Original Article

Low immunogenicity of vedolizumab determined by a simple drug-tolerant assay in patients with ulcerative colitis

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Vedolizumab is a humanized monoclonal antibody against the α4β7 integrin and is approved for treatment of inflammatory bowel diseases. In this study, we evaluated the immunogenicity of vedolizumab using a simple drug-tolerant assay developed in our laboratory. Serum vedolizumab trough levels and anti-vedolizumab antibody (AVA) levels were measured using new immunoassays in 37 patients with ulcerative colitis (UC) under vedolizumab maintenance therapy. The median vedolizumab trough level at week 30 was 16.0 μg/ml (interquartile range, 7.3–24.4). The vedolizumab trough level of the patients with clinical remission (partial Mayo score ≤1) was significantly higher than that of clinically active patients (16.7 μg/ml vs 6.8). The cut-off value of vedolizumab level predicting clinical remission at week 30 was 7.34 μg/ml. The median AVA level of patients under vedolizumab maintenance therapy was similar to that of healthy controls (*n* = 20) (0.032 μg/ml-c vs 0.022). One of 37 patients (2.7%) was judged to be AVA positive. There was no significant difference in serum AVA and vedolizumab trough levels between biologics-naïve (*n* = 19) and biologics-switched (prior anti-TNFα-exposed) patients (*n* = 18). In conclusion, the simple drug-tolerant assay developed in our laboratory demonstrated low immunogenicity of vedolizumab. Prior use of anti-TNFα drugs did not affect the immunogenicity of vedolizumab.

***Key Words*: vedolizumab, anti-vedolizumab antibody, therapeutic drug monitoring**

Inflammatory bowel diseases (IBDs), which include Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic intestinal inflammation mediated by dysregulated innate and adaptive immune responses.[1–3] While complete cure of IBD is difficult, various types of medications are available to induce and maintain clinical remission.[4] Among them, biologics have been approved for the treatment of moderate-to-severe UC and CD and these have markedly improved their management.[5]

Interaction between the α4β7 integrin expressed on the surface of memory T cells and the mucosal vascular addressin cell adhesion molecule-1 (MAdCAM1) on the gut endothelium plays a crucial role in the pathophysiology of IBD.[6] Infiltration of memory T cells into intestinal mucosa is initiated by their adhesion to the endothelium, and this process is mediated by the interaction of α4β7 integrin with MAdCAM1. Vedolizumab is a humanized IgG1 monoclonal antibody directed against the α4β7 integrin that blocks the binding of memory T cell to the gut endothelium, thereby preventing their infiltration into the mucosa.[6,7] Since MAdCAM1 is specifically expressed in the endothelium within the gastrointestinal (GI) tract and gut-associated lymphoid tissue,[8] the anti-inflammatory action of vedolizumab is restricted to the GI tract.[6] The efficacy and safety of vedolizumab in moderate-to-severe UC and CD were initially evaluated in three phase 3 clinical trials (GEMINI 1 for UC, GEMINI 2 and 3 for CD) and followed by multiple real-world cohort studies.[9–15] These indicated that vedolizumab is effective as the first- or second-line induction and maintenance therapy in UC and CD, and that there are no vedolizumab-specific safety concerns.

Therapeutic drug monitoring (TDM) is defined as the assessment of concentrations of drugs and anti-drug antibodies (ADAs) for optimizing biologic therapy. Based on the experiences with anti-tumor necrosis factor α (TNFα) drugs, TDM has been recognized as a useful strategy for optimizing the treatment of IBD patients.[16] TDM is helpful for objective analysis of potential reasons for therapeutic failure and for determining the next optimized treatment. While TDM has been extensively studied and applied when using anti-TNFα drugs,[16] its role in the optimization of vedolizumab remains unclear.

Vedolizumab was developed by the fusing of the antigen-recognizing domains of the mouse anti-human α4β7 monoclonal antibody Act-1 to a conventional human IgG1 scaffold domain.[17] In addition, two mutations were introduced into the Fc portion of vedolizumab to eliminate Fc-mediated cytotoxicity.[17] These molecular characteristics of vedolizumab have raised concerns that its immunogenicity could lead to the generation of anti-vedolizumab antibodies (AVA). However, there are few reports on the immunogenicity of vedolizumab.

