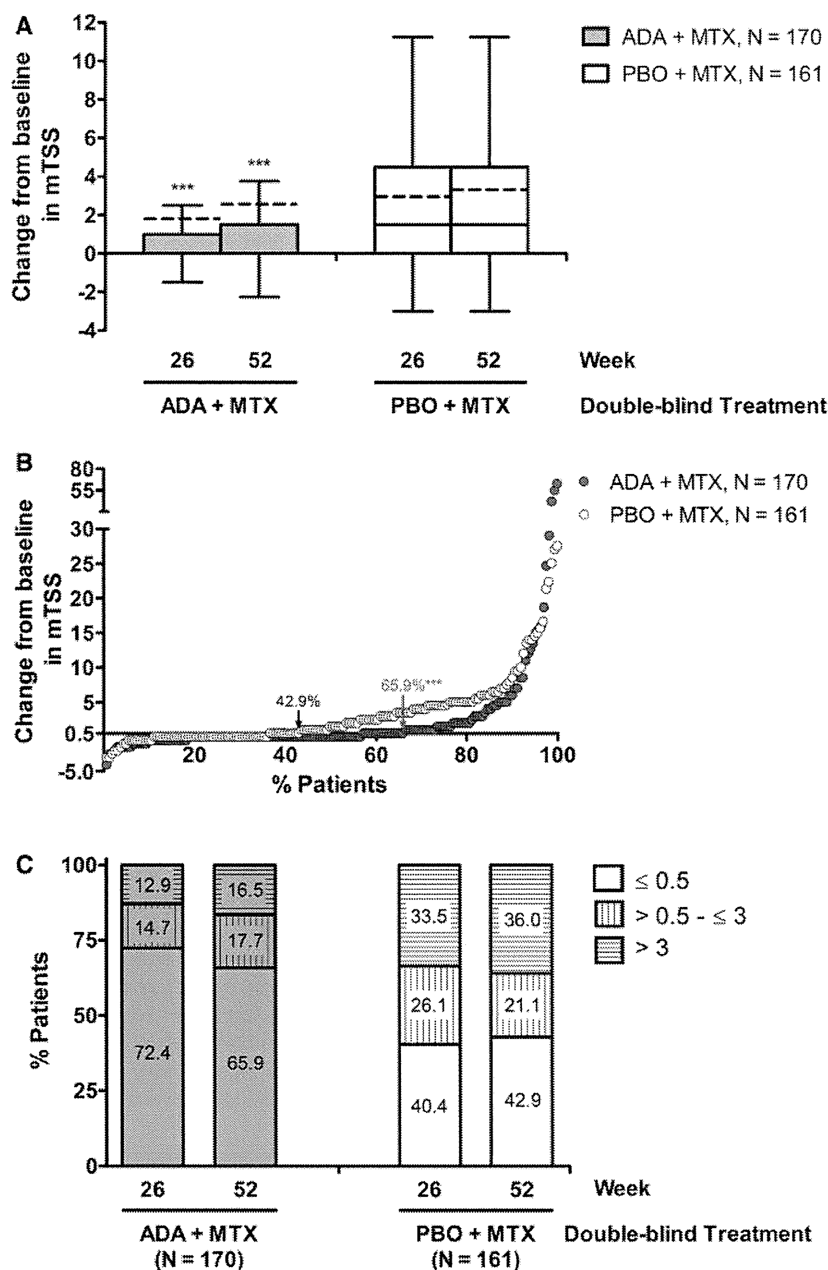
FIG. 3 Radiographic progression following up to 52 weeks of treatment with adalimumab (ADA) + MTX



(**A**) Box and whisker Tukey plot of change from baseline to week 26 or 52 in mTSS. Boxes represent interquartile range (25–75%); whiskers represent 1.5 times the interquartile range; line represents the median; dashed line represents the mean. (**B**) Cumulative distribution of change from baseline to week 52 in mTSS. (**C**) The percentages of patients in remission experiencing radiographic non-progression (ΔmTSS ⩽0.5), radiographic progression (ΔmTSS >0.5 to ⩽3.0) or clinically relevant radiographic progression (ΔmTSS >3.0) at the indicated time points. ***Statistical significance at the $P < 0.001$ level.

the reliability of prediction models that accurately classify patients based on pre-treatment characteristics is relatively poor [19, 20]. In this study population, an abnormal CRP baseline value was identified as an important predictor of damage accumulation in MTX-treated patients [13]. Expanded analyses combining baseline characteristics with genetic/biologic markers are needed to increase the accuracy that outcomes can be predicted.

Adalimumab + MTX treatment was generally well tolerated, and the safety profile was consistent with previously

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TABLE 2 Summary of adverse events

	Adalimumab + MTX (PY = 153.6), events (E/100 PY)	Placebo + MTX → adalimumab + MTX (PY = 78.9)[a], events (E/100 PY)
Any AE	740 (481.8)	439 (556.4)
AE at least possibly related to study drug	147 (95.7)	104 (131.8)
AE leading to discontinuation of study drug	12 (7.8)	5 (6.3)
Severe AE	4 (2.6)	1 (1.3)
Serious AE	17 (11.1)	10 (12.7)
Serious AE at least possibly related to study drug	8 (5.2)	4 (5.1)
Fatal AE	0	0
Infectious AE	189 (123.0)	116 (147.0)
Serious infectious AE	5 (3.3)	4 (5.1)
Opportunistic infection (excluding TB)	1 (0.7)	0
TB	0	0
Malignancy	0	0
Injection site reaction	29 (18.9)	22 (27.9)
Lupus-like syndrome	1 (0.7)	0
Allergic reaction	3 (2.0)	7 (8.9)
Haematological events	12 (7.8)	10 (12.7)
Elevated LFT level	65 (42.3)	30 (38.0)
Interstitial lung disease	1 (0.7)	2 (2.5)

[a]Patient-years (PY) are only reported for the period treated with adalimumab. AE: adverse event; E: events; TB: tuberculosis; LFT: liver function test.

reported studies of Japanese RA patients and consistent with global trials of TNF inhibitors in combination with MTX.

As with all studies, important limitations exist. At the time the study was conducted, the maximum approved dose of MTX was 8 mg/week and MTX was not allowed as the first-line DMARD in Japanese patients with RA. In 2011 the maximum approved MTX dose was increased to 16 mg/week and was also allowed to be prescribed as a first-line DMARD. Therefore the possibility exists that more aggressive MTX doses, typically used in western trials, may have more adequately limited progression of structural damage than the lower doses used in this study, although clinical trial data supporting the optimal dose of MTX in combination with TNF inhibitors is lacking. In addition to a lower allowable dose of MTX, the average weight of the studied Japanese population differentiates this study from other global adalimumab studies. Despite a lower mg/kg MTX dosing, clinical and radiographic outcomes of adalimumab + MTX were robust, indicating that lower doses of MTX in combination with adalimumab are capable of significantly reducing the signs and symptoms of RA.

Another important limitation is that the therapeutic regimen of the initial placebo + MTX group was switched to OL adalimumab + MTX at week 26 for all patients regardless of whether a good response was observed during the blinded period. Switching only those patients with an inadequate response to initial placebo + MTX may have yielded a better approximation of the harm of delayed adalimumab + MTX therapy, as was performed in the OPTIMA trial [12]. Furthermore, this analysis pooled patients who received rescue adalimumab + MTX therapy with those who were able to tolerate 26 weeks of placebo + MTX. Lastly, the relatively short follow-up time precluded long-term analysis of the socio-economic impacts and perhaps unique long-term benefit/risk profiles associated with the different treatment strategies for this population.

While the addition of OL adalimumab + MTX following the blinded period was able to improve clinical and functional outcomes comparable to patients initiated on combination therapy, undeniable benefits pertaining to the prevention of radiographic progression and associated joint damage exist. Early intervention with adalimumab + MTX is important to minimize irreversible structural damage in many Japanese patients with early, aggressive RA not previously treated with MTX.

Rheumatology key messages

- Japanese patients with aggressive RA benefit from early adalimumab combination therapy.
- The addition of adalimumab after 26 weeks of MTX monotherapy improves clinical and functional outcomes in patients with RA.
- Delayed treatment cannot recover radiographic damage occurring in the first 26 weeks in patients with RA.

