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V．研究成果の刊行物・別冊
（主なもの）

Mod Rheumatol (2008) 18:252–262
DOI 10.1007/s10165-008-0045-0

ORIGINAL ARTICLE

Clinical investigation in highly disease-affected rheumatoid arthritis patients in Japan with adalimumab applying standard and general evaluation: the CHANGE study

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Received: 26 July 2007 / Accepted: 2 January 2008 / Published online: 12 March 2008
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Abstract This multicenter, double-blind study evaluated the effects of three doses of adalimumab in Japanese patients with rheumatoid arthritis (RA). Patients were randomized to placebo ($n = 87$) or adalimumab 20 mg ($n = 87$), 40 mg ($n = 91$), or 80 mg ($n = 87$) every other week for 24 weeks. The primary efficacy endpoint was the American College of Rheumatology criteria for 20% improvement (ACR20) at Week 24. At Week 24, all adalimumab treatment groups achieved statistically significantly better ACR20 response rates (20 mg: 28.7%, $P < 0.05$; 40 mg: 44.0%, $P < 0.001$; and 80 mg: 50.6%, $P < 0.001$) versus placebo (13.8%), as well as statistically significantly greater ACR50 and ACR70 responses for the two higher adalimumab doses versus placebo. Rates of adverse events were comparable between the adalimumab groups and the placebo group, except for injection-site reactions, which occurred in more adalimumab-treated patients. Adalimumab 20, 40, and 80 mg were safe and effective in Japanese patients; however, the greatest responses occurred with the 40 and 80 mg doses. These results and comparable ACR20 responses in Western patients support adalimumab 40 mg every other week as the appropriate dosage to treat RA in Japanese patients.

Keywords Adalimumab · Japanese · Pharmacokinetics · Rheumatoid arthritis · Tumor necrosis factor (TNF) antagonist

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Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis, estimated to affect between 0.5 and 1.0% of the adult population worldwide [1]. Geographic differences in the incidence of RA in a particular country may be associated with genetic, environmental, or cultural factors [1]. Moreover, differences in ethnicity, medical status, and socioeconomic status may affect response to RA treatment [2].

In Japan, it is estimated that more than 700,000 patients within a population of 120 million have RA [3]. Of those afflicted, 70% are women and 10% are bedridden; the morbidity rate is estimated to be 0.5% [3, 4]. In Japanese patients, RA is an independent risk factor for mortality [5].

In the past decade, the treatment of RA has dramatically changed with the introduction of biologics that target inflammatory cytokines, such as tumor necrosis factor (TNF) [6–8]. Recently, the TNF antagonists infliximab (in combination with methotrexate [MTX]) and etanercept have demonstrated efficacy in Japanese patients with RA [2, 9–11]. Adalimumab is the first fully human monoclonal antibody that binds with high specificity to TNF [12]. Adalimumab has been well established in the treatment of patients with RA in multiple clinical trials conducted in North America [13] and in Europe, [14] both with and without concomitant MTX.

The objective of this study (CHANGE: Clinical investigation in Highly disease-affected rheumatoid Arthritis patients in Japan with Adalimumab applying staNdard and General Evaluation) was to evaluate the safety, efficacy, and pharmacokinetics of three different doses of subcutaneous adalimumab compared with placebo in Japanese patients with RA. In addition, this study was designed as a bridging study to compare efficacy and safety results with

those from a previously published study of adalimumab conducted in Western patients with RA [14].

Although adalimumab in Western regions of the world is primarily used in combination with MTX, this study evaluated adalimumab monotherapy and compared the results with the Western study in which adalimumab was also evaluated as monotherapy. Moreover, the combination of adalimumab and MTX was not used in this bridging study because of differences in MTX dosages used in Japan and Western countries. The maximum approved oral MTX dosage in Japan is 8 mg/week, which is much lower than the 15–20 mg/week dosage commonly used in Western countries [2, 8, 15, 16].

Patients and methods

Male and female patients aged 20 years or older were recruited from 68 sites in Japan. Eligible patients met the American College of Rheumatology (ACR) criteria for active RA, had failed treatment with at least one prior disease-modifying antirheumatic drug (DMARD), and had $\geq$10 swollen joints and $\geq$12 tender joints (excluding distal interphalangeal joints) at both the screening visit and baseline visit. Patients also had a C-reactive protein (CRP) concentration $\geq$2 mg/dl.