Screening of ADAs is frequently performed using standard immunoassays which reveal a low drug tolerance. These assays are able to detect only free ADA and unable to detect the ADA forming immune complexes with the drug (drug-sensitive assays),[18] leading to underestimation of the immunogenicity of the drug. In contrast, drug-tolerant assays can measure ADA that are bound to the drug.[18,19] In this study, we aimed to estimate the optimal concentration of serum vedolizumab predicting clinical remission and evaluate the immunogenicity of vedolizumab using a drug-tolerant assay developed in our laboratory.

Materials and Methods

Patients. Thirty-seven patients with UC were enrolled from January 2020 to December 2020. These patients were treated with vedolizumab at the Shiga University of Medical Science Hospital. The demographic characteristics of the study patients are described in Table 1. Healthy volunteers (*n* = 20) were enrolled to determine the background levels of assays.

The patients received intravenous infusion of vedolizumab

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Table 1. Demographic characteristics of the patients

Healthy controls (n = 20)	
Female/male	4/16
Age [years; median (range)]	35 (27–46)
Ulcerative colitis (n = 37)	
Female/male	21/16
Age [years; median (range)]	46 (21–80)
Disease type	
Left-side colitis	13
Total colitis	24
Medications	
5-ASA	30
Azathioprine/6-MP	21
Prednisolone	10
Biologics naïve	19
Switched from IFX	4
Switched from ADA	14

5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine; IFX, infliximab; ADA, adalimumab.

(300 mg/body) at day 1 and weeks 2 and 6 during induction therapy and were followed by intravenous vedolizumab every 8 weeks. A blood sample was collected before the infusion at week 30. At each visit, a partial Mayo score (pMayo score) (consisting of the Mayo score minus the endoscopy subscore;[20] range, 0 to 9, with higher scores indicating more active disease) was calculated. Clinical remission was defined as a pMayo score of 0 or 1.[21,22]

Ethics. The study protocol was approved by the institutional review boards of the Shiga University of Medical Science (permission No. 2019-308). All patients gave their written informed consent prior to their inclusion in this study. The registration number of the University Hospital Medical Information Network Center (UMIN) was 000045425.

Labeling of recombinant α4β7 integrin and vedolizumab. Biotin-labeling of recombinant α4β7 integrin (R&D Systems, Minneapolis, MN) was performed using a commercially available biotin-labeling kit (Dojindo Molecular Technologies Inc., Kumamoto, Japan). Horseradish peroxidase (HRP)-labeling of vedolizumab was performed using a commercially available HRP-conjugation kit (Solulink, San Diego, CA).

Measurement of serum vedolizumab concentrations. Serum vedolizumab levels were determined by an immunoassay, constructed according to the method described previously.[23] We used an avidin ELISA plate® (blocking-less type; Sumitomo Bakelite Co., Ltd, Tokyo, Japan), which is ready to use with a special coating to minimize non-specific protein binding. This plate was coated with biotinylated-recombinant α4β7 integrin (100 µl of 0.5 µg/ml) by incubation for 2 h. After extensive washing, a further blocking was performed with Block Ace® (DS Pharma Biomedical, Co., Ltd., Suita, Japan). After washing, samples (100 µl of 100-fold diluted serum) were incubated overnight at 4°C. Finally, the reacted vedolizumab was detected by HRP-labeled $F(ab')_2$ fragments of chicken anti-human IgG (×20,000 diluted; Thermo Fisher Scientific Co., Ltd., Waltham, MA). 3,3',5,5'-Tetramethylbenzidine (Nacalai Tesque, Kyoto, Japan) was used for color development.

Drug-tolerant assay for anti-vedolizumab antibodies. An immunoassay for anti-vedolizumab antibodies (AVAs) that works in the presence of vedolizumab (drug-tolerant assay) was developed according to the methods described previously.[23,24] Immune complexes of vedolizumab and AVA in samples were dissociated by treatment with 0.1 M glycine-HCl buffer (pH 2.7) and the IgG fraction was isolated using protein G beads. IgG was eluted and the concentration was adjusted to 20 µg/ml IgG with a carbonate-bicarbonate buffer (pH 9.6). Each well of a 96-well ELISA plate was coated with diluted IgG containing AVAs (100 µl) overnight. AVAs on the plate were detected by 3 h incubation with HRP-labeled vedolizumab (100 µl of 2.0 µg/ml). 3,3',5,5'-Tetramethylbenzidine was used for color development. The values were reported in µg/ml-calibrated (µg/ml-c) according to calibration standards using polyclonal goat anti-human IgG (MP Biomedicals, LLC, Solon, OH).