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ORIGINAL ARTICLE

Efficacy, safety, pharmacokinetics and immunogenicity of abatacept administered subcutaneously or intravenously in Japanese patients with rheumatoid arthritis and inadequate response to methotrexate: a Phase II/III, randomized study

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Abstract

Objective. To evaluate efficacy and safety of subcutaneous (SC) and intravenous (IV) abatacept and background methotrexate (MTX) in Japanese patients with rheumatoid arthritis (RA) and inadequate response to MTX (MTX-IR).
Methods. Double-dummy, double-blind study (NCT01001832); 118 adults with ≥ 10 swollen joints, ≥ 12 tender joints and C-reactive protein (CRP) ≥ 0.8 mg/dL randomized 1:1 to SC abatacept (125 mg weekly) with IV loading (~10 mg/kg on Day 1), or IV abatacept (~10 mg/kg monthly) for 169 days, both also receiving MTX (6–8 mg/week). Primary endpoint was Day 169 American College of Rheumatology (ACR)20 response; other efficacy endpoints, safety and immunogenicity were assessed.
Results. Similar proportions of patients achieved ACR20 responses at Day 169 with SC (91.5% [95% CI 81.3, 97.2]) and IV abatacept (83.1% [71.0, 91.6]). ACR50/70 responses, adjusted mean changes from baseline in Health Assessment Questionnaire–Disability Index scores and remission rates (28-joint Disease Activity Score [CRP] < 2.6) were also comparable between groups. Serious adverse event frequencies (5.1% vs. 3.4%) were similar with both formulations. One patient per group tested seropositive for immunogenicity. Weekly SC abatacept dosing achieved mean serum concentrations > 10 μg/mL (minimum therapeutic target).
Conclusions. SC abatacept demonstrated comparable efficacy and safety to IV abatacept, with low immunogenicity rates, in MTX-IR Japanese patients with RA.

Keywords

Rheumatoid arthritis, Japan, Abatacept, Subcutaneous injection, Intravenous infusion

History

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Introduction

Abatacept is a fully humanized soluble recombinant fusion protein consisting of the extracellular domain of human CTLA4 and the Fc domain of human immunoglobulin (Ig) G_1. It is the only therapy for rheumatoid arthritis (RA) that selectively modulates the CD28:CD80/86 co-stimulatory signal required for full T-cell activation, thereby modulating the production and activation of downstream inflammatory mediators [1]. In clinical studies, the use of intravenous (IV) abatacept has been well established in a variety of patient populations, including patients with early RA who are methotrexate (MTX) naïve [2], and those who are inadequate responders to MTX (MTX-IR) [3,4] or therapies targeting tumor necrosis factor [5]. The efficacy and safety of IV abatacept have also been studied in Japanese patients [6,7]. In an initial Phase I safety study, IV abatacept was well tolerated up to a dose of 16 mg/kg [6]. A Phase II study confirmed the safety of therapeutic doses (2 and 10 mg/kg) of abatacept in Japanese patients with RA who were MTX-IR and reported significantly greater treatment responses versus MTX alone over 24 weeks, with high proportions of patients achieving disease activity and physical function outcomes [7].

A subcutaneous (SC) formulation of abatacept is also available that provides an alternative, more convenient route of administration for patients with RA. SC abatacept has been studied in numerous multinational Phase II and III studies [8–12]. The ACQUIRE (Abatacept Comparison of SC versus IV in Inadequate Responders

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to MTX) study was a global, noninferiority study of almost 1500 patients treated with IV or SC abatacept. ACQUIRE established that weekly SC administration of abatacept had an efficacy, safety and tolerability profile similar to that of the IV formulation over 24 weeks [8]. Patient retention rates and immunogenicity were also comparable between SC and IV abatacept. Administration of fixed-dose SC abatacept (following a single IV loading dose) achieved serum trough abatacept concentrations higher than or equal to the weight-tiered monthly IV regimen, across all body weight subgroups. However, this study did not include Japanese patients. Here, we report the results of a multicenter, randomized, double-blind, double-dummy, Phase II/III study in Japanese patients. This study, designed to mirror ACQUIRE in a different patient population, was conducted to assess the efficacy, safety, pharmacokinetics and immunogenicity of SC and IV abatacept on background MTX in Japanese patients with active RA who were MTX-IR.

Patients and methods

Patient population

The study population consisted of Japanese adults (aged ≥ 20 years) with RA, as defined by the American Rheumatism Association [13] and the American College of Rheumatology (ACR) [14], who were MTX-IR. Eligible patients had to have received MTX for ≥ 3 months at a stable dose (6–8 mg/week) prior to entry, and to have ≥ 10 swollen/≥ 12 tender joints, and C-reactive protein (CRP) levels of ≥ 0.8 mg/dL. Patients treated with other disease-modifying antirheumatic drugs were required to undergo washout. Oral corticosteroid treatment was reduced to the equivalent of ≤ 10 mg of prednisone/day for 28 days prior to randomization. No intra-articular, IV or intramuscular corticosteroid injections were permitted within 28 days of randomization. All patients provided signed, written informed consent to participate.

Exclusion criteria were prior treatment with any biologic; pregnancy; other rheumatic disease or active vasculitis of a major organ system; current symptoms of severe, progressive or uncontrolled renal, hepatic, hematologic, gastrointestinal, pulmonary, cardiac, neurologic or cerebral disease; history of cancer within the past 5 years (excluding non-melanoma skin cancers cured by local resection); clinically significant drug or alcohol abuse; serious acute, chronic or recurrent bacterial infection; risk for tuberculosis (TB; evidence of current active TB; TB within the past 3 years; latent or previous TB that may not have been adequately treated); recently resolved herpes zoster; and current suspected, active, or latent bacterial or viral infection.

Study design

This multicenter, randomized, double-blind, double-dummy study was conducted across 34 sites (university hospitals, general hospitals and private clinics) in Japan (NCT01001832) between December 2009 and February 2011. The protocol and patient informed consent form received institutional review board/independent ethics committee approval, and the study was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice.

For the 24-week, double-blind period, all eligible patients were randomized in a 1:1 ratio (according to randomization numbers assigned using a central randomization system) to receive abatacept through two different routes of administration. One group received 125 mg SC abatacept injections weekly (administered with a single IV loading dose on Day 1, based on body weight as outlined below) and the other group received ~10 mg/kg IV abatacept (500, 750 and 1000 mg for patients weighing < 60 kg, 60–100 kg, and > 100 kg, respectively) on Days 1, 15 and 29, and every 28 days thereafter, until Day 141. A double-dummy design was utilized: thus, patients receiving IV abatacept also received SC placebo, and patients receiving SC abatacept also received IV placebo. MTX was continued at the same dose as at randomization (6–8 mg/week) in both arms. Stable low-dose oral corticosteroids and nonsteroidal anti-inflammatory drugs were permitted; high-dose corticosteroids (a short oral course or a single dose by other routes) were permitted at the investigator's discretion. After 24 weeks, participants could enter an open-label extension to receive weekly SC abatacept for an additional 1-year period.

The primary objective was to assess the efficacy of SC and IV abatacept based on ACR20 response (20% improvement in ACR criteria) after 24 weeks (Day 169). Secondary objectives were to assess the safety, pharmacokinetics and immunogenicity of abatacept during the 24-week, double-blind period. Other efficacy endpoints were assessed as exploratory objectives.

Efficacy assessments

The primary endpoint was the proportion of patients in each group achieving an ACR20 response at Day 169 [15]. Other efficacy endpoints included: ACR50 and -70 response rates at Day 169, and ACR20, -50, and -70 rates over time; adjusted change from baseline in Health Assessment Questionnaire–Disability Index (HAQ–DI) score and proportion of patients achieving a HAQ–DI response (improvement of ≥ 0.3 units in HAQ–DI score from baseline) at Day 169 [16,17]; change from baseline in 28-joint Disease Activity Score based on CRP (DAS28-CRP); and proportion of patients with Low Disease Activity State (LDAS) (defined as DAS28-CRP ≤ 3.2) and remission (defined as DAS28-CRP < 2.6) at Day 169.

Safety, pharmacokinetic and immunogenicity assessments

Safety assessments included the recording of adverse events (AEs) (classified according to Medical Dictionary for Regulatory Activities version 14.1 system organ class and preferred term) that occurred during the 169-day study period or within 56 days after the last dose of abatacept. The 56-day window covers 4 half-lives of abatacept. Blood samples for pharmacokinetic profiling (trough serum concentrations of abatacept [C_{min}]) and immunogenicity assessment were obtained prior to study drug administration on Days 1, 85 and 169. Patients who discontinued from the study had blood samples collected at 7, 28, 56 and 84, or 7, 28, 84 and 168 days after the last dose of treatment. For the first 60 patients randomized, blood samples were collected for the determination of additional pharmacokinetic parameters (peak serum concentration [C_{max}] and area under the serum concentration–time curve [$AUC_{(tau)}$]) prior to study drug administration on Days 1, 57, 85, 113, 120, 127, 134, 141 and 169, and also on Days 115, 116, 117 and 118. On Days 1 and 113, blood samples were also obtained at the end of IV infusion.

Serum levels of abatacept were assessed using a validated immunoassay, and pharmacokinetic parameters were derived by noncompartmental analysis. Serum levels of abatacept-specific antibodies were analyzed using an electrochemiluminescence immunoassay (Meso-Scale Discovery, Rockville, MD, USA), which differentiated between two antibody specificities: (i) IgG and/or junction region and (ii) CLTA4 and possibly Ig.

Sample size

Sample size determination was based on Japanese guidelines for the assessment of RA drugs in clinical studies [18], which recommend that data from 100 or more patients receiving treatment for at least 1 year are needed to assess long-term data. With an

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estimated discontinuation rate of 10%, the study aimed to include a total of 110 patients (55 each in the SC and IV groups).