Patients taking a DMARD, including MTX, must have discontinued DMARDs at least 28 days prior to study drug administration and returned for baseline visit within 42 days. Use of a live vaccine within 3 months; treatment with an investigational biologic, including anti-CD4 antibody, within 6 months; or prior treatment with any TNF antagonist or an alkylating agent was not permitted.

Exclusion criteria included patients with acute inflammatory joint diseases other than RA, active *Listeria* or tuberculosis, lymphoma, or leukemia, or any malignancy except for successfully treated nonmetastatic basal-cell carcinoma of the skin. Patients with positive serology for anti-human immunodeficiency virus antibody, hepatitis B virus surface antigen, or anti-hepatitis C virus antibody, ongoing or active infection, advanced or poorly controlled diabetes, or central nervous system demyelinating disorders were also excluded.

Patients with positive chest X-ray or strongly positive tuberculin skin test ($\geq$10 mm diameter of erythema and double redness/bullae/necrosis) could not be enrolled. Patients with a positive ($\geq$5 mm of induration), but not strongly positive, tuberculin skin test could be enrolled if receiving prophylactic isoniazid 300 mg daily at least three weeks prior to baseline.

A negative pregnancy test and use of reliable contraception were mandatory for women of childbearing potential. All patients were required to give written informed consent. This study was conducted in compliance with the study protocol, the standards of the Pharmaceutical Affairs Law, the Ministerial ordinance concerning Good Clinical Practice, and all other applicable regulatory requirements.

Study design

This was a Phase II/III, multicenter, double-blind, placebo-controlled trial comparing three different doses of adalimumab given as monotherapy performed from February 2004 through June 2005.

Patient eligibility was determined at screening and at baseline, during the period from 28 to 42 days prior to study drug administration for patients who required a washout period for DMARD therapy, and within 42 days prior to study drug administration for all other patients. Patients were randomly assigned in a 1:1:1:1 ratio to four treatment groups: 20 mg adalimumab every other week (eow), 40 mg adalimumab eow, 80 mg adalimumab eow, or placebo eow, administered by subcutaneous injection starting at Week 0 and continuing until Week 22. Study drug was administered by a physician or nurse supervised by an investigator.

Patients who experienced an increase in disease activity or who had less than 10% reduction in tender joint counts (TJC) and swollen joint counts (SJC) compared with baseline after at least eight weeks of treatment stopped study therapy with adalimumab/placebo and were switched to an open-label rescue treatment that could include higher doses of steroids, nonsteroidal antiinflammatory drugs, or conventional DMARDs. Patients completing 24 weeks of treatment, either double-blind or open-label rescue, had the option to enter an open-label extension study to receive 40 mg of adalimumab eow.

Efficacy assessment

The primary efficacy endpoint was ACR20 response rate at Week 24 for the adalimumab 40 and 80 mg groups compared with placebo. The comparison between ACR20 response rates at Week 24 for the adalimumab 20 mg group and the placebo group was a secondary endpoint. The ACR components were evaluated at Weeks 0 (predose), 2, 4, 8, 12, 16, 20, and 24. Additional secondary efficacy endpoints included ACR20 response rate at Week 12; ACR50 and ACR70 response rates at Weeks 12 and 24; individual components of the ACR response (including TJC and SJC) at Weeks 0 (baseline), 12, and 24; and the Health Assessment Questionnaire Disability Index (HAQ DI) at Weeks 0 (baseline), 12, and 24. Morning stiffness

was evaluated at Weeks 0 (predose), 2, 4, 8, 12, 16, 20, and 24; and rheumatoid factor (RF) was evaluated at Weeks 0 (predose), 12, and 24. In addition, ACR20 area under the curve (AUC) over the 24-week study period was determined. ACR20 AUC was defined as the sum of the duration that patients achieved an ACR20 response.

Pharmacokinetic analyses

Pharmacokinetic analyses included serum adalimumab concentrations and serum anti-adalimumab antibody (AAA) concentrations, which were determined using a validated enzyme-linked immunosorbent assay (ELISA) based on a double-antigen technique. The lower limit of quantitation for adalimumab and AAA were established at 2.5 and 0.5 ng/mL, respectively, in diluted serum. Because of interference of adalimumab concentrations with the AAA assay, AAA concentrations were analyzed only if the adalimumab concentration was less than 2 μg/mL.