Statistical analyses. Continuous variables were expressed as the median and interquartile (IQR). The Chi-square or Mann-Whitney U test were used to evaluate the association between two independent groups. The Spearman's correlation analysis was used to assess the association between clinical markers and vedolizumab trough levels. The cut-off values of vedolizumab concentration associated with clinical remission were determined using receiver operating characteristic (ROC) curve analysis. All statistical testing was performed at the 0.05 significance level.

Results

Validation of a newly developed immunoassay for vedolizumab concentration. Since major part of vedolizumab is human IgG, the most critical step in measurement of serum vedolizumab levels is prevention of non-specific binding of serum IgG. To check for effective prevention of non-specific serum IgG binding, we prepared vedolizumab standards (0, 5, 10, 20 µg/ml) using dilution by normal human serum. These standards were put on the current assay, and agreement between the prepared standards and the measurement results was confirmed (Fig. 1A). This means that the developed system can be used for measurement of serum vedolizumab levels. The background level obtained from healthy individuals was 0.44 µg/ml (median, IQR 0.37–0.86, n = 20) (Fig. 1B), and the vedolizumab trough level at week 30 was 16.0 µg/ml (median, IQR 7.3–24.4, n = 37) (Fig. 1B).

The median vedolizumab trough level of the patients with a partial Mayo score of ≤1 (clinical remission) was significantly higher than that of the patients with a pMayo score of >1 [median 16.7 µg/ml, IQR (12.6–26.6) vs 6.8 µg/ml (3.8–17.4)] (Fig. 2A). The cut-off value of vedolizumab concentration predicting a Mayo score of ≤1 (clinical remission) at week 30 was determined using receiver operating characteristic (ROC) curve analysis (Fig. 2B). Vedolizumab trough levels over 7.34 µg/ml were significantly associated with a Mayo score of ≤1 (clinical remission) at week 30 [area under the curve (AUC) 0.77, p = 0.016, sensitivity 0.67, specificity 0.89].

Evaluation of immunogenicity of vedolizumab using a drug tolerant assay. We produced a drug tolerant assay for serum AVAs according to the previously reported method for anti-infliximab antibodies in our laboratory.[24] In this assay, the immune complex of vedolizumab and AVAs was dissociated by acidic buffer treatment, and then AVA was detected by the peroxidase-labeled vedolizumab. The median AVA levels of healthy controls (n = 20) and vedolizumab-treated patients (n = 37) were 0.022 µg/ml-c (IQR 0.014–0.053) and 0.032 µg/ml-c (0.019–0.045), respectively (Fig. 3A). There was no statistical difference between the groups. The cutoff value for an AVA positive result was set at 0.25 µg/ml-c (mean + 3SD of healthy control values), and only one patient (2.7%) was positive for AVAs.

Vedolizumab trough levels and AVA levels were plotted according to whether the pMayo score was ≤1 (clinical remission) or not (Fig. 3B). There was no association between vedolizumab trough levels and AVA levels ($y = 1.97x + 16.7$, r = 0.12, p = 0.49, n = 37).