Statistical analysis

Efficacy and safety analyses were based on all randomized and treated patients (intent-to-treat [ITT] population). In addition, an analysis of the per-protocol (PP) population was planned to support the primary efficacy analysis.

No formal statistics were performed, but descriptive statistics were provided for all assessments, including point estimates and 95% CIs for the proportion of patients achieving ACR20, -50 and -70 responses, HAQ–DI responses, and DAS28-CRP, LDAS and remission rates in each treatment group. For the primary endpoint of ACR20 response at Day 169, the treatment difference between SC versus IV abatacept with 95% CIs was determined. Adjusted mean change from baseline in HAQ–DI and DAS28-CRP scores and their corresponding 95% CIs were calculated. All participants who prematurely discontinued the study were considered as ACR/HAQ–DI non-responders at all visits subsequent to discontinuation. In the assessment of physical function and disease activity, any missing values for HAQ–DI and DAS28-CRP score were imputed using the last observation carried forward, except for those for which only a baseline observation was available.

Safety data are presented as frequencies and summarized by treatment group. Summary statistics, including geometric mean and coefficient of variation (CV), abatacept C_{min}, C_{max} and $AUC_{(tau)}$ were obtained for the SC and IV treatment groups (for the interval between Days 113 and 141 [tau = 28 days] for IV-treated patients, and between Days 113 and 120 [tau = 7 days] for SC-treated patients). To assess the absolute bioavailability of SC abatacept, treatment groups were compared by one-way analysis of variance on the log-transformed $AUC_{(tau)}$. For immunogenicity analyses, the proportions of patients experiencing a positive response were summarized by treatment group.

Results

Patient disposition and baseline clinical characteristics

Of 118 patients included in the ITT population, 59 were randomized to receive SC abatacept and 59 to receive IV abatacept. No patients were excluded from the PP population, which was, therefore, identical to the ITT population. Overall, 2 patients (3.4%) treated with SC abatacept and 3 (5.1%) treated with IV abatacept discontinued prematurely from the study due to AEs; 113 patients (95.8%) completed the 24-week, double-blind period. Additionally, 1 patient in the SC abatacept group discontinued due to a serious AE (SAE) after the double-blind period but before entering the open-label extension (Figure 1).

The demographics and baseline disease characteristics of the two treatment groups were similar (Table 1). Duration of RA was longer in the SC versus IV group (mean [SD]: 7.5 [9.2] vs. 5.3 [7.3] years, respectively; median [range]: 3.0 [0–36.0] vs. 2.0 [0–33.0] years, respectively). The proportion of patients with disease duration of ≤ 2 years was 42.4% in the SC group and 50.8% in the IV group.

Clinical efficacy

ACR responses

For the primary endpoint, a similar proportion of patients receiving SC (91.5% [95% CI 81.3, 97.2]) and IV abatacept (83.1% [95% CI 71.0, 91.6]) achieved an ACR20 response at Day 169 (Figure 2). The estimated treatment difference in ACR20 response rate was 8.5% (95% CI − 9.3, 26.9). ACR20 response rates were comparable in both treatment groups at the first assessment (Day 15) (28.8% for SC and 22.0% for IV abatacept), and continued to increase comparably over the remainder of the 24-week study (Figure 2).

ACR50 and -70 responses in patients receiving either SC or IV abatacept also showed comparable changes over time (Figure 2), and response rates at Day 169 were similar between groups (ACR50: 66.1% [95% CI 52.6, 77.9] and 62.7% [95% CI 49.1, 75.0], respectively; ACR70: 37.3% [95% CI 25.0, 50.9] and 30.5% [95% CI 19.2, 43.9], respectively).

Physical function

At Day 169, adjusted mean changes from baseline in HAQ–DI score were similar in the SC and IV abatacept groups: − 0.62 (95% CI − 0.74, − 0.49) and − 0.61 (95% CI − 0.73, − 0.49), respectively. HAQ–DI responses were seen as early as the first assessment (Day 15) in 16.9% (95% CI 8.4, 29.0) and 23.7% (95% CI 13.6, 36.6) of patients in the SC and IV abatacept groups, respectively. Thereafter, the proportions of patients achieving a HAQ–DI response increased in both groups comparably, reaching 69.5% (95% CI 56.1, 80.8) for SC abatacept and 50.8% (95% CI 37.5, 64.1) for IV abatacept at Day 169.

Disease activity

At Day 169, the adjusted mean change from baseline in DAS28-CRP was similar for the SC and IV abatacept groups: − 2.97 (95% CI − 3.25, − 2.70) and − 2.75 (95% CI − 3.03, − 2.48), respectively. The proportion of patients achieving LDAS and remission increased over the 24-week study, with a response evident at the initial assessment (Day 15) and at subsequent time points in both treatment groups. At Day 169, LDAS was

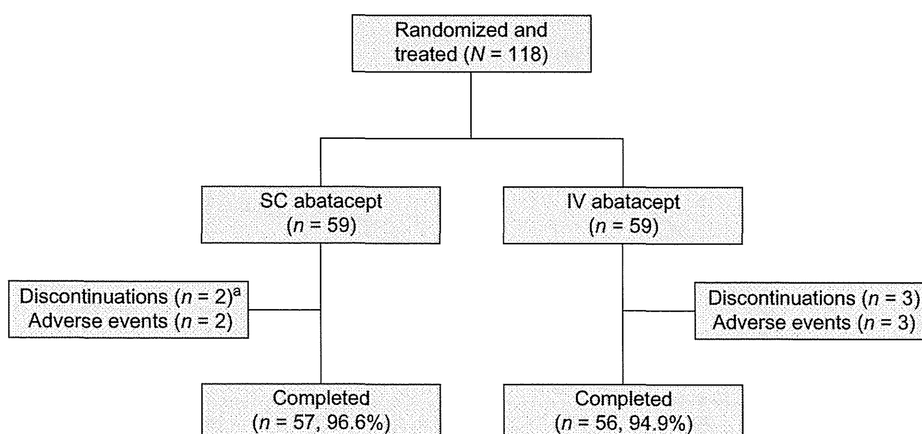

Figure 1. Disposition of patients randomized to SC or IV abatacept over 24 weeks. [a]One additional discontinuation occurred in the SC abatacept group after completion of the double-blind period but before the long-term extension; this case was not included in the count of patients who discontinued during the double-blind period, but was considered in the quantification of AEs leading to discontinuation.

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Table 1. Baseline[a] demographic and clinical characteristics.

Characteristic	SC abatacept, $n = 59$	IV abatacept, $n = 59$	Total $N = 118$
Age, mean years (SD)	56.1 ± 12.3	55.2 ± 13.6	55.6 ± 12.9
Female, *n* (%)	38 (64.4)	48 (81.4)	86 (72.9)
Japanese race, *n* (%)	58 (98.3)[b]	59 (100)	117 (99.2)
Weight, mean kg (SD)	56.4 ± 10.4	53.9 ± 10.0	55.1 ± 10.2
Weight, *n* (%)			
< 60 kg	40 (67.8)	44 (74.6)	84 (71.2)
60–100 kg	19 (32.2)	15 (25.4)	34 (28.8)
> 100 kg	0	0	0
Duration of disease, mean years (SD)	7.5 ± 9.2	5.3 ± 7.3	6.4 ± 8.3
Duration of disease category, *n* (%)			
≤ 2 years	25 (42.4)	30 (50.8)	55 (46.6)
> 2 to ≤ 5 years	7 (11.9)	11 (18.6)	18 (15.3)
> 5 to ≤ 10 years	10 (16.9)	6 (10.2)	16 (13.6)
> 10 years	17 (28.8)	12 (20.3)	29 (24.6)
Tender joint count, mean (SD)	20.9 ± 9.3	22.3 ± 9.9	21.6 ± 9.6
Swollen joint count, mean (SD)	16.4 ± 7.0	17.6 ± 7.2	17.0 ± 7.1
DAS28-CRP, mean (SD)	5.6 ± 0.8	6.0 ± 0.9	5.8 ± 0. 9
HAQ–DI score, mean (SD)	1.3 ± 0.7	1.3 ± 0.6	1.3 ± 0.7
MTX dose,[c] mean mg/week (SD)	7.3 ± 1.0	7.3 ± 1.0	7.3 ± 0.9

Includes all randomized and treated patients.
DAS28-CRP, 28-joint Disease Activity Score based on C-reactive protein level; HAQ–DI, Health Assessment Questionnaire–Disability Index; MTX, methotrexate; SD, standard deviation.
[a]Baseline is Day 1 of the study or last nonmissing pretreatment value.
[b]One patient was non-Japanese (Asian) but was living in Japan, and informed consent and all assessment procedures were conducted in an identical manner to those conducted to the Japanese patients. Thus, the patient was included in all assessments of the study.
[c]MTX dose (mg/week) is the calculated weekly total dose in the week ending at the first dose date of the double-blind period.