Blood samples for serum adalimumab concentrations were collected at Weeks 0 (immediately prior to dosing), 2, 4, 8, 12, 16, 20, and 24 (or following the last dose) and at the follow-up visit. Blood samples for AAA concentrations were collected at Weeks 0 (immediately prior to dosing), 4, 8, 12, 16, 20, and 24 (or following the last dose) and at the follow-up visit.

Safety assessment

Safety was evaluated on the basis of treatment-emergent adverse events (AEs). Laboratory tests, including hematology tests, clinical chemistry tests, and urinalysis, were conducted at screening; at Weeks 0 (predose), 2, 4, 8, 12, 16, 20, and 24 (or last dose); and at the follow-up visit. Vital signs and physical examinations were also evaluated. Comparisons were made of changes from Week 0 (predose) during treatment for all treatment groups.

Statistical analysis

To detect a difference of 25% in ACR20 response rates between the placebo group and the adalimumab 40 mg group, assuming an ACR response rate of 20% in the placebo arm and 45% in the 40 mg eow arm, a sample size of 74 patients per treatment group was estimated to be required to provide 80% power for a two-sided test (continuity corrected) with an alpha of 0.025. Therefore, taking the exclusion analysis into consideration, a total of 320 subjects (80 subjects per treatment group) needed to be equally allocated to one of the four treatments: 20 mg adalimumab, 40 mg of adalimumab, 80 mg adalimumab, or placebo.

Demographic and baseline characteristics were compared among the four treatment groups using the one-way analysis of variance test for continuous variables and the χ^2 test for categorical variables. All efficacy analyses were primarily performed on the full analysis set population, defined as all randomized patients who received at least one dose of study drug and from whom at least one assessment of efficacy under double-blind medication was available. Unless otherwise specified, statistical significance was set at $P = 0.05$ (two-sided) for all tests. The effect of study centers was analyzed; however, because there were not enough patients per treatment per center, study center was not adjusted in the statistical analyses.

The primary efficacy endpoint, ACR20 response rate at Week 24, was compared for the placebo group against that of the 40 and 80 mg eow adalimumab groups, using the Pearson χ^2 test. The Hochberg procedure was applied to control for multiplicity of testing. If both P values were less than 0.05, then the individual null hypotheses (no treatment difference between adalimumab and the placebo) were rejected. If one P value did not show significance at 0.05, then the other hypothesis was tested against and adjusted at the 0.025 level. If the test was significant at the adjusted 0.025 level, then the null hypothesis was rejected.

The response rate for ACR20 between the adalimumab 20 mg group and placebo at Week 24 was a secondary endpoint and was compared using the Pearson χ^2 test. For the additional secondary endpoints, ACR20 at Week 12 and ACR50 and ACR70 at Weeks 12 and 24, number of responders and response rates were presented in each group. Improvements in individual components of the ACR response at Week 0 (predose) and at Weeks 12 and 24 were presented as summary statistics for each treatment group, and comparisons between the placebo group and each adalimumab group were performed using analysis of covariance, with baseline values as covariates. For ACR20 AUC, summary statistics by treatment group were presented.

Patients who discontinued the study prior to Week 24 or who moved to the rescue arm following at least eight weeks of treatment were classified as nonresponders. In addition, the last observation carried forward post baseline was conducted for the following analyses: ACR20 response rate at Week 24; ACR50 and ACR70 at Weeks 12 and 24; ACR20 at Week 12; individual components of the ACR response at Weeks 0 (predose), 12, and 24; and ACR20 AUC. The last non-missing visit up to and including the Week-24 assessment for each patient was summarized as the endpoint visit.

All patients who received at least one dose of double-blind study medication were included in the safety analysis. Summary statistics included frequency tabulations and

were presented by the Medical Dictionary for Regulatory Activities, version 8.0, preferred term and system organ class for each treatment group.