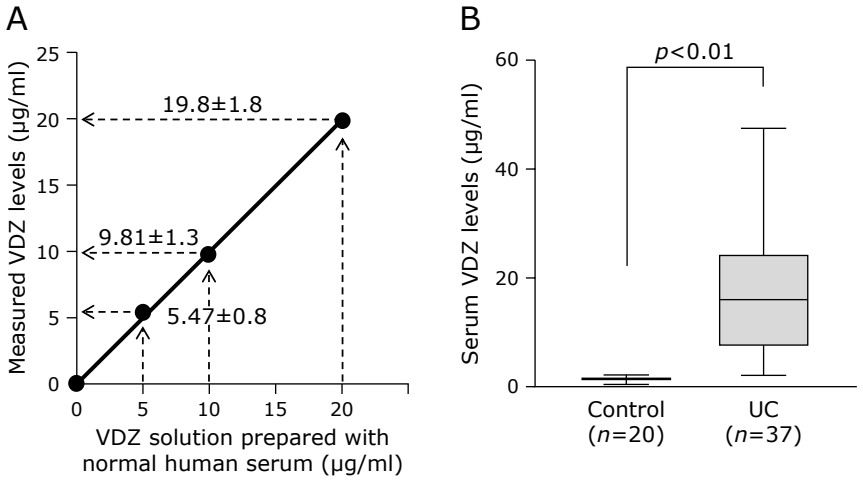

Fig. 1. Newly developed immunoassays for vedolizumab concentration. (A) To confirm effective prevention of non-specific serum IgG binding, we prepared vedolizumab standards (0, 1, 5, 10, 20 μg/ml) diluted by normal human serum. The measured results by the developed assay coincided with the prepared concentrations, indicating accurate measurement. Each point represents the mean of measured values (n = 25). (B) The background level determined by samples from healthy individuals was 0.44 μg/ml (median), and the trough level of vedolizumab at week 30 was 16.0 μg/ml (median).

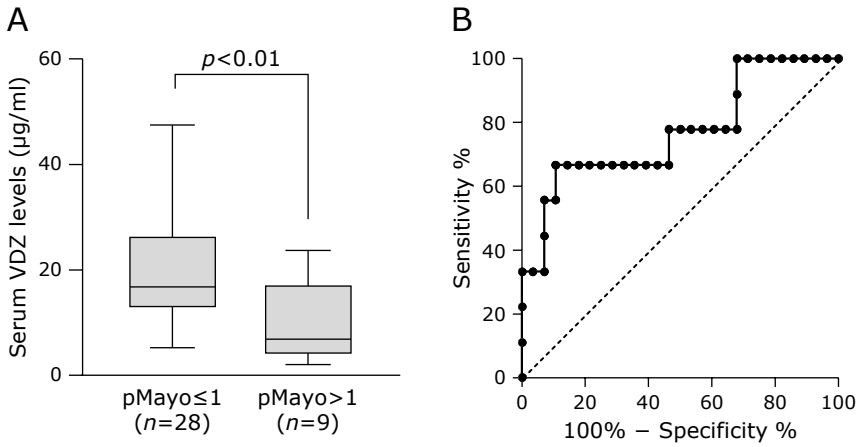

Fig. 2. Serum vedolizumab trough levels in UC patients. (A) The vedolizumab trough level of the patients with a partial Mayo (pMayo) score of ≤1 (clinical remission) was significantly higher than that of the patients with a pMayo score of >1 (p<0.01). The data are presented as median and IQR. (B) ROC analysis for setting optimal vedolizumab trough level predicting a pMayo score of ≤1 (clinical remission) at week 30. Vedolizumab trough levels over 7.34 μg/ml were significantly associated with a pMayo score of ≤1 (clinical remission) at week 30 (AUC 0.77, p = 0.016, sensitivity 0.67, specificity 0.89). pMayo, partial Mayo; ROC, receiver operating characteristic; AUC, area under the curve.

Association between biochemical markers and vedolizumab trough levels. CRP and erythrocyte sedimentation rate (ESR) were significantly lower in UC patients with vedolizumab trough levels ≥7.34 μg/ml than in patients with trough levels <7.34 μg/ml (Fig. 4A and B). Serum albumin levels were significantly higher in patients with vedolizumab trough levels ≥7.34 μg/ml than in patients with trough levels <7.34 μg/ml (Fig. 4C).

There was a significant and inverse correlation of vedolizumab trough levels with CRP (Fig. 5A). The vedolizumab trough level tended to be inversely correlated with ESR (p = 0.058, Fig. 5B), but this did not reach statistical significance (p = 0.058). There was no correlation between vedolizumab trough levels and serum albumin levels (Fig. 5C).

Effects of prior anti-TNFα drugs. This study included biologics-naïve patients (n = 19) and patients who had experienced anti-TNFα drugs and had then switched to vedolizumab (biologics-switched patients) (n = 18). There was no significant difference in serum AVA and vedolizumab trough levels between biologics-naïve and biologics-switched patients (Fig. 6A and B). There were no significant difference in pMayo score and CRP level between biologics-naïve and biologics-switched patients (Fig. 6C and D).

Discussion

In this study, we determined an optimal trough level of vedolizumab predicting clinical remission in UC patients and demonstrated the low immunogenicity of vedolizumab, using the simple immunoassays developed in our laboratory. The immunoassays used in this study are high throughput, relatively inexpensive and have no need for special analytical instruments such as high-performance liquid chromatography, and thereby might be applicable for routine clinical use.