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achieved by a similar proportion of patients in the SC and IV abatacept groups (70.2% [95% CI 56.6, 81.6] and 66.7% [95% CI 52.9, 78.6], respectively). The proportion of patients achieving DAS28-CRP remission at Day 169 was also similar: 50.9% (95% CI 37.3, 64.4) in the SC abatacept group and 40.4% [95% CI 27.6, 54.2] in the IV abatacept group.

Safety

Safety data for the SC and IV abatacept groups are summarized in Table 2. No deaths were recorded in either group. The most common AEs (≥ 5% in either group) were nasopharyngitis, stomatitis, pharyngitis, oropharyngeal pain, hypertension, rash, elevated alanine aminotransferase level (ALT), constipation, diarrhea, gastritis, periodontitis and upper respiratory tract inflammation. The four patients with elevated ALT were all in the IV group; ALT elevations were < 3× upper limit of normal and were not considered to be clinically significant. AEs leading to discontinuation were noted in two patients in the SC abatacept group (gastric cancer [classified as serious] in one patient and interstitial lung disease [classified as serious] and fungal infection, both in the second patient) and three patients in the IV abatacept group (extranodal marginal zone B-cell lymphoma of the mucosal-associated lymphoid tissue [MALT] type and organizing pneumonia [both considered serious], and increased blood creatine phosphokinase levels). One further patient in the SC abatacept group developed an SAE during the double-blind period (cryptococcal pneumonia), which led to discontinuation after completion of the double-blind period, but before the start of the open-label extension.

AEs of special interest

Infections, malignancies, autoimmune disorders, and infusion- and injection-related events were monitored as AEs of special interest.

The most common category of AEs reported overall were infections and infestations (SC abatacept 33.9% and IV abatacept 49.2%), which were mild or moderate in severity (Table 2). Nasopharyngitis was the most frequently reported infection (SC abatacept 15.3% and IV abatacept 27.1%). One (1.7%) patient in each group had a serious infection: cryptococcal pneumonia in a

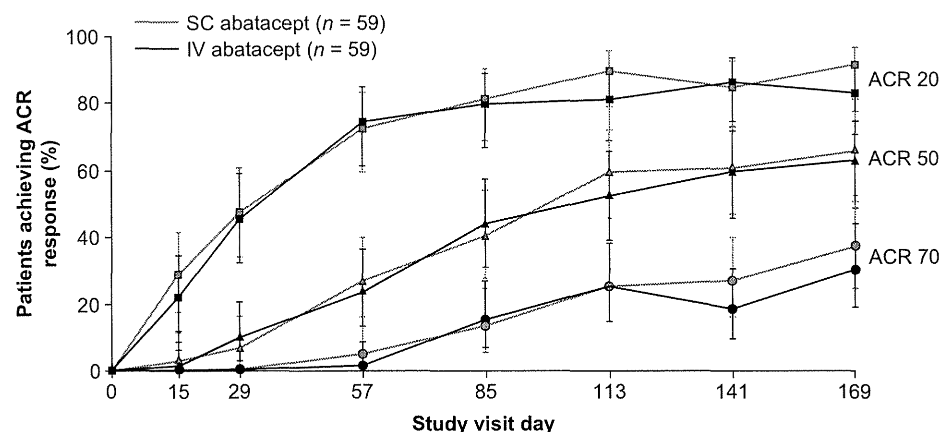

Figure 2. Proportion of patients (95% CI) receiving SC or IV abatacept achieving a 20%, 50% or 70% improvement in American College of Rheumatology (ACR) criteria. Error bars represent 95% CI.

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Table 2. Safety summary of AEs or SAEs.[a]

Events	Number (%) of patients	
	SC abatacept $n = 59$	IV abatacept $n = 59$
Deaths[b]	0	0
SAEs[c]	4 (6.8)	3 (5.1)
Treatment-related SAEs	3 (5.1)	2 (3.4)
Discontinued due to SAEs	3 (5.1)	1 (1.7)
AEs	45 (76.3)	49 (83.1)
Treatment-related AEs	31 (52.5)	35 (59.3)
Discontinued due to AEs	3 (5.1)[d]	3 (5.1)
AEs of special interest		
Infections and infestations	20 (33.9)	29 (49.2)
Serious infections	1 (1.7)	1 (1.7)
Malignancies	1 (1.7)	1 (1.7)
Autoimmune disorders	0	0
Acute infusional AEs	1 (1.7)	2 (3.4)
Peri-infusional AEs	1 (1.7)	4 (6.8)
Systemic SC injection reactions within 24 h	1 (1.7)	4 (6.8)
Local SC injection-site reactions	0	0

[a]Includes data up to 8 weeks after the last dose of the 24-week study.
[b]Includes any deaths reported during the 24-week study and those that occurred > 8 weeks after the last dose.
[c]Includes hospitalizations for elective surgical procedures.
[d]One patient had an SAE during the double-blind period, which led to discontinuation from the study after the double-blind period, but before the open-label extension; this patient was included only in the safety analysis.

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patient receiving SC abatacept that resulted in treatment discontinuation, and pneumonia in a patient receiving IV abatacept that required treatment interruption. Infections led to discontinuation in two patients in the SC abatacept group (fungal infection and cryptococcal pneumonia). Opportunistic infections were reported in two patients in the SC abatacept group; in addition to the cryptococcal pneumonia already described, one patient developed mild esophageal candidiasis, which did not result in treatment discontinuation or interruption.

Malignancy was observed in one patient (1.7%) in each group (gastric cancer in the SC abatacept group and extranodal marginal zone B-cell lymphoma [MALT type] in the IV abatacept group); both patients discontinued treatment.

No autoimmune AEs were reported during the 24-week treatment period.

Acute infusional AEs (defined as infusion reactions that occurred within 1 h of the start of IV administration) were reported in one patient (1.7%) in the SC abatacept group after the IV loading dose of abatacept on Day 1 (urticaria) and in two (3.4%) patients in the IV abatacept group (worsening of hypertension [$n = 1$] and a transient increase in blood pressure [$n = 1$]). Peri-infusional AEs (those occurring within 24 h after the start of IV administration) were observed in one patient (1.7%) in the SC abatacept group following IV abatacept loading dose (urticaria) and in four (6.8%) patients in the IV abatacept group (rash, increased blood pressure, headache and hypertension). All acute and peri-infusional AEs were classified as mild or moderate in severity and none required any alterations in study treatment.

No local SC injection-site reactions were reported in either treatment group. Systemic injection reactions (defined as a non-site-specific reaction within 24 h of SC injection) were observed in one patient (1.7%) in the SC abatacept group (urticaria) and in four patients (6.8%) in the IV abatacept group (dizziness, headache, rash, orthostatic hypotension). All systemic injection reactions were classified as mild in severity, and none led to early discontinuation.

Pharmacokinetic analysis

Geometric means for C_{max} and $AUC_{(tau)}$ following SC administration were 42.6 μg/mL (CV: 28.0%) and 5889.1 μg·h/mL (CV: 30.0%), respectively, compared with 277.4 μg/mL (35.0%) and 33,118.5 μg·h/mL (31.0%) following IV administration, after normalization for tau (Figure 3). Geometric mean C_{min} concentration at Day 169 was 36.0 μg/mL following weekly SC administration versus 16.7 μg/mL for monthly IV administration. Within the SC and IV abatacept groups, C_{min} was consistent across time points between 57 and 169 days. The absolute bioavailability of SC abatacept was 78.4%.

Immunogenicity

Of 59 patients receiving SC abatacept, one (1.7%) tested seropositive for anti-abatacept antibodies (anti-Ig and/or junction region antibody) on Day 85. Immunogenicity was transient (a single sample for this patient tested seronegative at Day 169) and did not lead to discontinuation of study drug.

Of 59 patients receiving IV abatacept, one patient (1.7%) tested seropositive for anti-CTLA4 and possibly Ig antibody on Day 169. This patient received their last dose of abatacept on Day 113 and discontinued from the study at Day 134 due to an SAE (extranodal marginal zone B-cell lymphoma [MALT type]). A sample collected from the patient 84 days after the last dose of abatacept was found to be seropositive; however, at 168 days after the last dose, the patient tested seronegative.

Patients who discontinued early were evaluated for immunogenicity after the last dose of the study drug. One patient treated with IV abatacept tested positive for anti-CTLA4 and Ig antibody on Day 85 after the last dose.

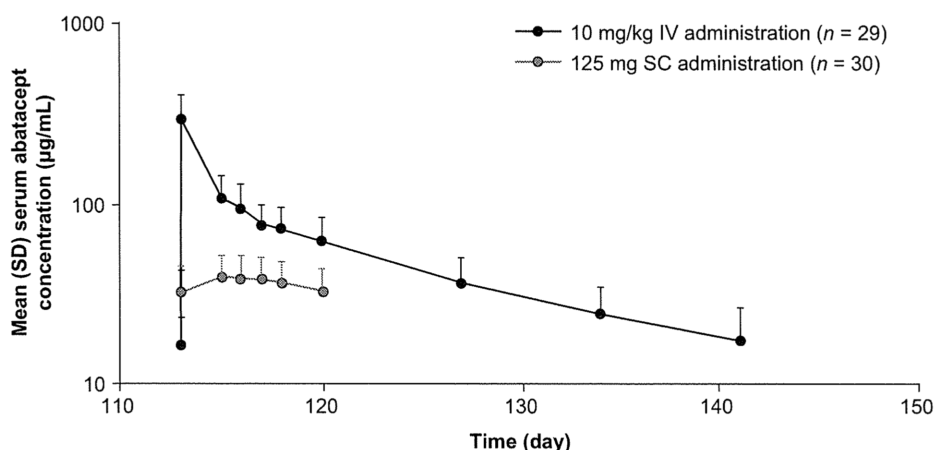

Figure 3. Mean (SD) abatacept serum concentration–time profiles following IV and SC administration of abatacept.