Results

A total of 482 patients were screened and 352 patients met the entry criteria and were randomized to the four treatment groups (placebo, $n = 87$; adalimumab 20 mg, $n = 87$; adalimumab 40 mg, $n = 91$; adalimumab 80 mg, $n = 87$). A total of 34 (9.7%) of 352 patients discontinued treatment (7, 7, 16, and 4 in the placebo and adalimumab 20, 40, and 80 mg groups, respectively) (Fig. 1). Three of the 34 discontinuations occurred during the rescue period. Discontinuations because of AEs occurred in 4 (4.6%), 5 (5.7%), 12 (13.2%), and 3 (3.4%) patients, in the placebo and adalimumab 20, 40, and 80 mg groups, respectively. Other reasons for early discontinuation included withdrawal of consent, protocol violations, and administrative reasons. A total of 107 patients received rescue medication after ≥8 weeks of double-blind study treatment (45, 26, 17, and 19 in the placebo and adalimumab 20, 40, and 80 mg groups, respectively). The three discontinuations that occurred during the rescue period occurred in one patient each from the original placebo, adalimumab 40 mg, and adalimumab 80 mg groups. A total of 312 (98.1%) of 318 patients who completed the study entered the extension study and 309 patients continued treatment with adalimumab.

Baseline demographics and disease-state characteristics are included in Table 1. For the overall study group, the mean age was 54.9 years, 79.5% of patients were female, the mean weight was 53.7 kg, the mean duration of RA was 9.5 years, and the mean TJC and SJC were 24.4 and 19.6, respectively. Baseline demographics were similar between groups. Baseline disease characteristics were consistent with what is generally observed in patients with RA with moderate to severe disease and were comparable between treatment groups with the following exceptions: the mean of the patient's global assessment of disease activity for the placebo group (64.6 mm) was lower than those for the adalimumab groups (20 mg: 73.1 mm; 40 mg: 71.2 mm; 80 mg: 75.7 mm; $P = 0.003$); the mean of HAQ DI for the placebo group (1.39) was lower than those for the adalimumab groups (20 mg: 1.57; 40 mg: 1.64; 80 mg: 1.77; $P = 0.010$); and the mean CRP for the 20 mg group (4.97 mg/dl) was lower than those for other groups (placebo: 5.86 mg/dl; 40 mg: 6.48 mg/dl; 80 mg: 6.56 mg/dl; $P = 0.020$).

All patients had previously used one or more DMARDs, and 91.5% (322/352) of the randomized patients had previously used two or more DMARDs. The most common previous DMARDs were MTX (87.2%), salazosulfapyridine (sulfasalazine: 73.9%), and bucillamine (67.6%). More than half (60.5%) of the patients used previous RA treatments other than DMARDs. The most common previous RA treatments other than DMARDs were prednisolone (29.3%), diclofenac (10.8%), and triamcinolone (8.5%).