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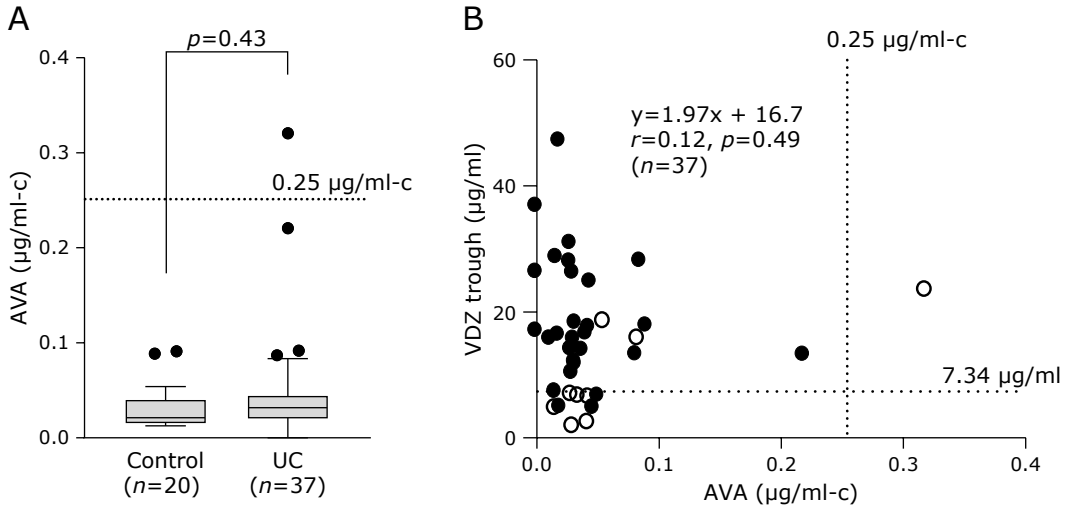

Fig. 3. Assays for serum anti-vedolizumab antibody. (A) The cutoff value for a positive result of anti-vedolizumab antibodies (AVAs) was determined to be 0.25 μg/ml-c (mean + 3SD of healthy controls, $n = 20$). One of the 37 UC patients (2.7%) was positive for AVAs. (B) Association of serum AVA levels with vedolizumab trough levels. There was no association between serum AVA and vedolizumab trough levels (y = 1.97x + 16.7, $r = 0.12$, $p = 0.5$, $n = 37$). Closed circle, pMayo score ≤1; open circle, pMayo score >1.