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Discussion

A variety of biologic agents for the treatment of RA are now available, administered via the SC or IV routes. The long-term efficacy and safety profile of IV abatacept in patients who are MTX-IR is well established [19–21]. An SC formulation of abatacept is now also approved in many countries including the USA, Japan and across Europe. The efficacy and safety profile of SC abatacept was previously shown to be equivalent to that of IV abatacept in MTX-IR patients with RA in ACQUIRE, a multinational, Phase IIIb, noninferiority study [8]. The aim of the study described here was to compare SC and IV abatacept in terms of efficacy, safety, pharmacokinetics and immunogenicity, in Japanese patients with moderate-to-severe active RA who were MTX-IR.

Similarities were observed between SC and IV abatacept in terms of the primary efficacy endpoint (ACR20 response rate on Day 169) and ACR20, -50 and -70 response profiles over time. Additionally, similar reductions in disease activity and improvements in physical function over 24 weeks were observed in the SC and IV groups. HAQ–DI responses and DAS28-CRP remission rates at Day 169 were numerically higher for SC than for IV abatacept, although, with overlapping CIs, the measures were comparable within the context of the variability potentially attributed to the small sample size. Similar results for safety were also observed between the two groups, and were consistent with the safety and tolerability profiles previously reported for SC and IV abatacept [22–25]. No local injection-site reactions were reported, and infusion reactions were infrequent and generally mild. Few patients discontinued due to AEs (~5% in each group), and retention rates were high overall (over 94% at Week 24). Fungal infection leading to abatacept discontinuation occurred in two patients in the SC abatacept group. In each case, the fungal infection was of moderate intensity. In RA, AEs of fungal infection are known for biologic therapies, and anti-tumor necrosis factors are subject to a US Food and Drug Administration black box warning for possible increased risk of developing fungal infections [26]. Fungal infections, such as candidiasis, have been observed previously with abatacept [8]. The ACQUIRE study demonstrated that there are no unusual infections associated with abatacept treatment [8]. In a network meta-analysis, IV abatacept was associated with statistically significantly fewer serious infections, including serious fungal infections, when indirectly compared with other biologics (odds ratio [95% CI]: 0.16 [0.06, 0.43] vs. certolizumab pegol; 0.39 [0.20, 0.77] vs. infliximab; 0.36 [0.15, 0.83] vs. tocilizumab) [27]. In a pooled safety analysis, rates of infections and of serious infections were numerically lower for SC versus IV abatacept (incidence rates [95% CI]: 53.91 [50.69, 57.33] vs. 75.68 [73.00, 78.44] and 1.94 [1.50, 2.50] vs. 2.87 [2.57, 3.19], events per 100 patient-years, respectively) [22,23]. The frequency of infections and serious infections were comparable for SC and IV abatacept in patients with up to 6 months of exposure to abatacept with concomitant MTX in the ACQUIRE study (31.8% vs. 30.7% and 0.7% vs. 1.4%, respectively) [8]. The results presented here are consistent with those from ACQUIRE in demonstrating similar efficacy and safety profiles for SC and IV abatacept.

As expected, systemic exposure (in terms of C_{max} and AUC) was higher following IV administration compared with SC administration. The absolute bioavailability of the SC formulation was 78.4% in this patient population. However, a fixed SC abatacept dosing regimen of 125 mg weekly achieved mean serum concentrations that were higher than the minimum therapeutic target of > 10 μg/mL. The steady-state C_{min} of abatacept was demonstrated to be consistent from Day 57 to Day 169.

The proportion of patients testing positive for immunogenicity was low in both SC and IV groups and, when detected, was transient. This is consistent with immunogenicity findings from the ACQUIRE study [8].

The results of this study should be interpreted within the limitations of a descriptive, short-term, 24-week study. The efficacy and safety of SC abatacept beyond this treatment duration in Japanese patients with RA are unknown, but are being studied in an open-label, 1-year extension of this study [28]. For a chronic disease such as RA, longer-term safety data are important. Although the study design and findings described here have many parallels with those from the ACQUIRE study, caution should be practiced in direct comparison of data from the two studies because of differences, including sample size, patient demographics, baseline disease characteristics and response to therapy. In addition, patients were not permitted MTX doses above 6–8 mg weekly. Although many rheumatologists in Japan initiate MTX at ~8 mg/week, the maximum MTX dose currently available in Japan is 16 mg weekly. As such, the MTX dose given in this study may be different to doses used in other clinical trials, and this may have implications for indirect, interstudy comparisons. These data also indicate that clinical efficacy can be achieved with low-dose MTX in combination with SC or IV abatacept in many patients, which could be important for use in clinical practice.

Overall, these data indicate that the safety and efficacy of SC abatacept in Japanese patients are consistent with those observed in the global population [8]. Abatacept administered via the SC route demonstrated efficacy and safety profiles similar to those of IV abatacept, in Japanese patients with RA who were MTX-IR. Fixed SC dosing of abatacept at 125 mg/week achieved mean serum concentrations above the minimum therapeutic target of > 10 μg/mL. Immunogenicity rates were low and transient for both SC and IV abatacept. The efficacy and safety of IV abatacept in Japanese patients are well documented; findings reported here support the inclusion of SC abatacept as an option for the treatment of RA in Japanese patients who are MTX-IR.

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Conflict of interest

M. Iwahashi has received lecture fees from Bristol-Myers K.K., Chugai Pharmaceutical Co., Ltd, Pfizer Japan Inc., Mitsubishi Tanabe Pharma Corporation, Janssen Pharmaceutical K.K., Abbott Japan Co., Ltd, Astellas Pharma Inc., Santen Pharmaceutical Co., Ltd and Asahi Kasei Pharma

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Corporation. K. Amano has received lecture fees from AbbVie GK., Astellas Pharma Inc., Bristol-Myers K.K., Chugai Pharmaceutical Co., Ltd, Mitsubishi Tanabe Pharma Corporation, Eisai Co., Ltd and Pfizer Japan Inc. T. Nakano has received lecture fees from Eisai Co., Ltd, Pfizer Japan Inc., Abbott Japan Co., Ltd, Daiichi Sankyo Company Limited, Astellas Pharma Inc., Ono Pharmaceutical Co., Ltd, UCB Japan Co. Ltd and Mitsubishi Tanabe Pharma Corporation. T. Takeuchi has received grants from Abbott Japan Co., Ltd, Astellas Pharma Inc., Bristol-Myers K.K., Chugai Pharmaceutical Co., Ltd, Daiichi Sankyo Company, Limited, Eisai Co., Ltd, Mitsubishi Tanabe Pharma Corporation, Pfizer Japan Inc., Sanofi K.K., Santen Pharmaceutical Co., Ltd, Takeda Pharmaceutical Company Limited, Teijin Pharma Limited, AbbVie GK., Asahi Kasei Pharma Corporation and Taisho Toyama Pharmaceutical Co., Ltd; lecture fees from Abbott Japan Co., Ltd, Bristol-Myers K.K., Chugai Pharmaceutical Co., Ltd, Eisai Co., Ltd, Janssen Pharmaceutical K.K., Mitsubishi Tanabe Pharma Corporation, Pfizer Japan Inc., Takeda Pharmaceutical Company Limited., Astellas Pharma Inc. and Daiichi Sankyo Company Limited; consulting fees from AstraZeneca K.K., Eli Lilly Japan K.K., Novartis Pharma K.K., Mitsubishi Tanabe Pharma Corporation, Asahi Kasei Pharma Corporation, AbbVie GK and Daiichi Sankyo Company Limited. A. Yamazaki is a paid employee of Bristol-Myers K.K. and owns Bristol-Myers Squibb stock. C. Karyekar is a paid employee of Bristol-Myers Squibb and owns Bristol-Myers Squibb stock. H. Inoue, T. Matsubara, T. Tanaka, T. Kanamono, S. Uchimura and T. Izumihara have nothing to disclose.