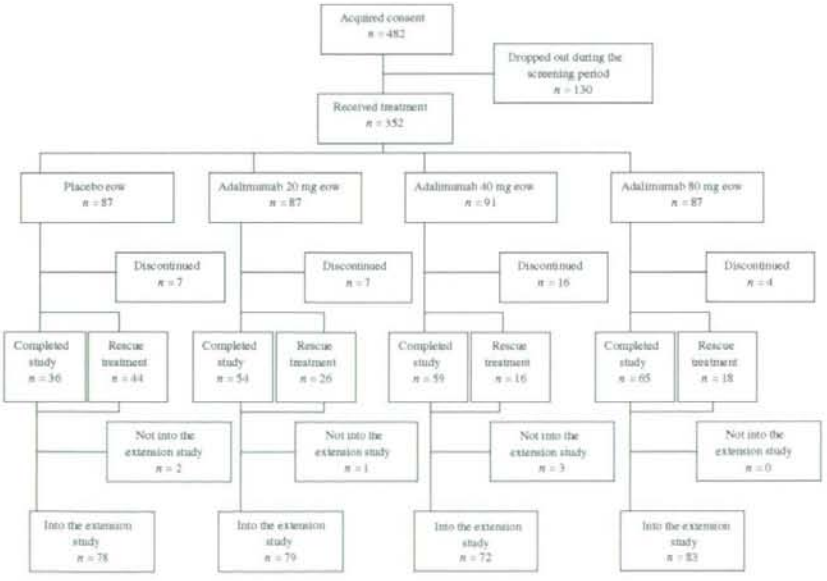

Fig. 1 Disposition of patients

Table 1 Baseline demographics and disease characteristics

	Placebo ($n = 87$)	20 mg ($n = 87$)	40 mg ($n = 91$)	80 mg ($n = 87$)	All doses ($n = 265$)
Mean age (year)	53.4 ± 12.8	54.8 ± 12.5	56.9 ± 10.3	54.3 ± 10.9	55.3 ± 11.3
Female, *n* (%)	67 (77.0)	69 (79.3)	72 (79.1)	72 (82.8)	213 (80.4)
Body weight (kg)	53.7 ± 10.1	55.2 ± 8.6	53.6 ± 9.6	52.4 ± 10.5	53.7 ± 9.7
Duration of RA (year)	8.4 ± 8.2	10.0 ± 7.7	9.9 ± 7.9	9.5 ± 8.3	9.8 ± 8.0
TJC	23.7 ± 8.8	24.6 ± 11.1	24.4 ± 10.7	24.9 ± 10.7	24.6 ± 10.8
SJC	19.3 ± 7.0	19.2 ± 8.4	19.1 ± 7.3	20.8 ± 7.9	19.7 ± 7.9
Physician's global assessment of disease activity VAS (mm)	74.1 ± 15.6	72.3 ± 15.6	76.2 ± 14.7	76.4 ± 16.4	75.0 ± 15.6
Subject's global assessment of disease activity VAS (mm)	64.6 ± 22.9	73.1 ± 19.2	71.2 ± 19.7	75.7 ± 19.3	73.3 ± 19.4
Subject's assessment of pain VAS (mm)	62.7 ± 22.8	69.0 ± 21.3	68.1 ± 21.0	70.4 ± 21.4	69.2 ± 21.2
HAQ DI	1.39 ± 0.75	1.57 ± 0.78	1.64 ± 0.70	1.77 ± 0.74	1.66 ± 0.74
CRP (mg/dl)	5.86 ± 3.30	4.97 ± 3.42	6.48 ± 4.45	6.56 ± 3.87	6.01 ± 4.00
Duration of morning stiffness (min)	195.5 ± 329.4	216.7 ± 367.6	193.3 ± 317.6	202.3 ± 330.6	203.9 ± 337.7
Any morning stiffness, *n* (%)	75 (86.2)	70 (81.4)	70 (76.9)	76 (88.4)	216 (82.1)
Positive rheumatoid factor, *n* (%)	75 (86.2)	79 (90.8)	81 (89.0)	81 (93.1)	241 (90.9)

Data are presented as mean ± SD unless otherwise noted

VAS visual analog scale

Primary efficacy: ACR20 at Week 24

At Week 24, adalimumab demonstrated dose-dependent increases in ACR20 response rates and all adalimumab treatment groups achieved statistically significantly higher ACR20 responses (28.7% in the 20 mg group, $P < 0.05$; 44.0% in the 40 mg group, $P < 0.001$; and 50.6% in the 80 mg group, $P < 0.001$) compared with the placebo group (13.8%) (Table 2).

ACR20, ACR50, and ACR70 response rates

Similarly, ACR20 response rates at Week 12 were statistically significantly greater in all adalimumab treatment groups compared with placebo (Table 2). ACR50 and ACR70 responses were statistically significantly greater in all adalimumab treatment groups compared with placebo at Weeks 12 and 24, except for the ACR50 response for the 20 mg group at Week 24 and the ACR70 response for the 20 mg group at Week 12 (Table 2). Overall, the increases in adalimumab ACR20, ACR50, and ACR70 response rates were time dependent (Fig. 2). All adalimumab treatment groups showed ACR20 response rates exceeding 30% at Week 2. For the 40 and 80 mg groups, ACR20 response rates were maintained during the study. However, for the 20 mg group, ACR20 responses fell gradually from Week 16 until the end of the study. In general, ACR50 and ACR70 responses had the tendency to increase with time in each treatment group (Fig. 2).

Table 2 ACR20, ACR50, and ACR70 response rates at Week 12 and Week 24

	Placebo $n = 87$ *n* (%)	Adalimumab		
		20 mg $n = 87$ *n* (%)	40 mg $n = 91$ *n* (%)	80 mg $n = 87$ *n* (%)
ACR20				
Week 12	11 (12.6)	39 (44.8)[a]	39 (42.9)[a]	47 (54.0)[a]
Week 24	12 (13.8)	25 (28.7)[a]	40 (44.0)[b]	44 (50.6)[b]
ACR50				
Week 12	3 (3.4)	16 (18.4)[a]	19 (20.9)[a]	23 (26.4)[a]
Week 24	5 (5.7)	14 (16.1)	22 (24.2)[a]	28 (32.2)[a]
ACR70				
Week 12	1 (1.1)	6 (6.9)	15 (16.5)[a]	10 (11.5)[a]
Week 24	1 (1.1)	9 (10.3)[a]	11 (12.1)[a]	13 (14.9)[a]