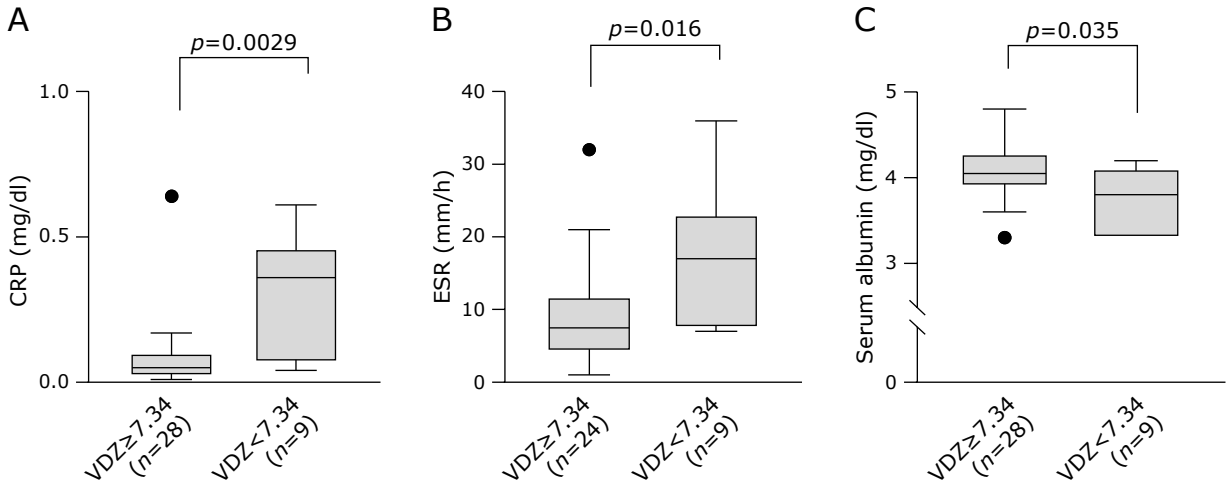

Fig. 4. Association between vedolizumab trough levels and biochemical markers. (A, B) CRP and ESR levels were significantly lower in patients with vedolizumab trough levels of ≥7.34 μg/ml than in patients with vedolizumab trough levels of <7.34 μg/ml. (C) Serum albumin levels were significantly higher in patients with vedolizumab trough levels of ≥7.34 μg/ml than in patients with trough levels of <7.34 μg/ml. The data are presented as median and IQR.

The appearance of ADA leads to a subtherapeutic serum drug level, resulting in the insufficient efficacy of biologics. Recent studies have recommended to test for ADAs when the patients reveal no or poor response to biologics, particularly when anti-TNFα drugs are used.[16,18] Under such situations, the importance of drug-tolerant assay for ADA testing has been recognized. In most assays, ADAs are detected by a labeled variant of the drug. However, ADAs usually form a drug-ADA immune complex in the serum and this interferes with the detection by the labeled drug. Early assay formats did not include the dissociating step of drug ADA complexes (drug-sensitive assay) and led to underestimated results due to detection of only free ADAs. The drug-tolerant assay includes the dissociation step by acidic buffer treatment and allows the measurement of ADA bound to the drugs.[18,19] However, there are few reports on the immunogenicity of vedolizumab using drug-tolerant assays.[25–27]

We initially assumed that mouse-derived components of vedolizumab may exert immunogenicity and easily induce AVA generation. However, the results in this study showed that only one of the 37 patients (2.7%) was positive for AVAs, indicating an extremely low immunogenicity of this drug. The low immunogenicity of vedolizumab may be supported by the results in our previous studies using the same formats of drug-tolerant assays for infliximab (27.6% positive for ADA) and adalimumab (35.0% positive).[13,23] The low immunogenicity of mouse components of vedolizumab was also demonstrated by the fact that when developing an immunoassay for vedolizumab concentrations, anti-mouse IgG antibodies could not detect vedolizumab and we used anti-human IgG antibodies for detection.

The results in this study are consistent with the few prior reports that have evaluated AVAs using drug-tolerant assays, although the used assays and the sampling period in the disease course are different. Wyant *et al.*[28] evaluated the samples of GEMINI 1 and 2 studies using a drug-tolerant assay and reported

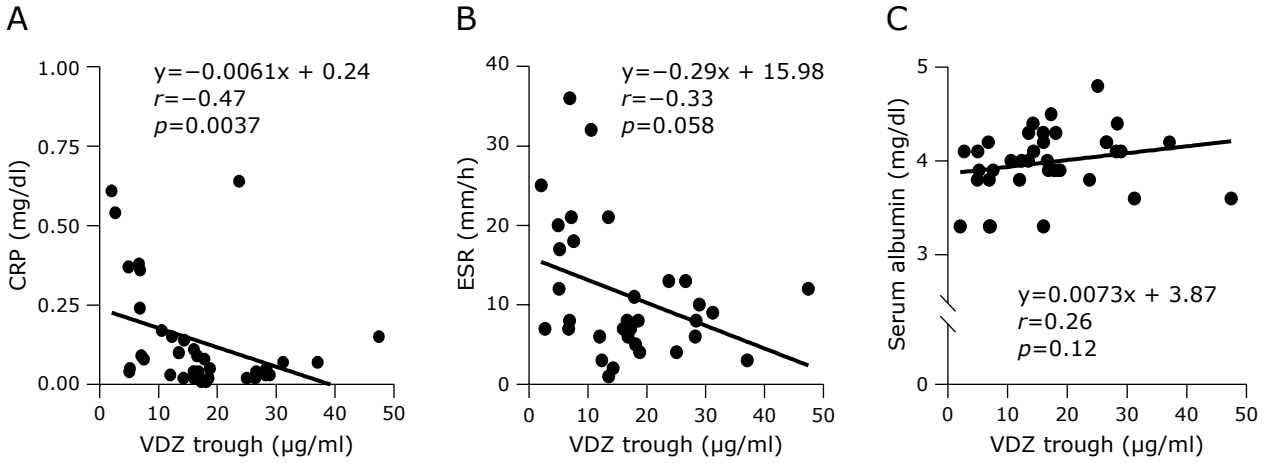

Fig. 5. Relationship of vedolizumab trough levels with biochemical markers. (A) Serum CRP was inversely and significantly correlated with vedolizumab trough levels. (B) ESR was tended to be inversely correlated with vedolizumab trough level, but this did not reach statistical significance ($p = 0.058$). (C) There was no correlation between vedolizumab trough levels and serum albumin levels.