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REVIEW

The gut microbiota and inflammatory bowel disease

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Abstract Inflammatory bowel disease (IBD) is a chronic and relapsing inflammatory disorder of the gut. Although the precise cause of IBD remains unknown, the most accepted hypothesis of IBD pathogenesis to date is that an aberrant immune response against the gut microbiota is triggered by environmental factors in a genetically susceptible host. The advancement of next-generation sequencing technology has enabled identification of various alterations of the gut microbiota composition in IBD. While some results related to dysbiosis in IBD are different between studies owing to variations of sample type, method of investigation, patient profiles, and medication, the most consistent observation in IBD is reduced bacterial diversity, a decrease of Firmicutes, and an increase of Proteobacteria. It has not yet been established how dysbiosis contributes to intestinal inflammation. Many of the known IBD susceptibility genes are associated with recognition and processing of bacteria, which is consistent with a role of the gut microbiota in the pathogenesis of IBD. A number of trials have shown that therapies correcting dysbiosis, including fecal microbiota transplantation and probiotics, are promising in IBD.

Keywords Inflammatory bowel disease · Ulcerative colitis · Crohn's disease · Dysbiosis

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Introduction

Inflammatory bowel disease (IBD) is a disorder characterized by chronic and relapsing intestinal inflammation and is mainly defined as either ulcerative colitis (UC) or Crohn's disease (CD). Although the cause of IBD remains unknown, genetic background is considered to be involved in the pathophysiology of IBD because a number of disease susceptibility genes have been identified. The rapid increase in the incidence of IBD, however, cannot be explained by genetic factors alone, and environmental factors must also be essential to its development.

The involvement of the gut microbiota in the pathophysiology of IBD has recently been highlighted. Several lines of evidence suggest an essential role of the gut microbiota in intestinal inflammation. (1) In murine models of IBD such as IL-10-deficient mice and the $CD45Rb^{high}$ transfer model, where transferred naïve helper T cells cause microbiota-dependent intestinal inflammation in immune-deficient recipients such as $Rag2^{-/-}$ mice, germ-free animals do not develop colitis. (2) Diversion of the fecal stream ameliorates intestinal inflammation in CD [1]. (3) Antibiotics are, to a certain degree, effective for the treatment of IBD [2]. (4) Antibiotics such as metronidazole and ciprofloxacin are also effective for anal lesions and prevention of postoperative recurrence in CD [3]. (5) Many of the reported IBD susceptibility genes are associated with recognition and processing of microbes [4].

Given these observations, the most accepted hypothesis of IBD pathogenesis to date is that an aberrant immune response against the gut microbiota is triggered by environmental factors in a genetically susceptible host. In this review, we will discuss abnormalities of the gut microbiota observed in IBD, their contribution to the pathogenesis of IBD, and related therapeutic applications.

Alteration of the gut microbiota in IBD

Dysbiosis

The host provides a nutrient-rich environment and residence for the gut bacteria, and in turn, they contribute to the host by producing short-chain fatty acids and essential vitamins. This mutual relationship between the host and the gut bacteria is called symbiosis. Recent advancement of next-generation sequencing techniques has enabled culture-independent analysis of the gut microbiota, revealing that an altered balance of the gut microbiota constituents, rather than specific pathogens, is involved in the pathophysiology of several diseases. This shift in the balance of the gut microbiota is referred to as dysbiosis.

More than 90 % of the human gut microbiota is composed of four major phyla. The Firmicutes (49–76 %) and Bacteroidetes (16–23 %) phyla dominate, followed to a much less extent by the Proteobacteria and Actinobacteria phyla [5, 6]. The Firmicutes phylum is mainly composed of the *Clostridium* XIV and IV groups.

Various alterations of the gut microbiota have been reported in IBD patients (Table 1). Most studies have shown reduced diversity of the gut microbiota in IBD patients [6–9]. The most consistent observations of altered composition of the gut microbiota in IBD patients are a reduction in Firmicutes and an increase in Proteobacteria [6, 7, 10–12]. The reduced diversity of the gut microbiota observed in IBD patients is largely due to a decline in the diversity of Firmicutes. Among Firmicutes, a decrease in the *Clostridium leptum* groups, especially *Faecalibacterium prausnitzii*, has been reported in many studies [7, 13, 14]. Results related to Enterobacteriaceae, *Bacteroides*, *Bifidobacteria* species, *Lactobacillus* species, and *Escherichia coli* are not consistent among studies [15, 16]. Various factors may explain the between-study discrepancies: (1) sample source (biopsy or stool), (2) sampling location (inflammatory or noninflammatory sites), (3) disease activity (active or quiescent), (4) medication, (5) diet, (6) age, (7) smoking, and (8) methods used to analyze the microbiota.

While the gut microbiota in healthy subjects shows little temporal change, the gut microbiota in IBD patients is unstable. The composition of the gut microbiota differs between active and quiescent stages. Furthermore, a study that longitudinally examined the gut microbiota in IBD patients for a year demonstrated that the gut microbiota was unstable even in UC patients in remission [17]. Before relapse of UC, normal anaerobic bacteria such as *Bacteroides*, *Escherichia*, *Eubacterium*, *Lactobacillus*, and *Ruminococcus* are decreased and the diversity of the gut microbiota is also reduced [18]. In CD patients, dysbiosis is observed even in patients with remission. Medication also affects the composition of the gut microbiota. Mesalazine, for example, reduces the total bacterial number to almost half [19]. Bowel rest, which is a treatment option in CD, changes the composition of the gut microbiota. Antibiotics dramatically amplify the dysbiosis of CD [20].

The distribution of the gut microbiota should be taken into account when interpreting dysbiosis. For example, the composition of the microbiota is significantly different between fecal and mucosal samples [5, 21]. Mucosal samples are reported to be superior in order to detect dysbiosis [20]. The mucosa-associated microbiota is increased in IBD compared with healthy subjects [22, 23]. It is tempting to speculate that the mucosa-associated microbiota is physiologically more important in IBD than luminal microbiota because of the close contact of mucosa-associated bacteria with the intestinal surface. It has also been reported that the gut microbiota is different in the same individual between inflammatory and noninflammatory sites [24]. The dysbiosis observed in noninflammatory sites may be more representative of a causative composition because the dysbiosis observed in inflammatory sites may be affected by inflammation.

It remains controversial whether dysbiosis is a cause or consequence of intestinal inflammation in IBD. Comparison of the gut microbiota composition of IBD patients with that of their unaffected relatives, who are likely to share genetic and environmental background, is useful to provide evidence relevant to this fundamental question. Compositional change of the gut microbiota was not consistent between UC patients and their unaffected twins [25]. In contrast, a decrease in *F. prausnitzii* was reported to be observed in both UC patients and their first-grade relatives [26]. Unaffected relatives of CD patients also had dysbiosis, although it was different from the dysbiosis observed in CD patients [27]. Furthermore, it was reported that the genetic status of *NOD2* and *ATG18L* genes, which are two major CD susceptibility genes, was associated with alteration of the gut microbiota [28]. These results suggest that dysbiosis is caused by genetic and environmental factors, rather than being a consequence of inflammation.

There have been attempts to utilize dysbiosis as a diagnostic tool or biomarker [9]. To date, there are no microbial constituents specific to UC or CD, because interindividual variations are much larger than inter-disease differences [6]. Several studies have suggested the possibility of using the gut microbiota as a biomarker. Firmicutes, for example, was increased in UC patients who responded to mesalazine [19]. The relapse rate was lower in postoperative CD patients who had a similar composition of gut microbiota to healthy controls than in those with dysbiosis [29]. In the largest CD microbiota cohort so far, comparing 447 newly diagnosed pediatric CD patients with 221 healthy controls, Gevers et al. proposed a "dysbiosis index," which was shown to be associated with clinical disease severity assessed using the Pediatric Crohn's Disease Index [20]. They also reported that profiles of the gut microbiota were able to be utilized as a diagnostic marker of CD and were also useful to predict the severity at 6 months.

Table 1 Metagenomic analysis of the gut microbiota in inflammatory bowel disease

Sample source	Sample no.			Diversity	Bacterial no.	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria	Ref.
	CD	UC	HC							
Stool	6	–	6	↓ in CD		↓ in CD	→ in IBD			[7]
Biopsy	6	5	5			↓ in CD	↑ in CD	↑ in IBD	↑ in CD	[10]
Surgical tissue	35	55	34		↓ in IBD	↓ Lachnospiraceae ↑ *Bacillus*	↓ in IBD	↑ Bifidobacteriaceae in cCD ↑ Coriobacteriaceae in cCD	↑ in IBD	[6]
Stool	29	16	35	↓ in CD	↓ in CD	↑ in cCD, ↓ in iCD ↑ Ruminococcaceae in cCD ↓ Ruminococcaceae in iCD			↑ Enterobacteriaceae in CD	[8]
Biopsy	6	6	5	↓ in IBD	↓ in CD	↓ in IBD → *F. prausnitzii* in IBD	↑ in IBD		↑ Enterobacteriaceae in CD	[24]
Biopsy Stool	121	75	27			↓ in CD		↑ in IBD	↑ Enterobacteriaceae in CD	[49]
Endoscopic lavage	16	16	32	↓ in IBD		↓ in IBD ↓ *F. prausnitzii* in IBD			↑ in IBD	[9]
Stool	21	34	21		→	↓ *C. coccoides* and *C. leptum* in IBD ↑ *Lactobacillus* in CD ↓*F. prausnitzii* in IBD		↑ *Bifidobacterium* in UC	↑ *E. coli* in CD	[14]
Biopsy	29	15	21		↓ in IBD	↓ *C. coccoides* in CD ↓ *C. leptum* in IBD ↑ *Lactobacillus* in CD ↓ *F. prausnitzii* in IBD	↑ in IBD	↓ Bifidobacteriaceae in CD	↑ *E. coli* in IBD	[14]
Biopsy and stool	447	–	221	↓ in CD		↓ Clostridiales in CD	↓ in CD		↑ Enterobacteriaceae in CD	[20]

CD Crohn's disease, *UC* ulcerative colitis, *IBD* inflammatory bowel disease, *iCD* ileal CD, *cCD* colonic CD

This report encourages further efforts to use gut microbial profiles as a diagnostic tool or biomarker for disease activity, prognosis, and response to treatment.