ACR20 at Week 24 for adalimumab 40 and 80 mg groups compared with placebo was the primary efficacy endpoint

[a] $P < 0.05$ versus placebo based on χ^2 test

[b] $P < 0.0001$ versus placebo based on χ^2 test

ACR core components

For both the 40 and 80 mg groups, all individual ACR components showed statistically significant improvement compared with the placebo group ($P < 0.05$) at Week 24, with the exception of patient's assessment of pain for the 80 mg group and HAQ DI for the 40 and 80 mg groups (Table 3). For the 20 mg group, there was only significant

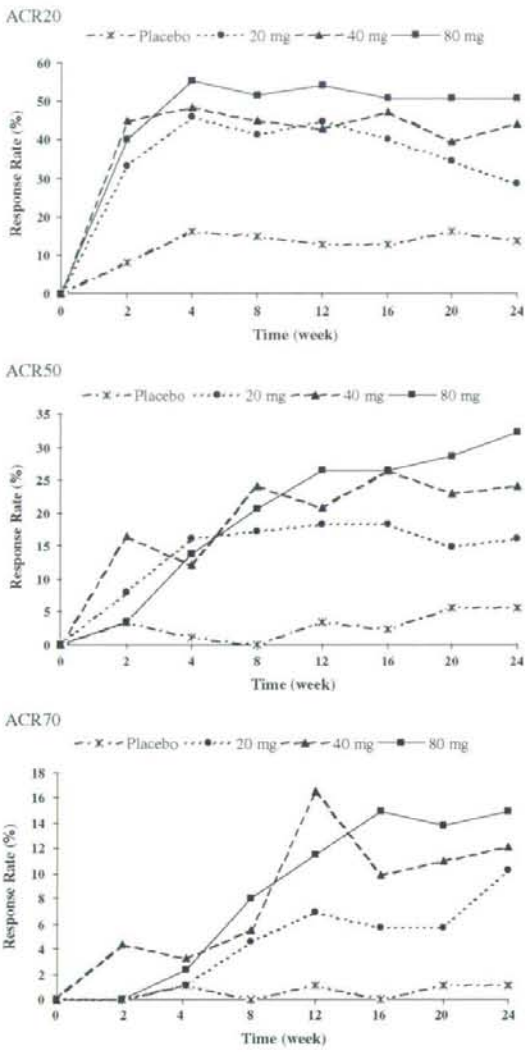

Fig. 2 ACR20, ACR50, and ACR70 response rates over time

improvement in SJC compared with the placebo group at Week 24. After adjusting for lower baseline CRP levels in the 20 mg group, results of subgroup analyses found that CRP had a statistically significant relationship to the ACR20 response rate (odds ratio: 0.9); however, its effect on the primary efficacy endpoint evaluation was small. ACR20 response rates in all adalimumab groups with the adjustments were still statistically significantly greater compared with placebo response rates, as achieved without the adjustments.

Morning stiffness

At Week 24, greater percentages of patients in each adalimumab treatment group reported complete resolution of baseline morning stiffness (17.1% in the 20 mg group, 22.9% in the 40 mg group, and 25.0% in the 80 mg group) compared with the placebo group (6.7%). At Week 24, the mean observed duration of morning stiffness compared with baseline decreased by 55.8, 87.6, and 137.0 min in the 20, 40, and 80 mg adalimumab groups, respectively, versus 5.9 min in the placebo group.

RF status

Of the RF-positive patients at baseline, 3.4, 6.0, 9.8, and 6.7% of patients in the placebo and adalimumab 20, 40, and 80 mg groups, respectively, shifted to RF-negative at Week 24, with no significant differences between groups. Of the RF-negative patients at baseline, two patients (one in the placebo group and one in the adalimumab 40 mg group) shifted to RF-positive at Week 24.

Pharmacokinetic analysis

Serum trough concentrations of adalimumab reached a steady state approximately by Week 8 and remained relatively constant through Week 24 (Fig. 3a). Serum adalimumab concentrations were lower in the lower dose groups (20 and 40 mg) compared with the 80 mg group.