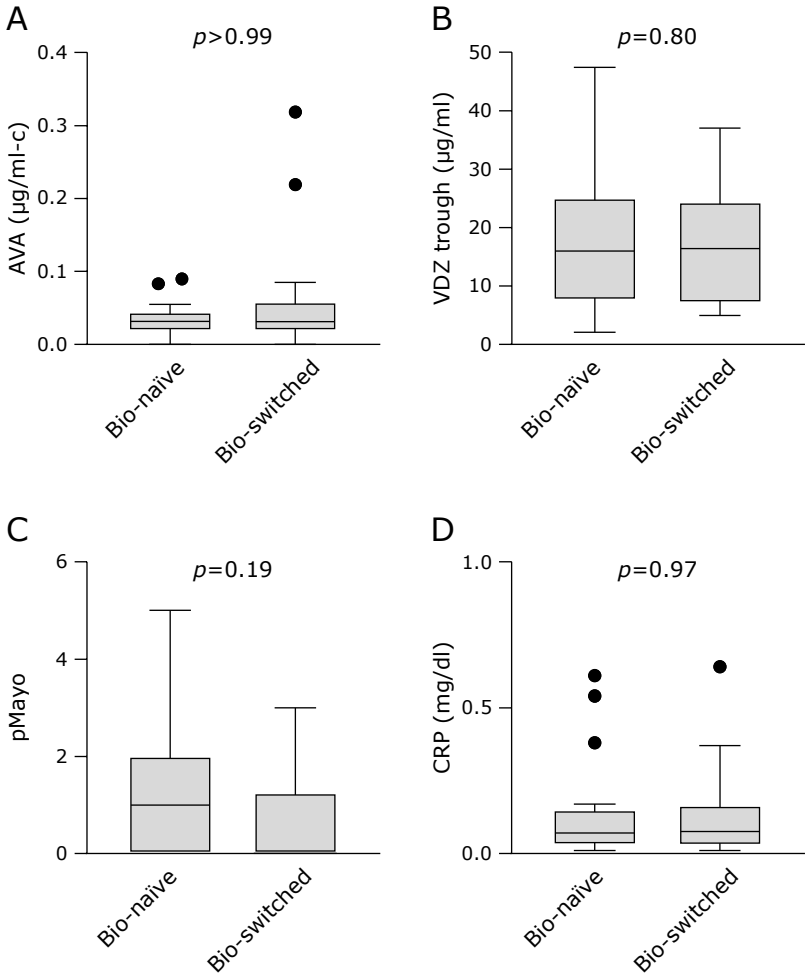

Fig. 6. Influence of prior use of anti-TNFα drugs to AVA and vedolizumab trough levels. (A, B) There was no difference in serum AVA and vedolizumab trough levels between anti-TNFα-experienced (bio-switched) patients ($n = 18$) and biologics-naïve (bio-naïve) patients ($n = 19$). (C, D) The partial Mayo score and CRP levels were similar between the two groups. The data are presented as median and IQR.

that 6% patients (86/1,427) were positive for AVAs. In other studies of drug-tolerant assays, AVAs were positive in 1.7–3.0% of IBD patients under induction or maintenance therapy.[25,29] Bian *et al.*[26] demonstrated that 3 of 179 patients (1.7%) were positive for AVAs at the induction phase but that ADAs were transient and disappeared on serial measurement in all patients.

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Thus, these observations indicate a low immunogenicity of vedolizumab. Based on the experiences with anti-TNFα drugs, immunomodulator use was considered to prevent the appearance of ADAs.[30] However, the low immunogenicity of vedolizumab suggests that there is no need for systemic immunosuppression with immunomodulators. Since immunomodulator use might be associated with tumorigenesis such as skin cancer and lymphoma,[31] the low immunogenicity as well as gut selective immunosuppression are major advantage of vedolizumab. It should be confirmed whether immunomodulators are required in the long-term use of vedolizumab in the future.