Specific bacteria associated with IBD

There have been no specific pathogens yet identified that fulfill Koch's postulates. There are, however, several specific bacteria that are associated with IBD. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes chronic granulomatous ileitis (Johne's disease) in cattle and sheep, which shares some pathological features with CD. In addition, because MAP has been found in commercial milk, it is suspected as a causative pathogen of CD. MAP was detected in CD patients using mucosal PCR, and the positive rates of serum anti-MAP antibody were higher in CD patients than in healthy controls or UC patients [30]. However, a clinical trial of a 2-year administration of antituberculosis drugs to CD patients showed no efficacy [31]. Adhesive-invasive *E. coli* (AIEC), which can adhere to and invade the intestinal epithelial cells, colonize the ileal mucosa of CD [32]. AIEC also replicate in macrophages and stimulate TNFα production from macrophages. It was observed that *Fusobacterium varium* attaches to inflamed regions in UC and invades the mucosa at ulcers [33]. Serum titers for anti-*F. varium* antibodies were higher in UC patients compared with healthy controls [34]. *F. varium* produces butyrate, and rectal administration of butyrate has been shown to cause mucosal damage in mice [35]. The pathological consequence of butyrate production by *F. varium*, however, needs to be examined more extensively because butyrate has diverse effects on the intestinal homeostasis including Treg induction in the gut and energy supply to the intestinal epithelial cells. The combination therapy of amoxicillin, tetracycline, and metronidazole (to which *F. varium* is sensitive) for 2 weeks showed efficacy in active UC patients, suggesting the possible pathogenic role of *F. varium* [36].

The role of the gut microbiota in IBD revealed by susceptibility genes

The association of IBD susceptibility genes with bacteria has recently been highlighted. The development of the genome-wide association study has contributed greatly to the

identification of more than 160 IBD susceptibility genes to date [4]. The physiological functions of these genes are categorized into several groups relating to (1) acquired immunity (*IL23R*, *IL12B*, *JAK2*, *STAT3*), (2) bacterial recognition and processing (*NOD2/CARD15*), (3) autophagy (*ATG16L*, *IRGM*, *ATG5*), and (4) mucosal barrier (*ECM1*, *CDH1*, *LAMB1*) [37]. Many of the CD susceptibility genes are associated with bacterial recognition and processing, and many of the UC susceptibility genes are related to mucosal barrier function, suggesting that impaired handling of bacteria or disruption of the mucosal barrier function leads to breakdown of tolerance against the commensal bacterial in the gut in CD and UC, respectively.

NOD2/CARD15 was the first reported CD susceptible gene and shows the strongest association with CD. The function of the NOD2 protein has been extensively studied. NOD2 is an intracellular receptor for muramyl dipeptide (MDP), a component of the cell wall of gram-positive bacteria, and is expressed in intestinal epithelial cells and monocytes/macrophages. While *Nod2*-deficient mice do not develop spontaneous colitis, the bacterial load in the gut is increased in these mice. CD patients with *NOD2* mutations demonstrate diminished production of antimicrobial peptides (AMPs) from Paneth cells [38] as well as reduced production of the anti-inflammatory cytokine IL-10 from peripheral mononuclear cells [39]. NOD2 stimulation with MDP induces autophagy [40], which regulates replication of intracellular bacteria and is also involved in bacterial antigen presentation in the infected cells.

Several autophagy-related genes have also been reported as CD susceptibility genes. Autophagy is an intracellular process that is involved in degradation and recycling of proteins when cells are in starvation. Autophagy is also involved in the handling of intracellular pathogens. *ATG16L* is a susceptibility gene for CD and is essential for autophagosome formation. Interestingly, it has been shown that NOD1 and NOD2 sense bacterial invasion into the cell and recruit ATG16L to the site of bacterial entry, which triggers autophagy. The intracellular bacteria are then processed through autophagy [40]. This close association between NOD2 and ATG16L suggests the importance of this pathway in the pathophysiology of CD.

Studies on IBD susceptibility genes have revealed the essential role of Paneth cells in CD. Paneth cells reside at the bottom of the intestinal crypts and produce AMPs. The important role of Paneth cells in the regulation of the gut microbiota and the intestinal immune system is shown by the observation that genetically engineered mice overexpressing α-defensin, one of the AMPs, in intestinal epithelial cells had a reduced number of segmented filamentous bacteria (SFB) in the gastrointestinal tract, resulting in impaired Th17 development in the gut [41]. Abnormalities in the size, number, and distribution of granules in Paneth cells, which contain AMPs, have been observed in CD. These morphological abnormalities were reported to be more frequent in CD patients with *NOD2* or *ATG16L* mutations [42]. Mice harboring the same *Atg16l* mutation as CD patients develop similar morphological abnormalities of Paneth cells after murine *Norovirus* infection and become susceptible to dextran sodium sulfate (DSS)-induced colitis [43]. These results provide a good example of the complex interaction between a genetic factor and an environmental factor in the development of intestinal inflammation. Mice deficient in *Xbp1*, which is an essential molecule for endoplasmic reticulum stress and is a CD susceptibility gene, also have impaired autophagy induction in Paneth cells and develop spontaneous ileitis [44]. These results suggest that autophagy in Paneth cells is critical for maintaining gut homeostasis, probably through regulation of the gut microbiota by AMP production. Impaired Paneth cell function may be an essential element in the development and perpetuation of intestinal inflammation in CD.

How does dysbiosis lead to intestinal inflammation?

It is well known that different commensal bacteria induce distinct types of colitis in IL-10-deficient mice. A mono-association study, in which a single strain of bacteria was inoculated into germ-free IL-10-deficient mice, demonstrated that *E. coli* induced cecal inflammation, *Enterococcus faecalis* induced distal colitis, and *Pseudomonas fluorescens* did not cause colitis [45]. It was also reported that the presence of *Helicobacter hepaticus*, a species of commensal bacteria, exacerbated colitis in IL-10-deficient mice. These results show that alteration of the composition of the gut microbiota can cause distinct intestinal immune responses even in a host with the same genetic background, suggesting that dysbiosis can modulate the immune response in the gut.

Garrett et al. [46] reported that mice deficient in both *Tbx21/T-bet*, which is an essential transcription factor for Th1 differentiation, and *Rag*, which is indispensable for the acquired immune system, developed spontaneous UC-like colitis, which was ameliorated by the administration of antibiotics. Importantly, wild-type mice co-housed with colitic *T-bet/Rag* double knockout mice also developed similar colitis, suggesting that a dysbiotic gut microbiota is communicable and can cause intestinal inflammation without genetic manipulation.

Functional changes in the gut microbiota resulting from dysbiosis may be involved in the pathophysiology of IBD. The number of genes harbored in the gut microbiota is 100 times greater than that in the human genome [5, 47, 48]. Metabolites of the gut microbiota contribute to epithelial cell function, energy balance, and the immune system of the host. A metagenomic analysis of the gut microbiota showed a decrease in genes responsible for carbohydrate and amino acid metabolism and an increase in those in the oxidative

stress pathway, in IBD patients [49], raising the possibility that oxidative stress from the gut microbiota causes intestinal inflammation in IBD patients. A specific metabolite of the gut microbiota is also likely to be involved in the pathophysiology of IBD. The gut microbiota metabolizes nonabsorptive dietary fiber and produces short-chain fatty acids such as butyrate and propionate. Commensal bacteria-derived butyrate induces the differentiation of colonic regulatory T cells in mice [50]. Butyrate is also an important energy source for intestinal epithelial cells and increases production of mucin and AMPs [13]. The concentrations of butyrate in feces have consistently been shown to be decreased in IBD patients. Consistently, *F. prausnitzii*, a species of butyrate-producing bacteria, has also been observed to be decreased in IBD [16]. It is possible that the decreased level of butyrate in the gut contributes to inducing intestinal inflammation. Another example of a functional alteration of the gut microbiota in IBD is the increase of sulfate-reducing bacteria (SRB) in UC [51]. SRB produce hydrogen sulfide, which is toxic to the intestinal epithelial cells and can cause mucosal inflammation.