The percentages of patients who had at least one AAA-positive serum sample from the start of study drug treatment until 30 days after the last dose were 40.2, 44.0, and 26.4% in the 20, 40, and 80 mg adalimumab groups, respectively. The distribution of serum AAA-positive concentrations is shown in Fig. 3b. The majority of patients who were positive for AAA had relatively low titers. Mean serum adalimumab concentrations over time stratified by AAA status are depicted in Fig. 3c. Concentration-versus-time curves normalized once AAA status was considered.

For ACR20 response rate at Week 24, the AAA-positive patients (14.3, 27.5, and 34.8%) in the adalimumab 20, 40, and 80 mg groups showed lower response rates than AAA-negative patients (38.5, 56.9, and 56.3%), respectively. ACR50 response at Week 24 showed a similar tendency (AAA-positive [5.0–13.0%] and AAA-negative [19.2–39.2%]).

Table 3 Summary of individual ACR components: changes from baseline to Week 12 and Week 24

ACR components visit	Placebo $n = 87$ Mean ± SD	Adalimumab 20 mg $n = 87$ Mean ± SD	Adalimumab 40 mg $n = 91$ Mean ± SD	Adalimumab 80 mg $n = 87$ Mean ± SD
TJC				
Baseline (value)	23.7 ± 8.8	24.6 ± 11.1	24.4 ± 10.7	24.9 ± 10.7
Study week 12	−0.6 ± 10.6	−8.3 ± 10.2[a]	−11.2 ± 12.2[a]	−10.1 ± 11.1[a]
Study week 24	−0.5 ± 10.9	−6.6 ± 11.4	−10.7 ± 12.3[a]	−10.0 ± 13.3[a]
SJC				
Baseline (value)	19.3 ± 7.0	19.2 ± 8.4	19.1 ± 7.3	20.8 ± 7.9
Study week 12	−1.6 ± 7.2	−6.8 ± 7.7[a]	−8.1 ± 9.3[a]	−8.7 ± 8.6[a]
Study week 24	−1.8 ± 7.4	−5.9 ± 7.6[a]	−8.2 ± 8.8[a]	−8.7 ± 9.4[a]
Physician's global assessment of disease activity (VAS)				
Baseline (value)	74.1 ± 15.6	72.3 ± 15.6	76.2 ± 14.7	76.4 ± 16.4
Study week 12	−7.1 ± 19.7	−23.0 ± 24.9[a]	−31.3 ± 26.6[a]	−31.9 ± 29.2[a]
Study week 24	−8.0 ± 21.8	−20.1 ± 27.8	−30.3 ± 24.8[a]	−31.0 ± 31.6[a]
Patient's global assessment of disease activity (VAS)				
Baseline (value)	64.6 ± 22.9	73.1 ± 19.2	71.2 ± 19.7	75.7 ± 19.3
Study week 12	2.1 ± 21.8	−19.9 ± 26.1[a]	−19.1 ± 29.0[a]	−25.9 ± 28.9[a]
Study week 24	2.6 ± 23.5	−16.6 ± 27.0	−19.9 ± 31.0[a]	−25.8 ± 31.6[a]
Patient's assessment of pain (VAS)				
Baseline (value)	62.7 ± 22.8	69.0 ± 21.3	68.1 ± 21.0	70.4 ± 21.4
Study week 12	2.3 ± 23.5	−17.3 ± 27.6	−17.2 ± 28.2[a]	−20.5 ± 29.4[a]
Study week 24	3.5 ± 25.4	−12.8 ± 26.0	−17.4 ± 27.9[a]	−20.3 ± 33.3
HAQ DI score				
Baseline (value)	1.4 ± 0.7	1.6 ± 0.8	1.6 ± 0.7	1.8 ± 0.7
Study week 12	0.1 ± 0.6	−0.2 ± 0.5[a]	−0.3 ± 0.6	−0.4 ± 0.5[a]
Study week 24	0.1 ± 0.6	−0.2 ± 0.5	−0.2 ± 0.6	−0.4 ± 0.6
CRP (mg/dl)				
Baseline (value)	5.9 ± 3.3	5.0 ± 3.4	6.5 ± 4.4	6.6 ± 3.9
Study week 12	0.2 ± 2.9	−0.6 ± 3.1	−1.6 ± 4.4	−2.3 ± 3.9[a]
Study week 24	0.1 ± 3.2	−0.5 ± 3.2	−1.6 ± 4.1[a]	−2.3 ± 4.0[a]

