.[38] In patients with another immunologic disease – spondyloarthritis – anti-TNF-α agents can inhibit the

immunogenicity of the influenza A(H1N1)pdm09 vaccine.[39] Taken together, these findings suggest that immunosuppressive therapy independently inhibits the immunogenicity of the trivalent influenza vaccine in both pediatric and adult IBD patients.[40] Immunologic studies must be performed to evaluate the mechanism underlying the effect of these immunosuppressive therapies on the immunogenicity of vaccines in IBD patients. Investigations of host immunologic potential (i.e., T cells) and antibody formation response (i.e., B cells) are also needed.[17,41]

As for side effects, there was no significant difference in the proportion of each symptom between the IS and NIS groups. In addition, vaccination did not influence IBD activity in the participants, consistent with results of a previous study.[20] Thus, the trivalent influenza vaccination appears to be safe for adult IBD patients, regardless of whether they are receiving immunosuppressive treatment.

This is the first study to show that immunosuppressive therapy (AZA/6MP and/or IFX) inhibits the immunogenicity of the trivalent influenza vaccine in adult IBD patients. This study, however, has some limitations. We did not analyze the influence of dosage and treatment duration for each immunosuppressive drug. Further, the number of participants treated with corticosteroids or combination therapy (AZA/6MP and IFX) was relatively small, which could influence the results of statistical analysis. In addition, healthy controls were not included for comparison. However, the comparisons between the IS group and NIS group should be clinically important with reference to past notable investigations.[17,20,23] Moreover, the NIS group of IBD patients is an appropriate control group to reveal the influence of immunosuppressive therapy on immune responses to the trivalent influenza vaccine.

To optimize the trivalent influenza vaccination for adult IBD patients treated with immunomodulators and/or anti-TNF-α agents, more effective vaccination methods such as dual vaccinations with booster doses must be established for these patients. Immunogenicity of other types of inactivated vaccines such as the pneumococcal vaccine is also inhibited in IBD patients treated with immunomodulators and/or anti-TNF-α agents.[42] Booster doses of the hepatitis B vaccine, another inactivated vaccine, are recommended.[43] Optimization with a booster dose of the trivalent influenza vaccine is also considered to enhance efficacy in patients with rheumatoid arthritis who are additionally receiving treatment with immunosuppressive drugs.[44,45] We are currently investigating the efficacy of a booster dose of the trivalent influenza vaccine in adult IBD patients undergoing immunosuppressive therapy (UMIN000009259). Furthermore, the development of personalized vaccination plans according to pre-vaccination antibody titer, treatment drugs, and immunologic potential, is expected in the near future.[46]

In conclusion, treatment with infliximab and/or immunomodulators inhibits the immune response to trivalent influenza vaccination in adult IBD patients. These findings should contribute to the development of optimized and personalized influenza vaccines.

Conflict of interest

No conflicts of interest exist.

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