Recent studies have revealed that specific bacteria control the intestinal immune system. SFB, for example, induce Th17 cells in the murine intestine [52]. Although the human counterpart of SFB has not yet been identified, SFB-like organisms were observed in six out of six surgical specimens from UC patients [53]. These SFB-like organisms were not observed in surgical specimens from CD patients. In non-IBD controls, SFB-like organisms were observed in three out of six specimens, with a much lower density compared with UC. These are interesting observations because Th17 cells were reported to be increased in IBD. The physiological role of these SFB-like organisms requires further investigation.

The number of bacteria in the mucus layer is increased in IBD [22], suggesting impaired mucosal barrier function. This is consistent with the fact that many of the UC susceptibility genes are related to mucosal barrier function. Furthermore, bacteria that can degrade mucins in the mucus layer and utilize it as an energy source, for example *Ruminococcus gnavus* and *Ruminococcus torques*, are increased in IBD. These bacteria help other bacteria reside in the mucus layer by providing degraded mucins as nutrients.

IBD therapies targeting the gut microbiota

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is a treatment to restore abnormal microbial composition of the gut by introducing fecal microbiota obtained from a healthy donor into a diseased individual. The results of a randomized study to compare FMT with antibiotics for recurrent *Clostridium difficile* infection were striking [54]. Resolution of *C. difficile*-associated diarrhea was observed in 13 of 16 patients (81 %) in the FMT group, compared with four out of 13 patients (31 %) in the antibiotics group.

FMT has been highlighted as a treatment to correct dysbiosis in IBD. The first implementation of FMT in UC was reported in 1989 [55]. One of the authors of the paper received FMT for his continuously active UC, resulting in drug-free remission. A recent systematic review identified 18 UC patients without *C. difficile* infection who were treated with FMT [56]. Thirteen out of the 18 patients experienced disease resolution; however, selection bias should be considered because all of the cases were from case reports or small case series.

Two prospective studies of FMT for adult UC patients were recently published [57, 58]. Both of these studies longitudinally analyzed the change of bacterial composition in FMT recipients. Unexpectedly, none of the combined 11 patients in the two studies achieved clinical remission after FMT. In contrast, a significant change in the gut microbiota composition was observed in most of the patients. One paper reported that the successful colonization of donor microbiota was correlated with clinical improvement in one patient, but the other study did not confirm this finding. Both of the papers reported that the alteration of gut microbiota was temporary in most patients, suggesting the necessity of periodically repeated transplantation to maintain the altered gut microbiota.

A phase I trial of FMT for 10 pediatric UC patients with mild-to-moderate activity has recently been completed, reporting no serious adverse events and a high rate of clinical response (79 %) within 1 week [59]. This result is in contrast to the above-mentioned studies. One possible reason to explain this discrepancy is that a certain population of UC patients, but not all, may benefit from FMT. Interestingly, Angelberger et al. identified phylotypes that are indicative of disease severity and FMT success, specifically over-presentation of Enterobacteriaceae and under-repression of Lachnospiraceae [57]. This is an important factor in selecting a subgroup of UC patients that may be responsive to FMT.

Probiotics

Probiotics are preparations utilizing live bacteria that can be beneficial to human health. Several reports have shown the efficacy of various probiotic bacteria for IBD (Table 2). Efficacy of probiotics was studied more extensively in UC than CD. VSL#3 is a freeze-dried preparation containing eight different lactic acid bacteria (*Lactobacillus acidophilus*, *L. bulgaricus*, *L casei*, *L. plantarum*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *B. infantis*, and *B. longum*). Two double-blinded placebo-controlled trials

Table 2 Randomized controlled trials of probiotics for inflammatory bowel disease

Probiotics	Disease	Endpoint	Groups and subject no.	Duration	Conclusion	Ref.
VSL#3	UC	Induction	Conventional therapy+VSL#3, 77 Conventional therapy+placebo: 70	12 weeks	Effective	[63]
	UC	Induction	Conventional therapy+VSL#3, 71 Conventional therapy+placebo, 73	8 weeks	Effective	[62]
	UC	Induction Maintenance	Steroid/mesalazine+VSL#3, 14 Steroid/mesalazine+placebo, 15	1 year	Effective	[64]
	Pouchitis	Maintenance	VSL#3, 20 Placebo, 20	9 months	Effective	[60]
	Pouchitis	Maintenance	VSL#3, 20 Placebo, 16	12 months	Effective	[61]
Nissle 1917	UC	Induction	Steroid+mesalazine, 57 Steroid+*Nissle* 1917, 59	12 weeks	Equivalent to mesalazine	[66]
	UC	Maintenance	*Nissle* 1917, 162 Mesalazine, 165	12 months	Equivalent to mesalazine	[67]
	UC	Maintenance	Mesalazine, 50 *Nissle* 1917, 53	12 weeks	Equivalent to mesalazine	[68]
Lactobacillus GG	CD	Maintenance	Conventional therapy+*Lactobacillus* GG, 39 Conventional therapy+placebo, 36	~2 years	Not effective	[71]
	UC	Maintenance	*Lactobacillus* GG, 65 *Lactobacillus* GG+mesalazine, 62 Mesalazine, 60	12 months	Equivalent to mesalazine	[70]
Bifidobacteria-fermented milk (BFM)	UC	Induction	Conventional therapy+BFM, 10 Conventional therapy+placebo, 10	12 weeks	Effective	[74]
	UC	Maintenance	Conventional therapy+BFM, 11 Conventional therapy, 10	12 months	Effective	[75]
Bifidobacterium longum/Synergy 1	UC	Induction	*Bifidobacterium longum*/Synergy 1, 9 Placebo, 9	1 month	Effective	[73]

have shown the efficacy of VSL#3 in the prevention of recurrence in patients with chronic relapsing pouchitis [60, 61]. Two randomized controlled clinical trials showed that addition of VSL#3 to conventional therapy was more efficacious in remission induction in active UC patients [62, 63]. Another randomized controlled trial compared the efficacy of addition of VSL#3 to mesalazine and steroids in 29 newly diagnosed pediatric UC patients with addition of a placebo [64]. The remission rates at 4 weeks were significantly higher in the VSL#3 group (92.8 % (13/14)) than in the placebo group (36.4 % (4/15)). Furthermore, the recurrence rates at 1 year were 36.4 % (3/14) and 73.3 % (11/15) in the VSL#3 and placebo groups, respectively. These results show that VSL#3 is effective for induction of remission as well as its maintenance in UC patients. The mechanism of the anti-inflammatory effect of VSL#3 is not yet fully understood but an increase in regulatory T cells in the gut and upregulation of mucosal alkaline sphingomyelinase have been reported [65].

Nissle 1917, a nonpathogenic *E. coli* strain, showed efficacy in maintenance of remission in UC equivalent to mesalazine [66–68]. *Nissle 1917* inhibits IL-8 production stimulated by TNFα from the epithelial cells [69]. *Lactobacillus GG* can be effective for maintenance of remission in UC patients [70], but not in CD patients [71, 72]. A pilot study demonstrated that *Bifidobacterium longum/Synergy 1* improved intestinal inflammation in active UC patients [73]. *Bificobacteria*-fermented milk also demonstrated efficacy in induction and maintenance of remission in UC patients [74, 75].

We reported that *Clostridium butyricum* (CB), which is used as a probiotic for patients with functional gastrointestinal disorders in a clinical setting, suppresses intestinal inflammation in murine IBD models [76]. CB potently induced the anti-inflammatory cytokine IL-10 from colonic mucosal macrophages. This IL-10 production was dependent on the Toll-like receptor 2/MyD88 pathway. The effect of CB was abolished in IL-10-deficient mice, suggesting that the anticolitic effect of CB was due to IL-10. Interestingly, heat-killed CB also induced IL-10 from macrophages, strongly indicating that bacterial-derived products are responsible for IL-10 induction. A clinical trial to examine the anticolitic effect of CB in IBD patients is warranted.

Closing remarks

The gut immune system is separated from an enormous number of bacteria only by a single layer of epithelial cells. It is thus tempting to speculate that the gut microbiota is involved in the pathophysiology of IBD. The advancement of next-generation sequencing technology has revealed a range of alterations of the gut microbiota in IBD. However, it remains unclear whether the dysbiosis observed in IBD is a cause or a consequence of intestinal inflammation. To answer this essential question, studies to examine longitudinal changes of the microbiota in a large number of IBD patients, especially newly diagnosed patients, are necessary. Furthermore, little information is available to how dysbiosis regulates the gut immune system. Understanding the complex relationship between the gut immune system and the microbiota should lead to further elucidation of the pathogenesis of IBD and development of curative treatments. Utilizing the gut microbiota as a diagnostic tool or biomarker is also an attractive idea. To this end, a disease-specific or activity-specific core microbiome should be identified. The gut microbiota is composed not only of bacteria but also of viruses and fungi. It is important to include viruses and fungi in any investigation of the gut microbiota. Furthermore, functional analysis of the gut microbiota in IBD is warranted. Because a majority of the gut bacteria are unable to be cultured, gnotobiotic approaches will be an important tool to investigate the function of the gut microbiota. The gut microbiota represents a "gold mine" for both clinical and basic IBD research.

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