Due to the low immunogenicity of mouse components of vedolizumab, the immunoassay for vedolizumab concentrations was constructed according to the methods for human IgG biologics such as adalimumab and ustekinumab.[23,32] The new immunoassay reported that the median trough level of vedolizumab at week 30 was 16.0 μg/ml. The median vedolizumab trough level of the patients with a partial Mayo score of ≤1 (clinical remission) was significantly higher than those of the patients with a pMayo score of >1 (median 16.7 μg/ml vs 6.8). These are consistent with the findings in the meta-analysis of vedolizumab-treated UC patients where vedolizumab trough concentration during maintenance therapy was significantly higher in patients with clinical remission (median 14.3 μg/ml) than in active patients (10.5 μg/ml).[33] They proposed a therapeutic target range of 12 to 20 μg/ml of vedolizumab for achieving clinical remission.[33] In this study, the cut-off value of vedolizumab level predicting Mayo score of ≤1 was calculated to be 7.34 μg/ml, and this was relatively lower than the value reported by Ungaro *et al.*[25] (10.1 μg/ml). This might be associated with different backgrounds and the sample size of enrolled patients. We observed significant inverse correlations between vedolizumab trough levels and laboratory markers such as CRP and ESR and there was a significantly positive correlation with serum albumin, indicating that higher vedolizumab trough levels are associated with better biochemical outcome as well as clinical outcome.

Some studies have shown that prior use of anti-TNFα drugs is associated with lower therapeutic effects or treatment failure with vedolizumab,[11,14,34] whereas others have reported that the response to vedolizumab is independent of previous anti-TNFα failure.[35–37] Although the development of ADAs is a main factor contributing to the poor response of biologics,[23,24,38] there are a few studies on how prior use of anti-TNFα drugs affect AVA generation and vedolizumab trough levels. Using the new immunoassays, we investigated the effects of prior use of anti-TNFα drugs on AVA generation and vedolizumab trough levels at week 30. As shown in Fig. 6, the AVA levels and vedolizumab trough levels at week 30 were similar between the bio-naïve and bio-switched (prior use of anti-TNFα drugs) groups. A similar observation has been recently reported by Costable *et al.*[39] They analyzed anti-TNFα ADA positive ($n = 41$) and negative ($n = 22$) IBD patients using a drug-tolerant assay and found no significant difference in the rates of anti-vedolizumab ADA development between the two groups (2.7% vs 0.9%).[39] Despite limited data, these results suggest that prior use of anti-TNFα drugs does not lead to the easy development of AVAs and does not affect vedolizumab trough levels. This can be explained by the low immunogenicity of vedolizumab. Reflecting these pharmacokinetic results, there were no differences in clinical and biochemical outcomes (pMayo score and CRP) between the two groups. However, these findings should be prospectively confirmed in a much larger scale study in the future.

The strengths of the current study include the development of new simple immunoassays for vedolizumab and AVAs, which can be easily applied to routine clinical use. However, there are several limitations. First, our study is retrospective in design, which may lead to an increased risk of selection bias. Second, backgrounds of prior anti-TNFα treatment such as types of drug and exposure duration were not consistent in anti-TNFα-treated patients. Finally, our analysis was performed in a single center and limited by the sample size, and subsequent studies with larger cohorts are necessary to confirm our findings.

In conclusion, the immunogenicity of vedolizumab is extremely low as compared to infliximab and adalimumab, using the drug-tolerant immunoassays developed by the same format in our laboratories. The low immunogenicity of vedolizumab may be advantageous for IBD patients as there is no need for concomitant thiopurine use to prevent the generation of ADAs. Low generation of ADAs may lead to maintaining remission over a long period and little interference with the therapeutic effects of next biologics. The TDM approach has become standard for anti-TNFα drugs, but the need for such an approach remains unclear in vedolizumab. To improve our ability to optimize vedolizumab use, more pharmacokinetic data on vedolizumab should be accumulated in a large-scale study.

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Conflict of Interest

AA received lecture fee from Janssen, Takeda, AbbVie, and Tanabe-Mitsubishi. All other authors declare that they have no conflict of interest in this study.

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