Data based on the full analysis set with last observation carried forward

[a] $P < 0.05$ compared with placebo based on analysis of covariance

Safety

All patients who received at least one injection of study drug and at least one postbaseline safety evaluation ($n = 352$) were included in the safety analysis. Overall, 81.6, 92.0, 98.9, and 93.1% of patients in the placebo and the adalimumab 20, 40, and 80 mg groups, respectively, reported at least one treatment-emergent AE during the double-blind study period (Table 4). The number of AEs that occurred in the 40 and 80 mg groups were statistically significantly greater compared with the placebo group ($P < 0.05$ for both groups) (Table 4). The majority of AEs were mild or moderate in intensity. The most common treatment-emergent AEs across all treatment groups were nasopharyngitis, injection site erythema, anti-dsDNA antibody–positive reaction, and anti–nuclear antibody–positive reaction. Injection-site reaction was reported by a statistically significantly greater percentage of patients in each of the adalimumab treatment groups compared with placebo (Table 4). However, most of these reactions, although reported frequently, were generally mild or moderate in nature and rarely led to discontinuation of treatment. Also, injection-site reactions tended to decrease over time. Three patients in the adalimumab 40 mg group discontinued the study because of injection-site reactions. Severe AEs were reported at similar rates in the adalimumab groups and the placebo group (Table 4).

Infectious AEs were also reported at similar rates in the adalimumab groups and the placebo group. A total of 44 infectious AEs were reported by 36.8% (32/87) of placebo-treated patients and 161 infectious AEs were reported by 40.8% (108/265) of the adalimumab-treated patients. The

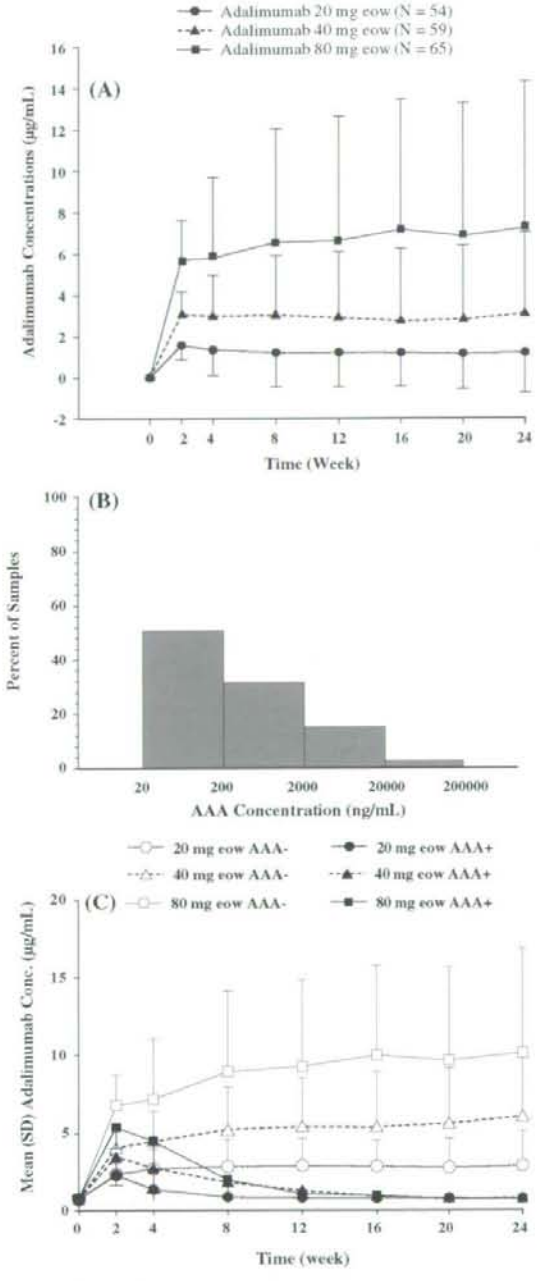

Fig. 3 **a** Mean (SD) serum adalimumab concentrations over time. **b** Distribution (%) of samples with positive serum anti-adalimumab antibody (*AAA*) concentrations. **c** Mean (SD) predose serum adalimumab concentrations (μg/mL) over time in Japanese patients with rheumatoid arthritis who completed the 24-week study with every-other-weekly adalimumab treatment stratified by AAA status

most common infectious AE, nasopharyngitis, was reported by 15.8% of the patients in all of the adalimumab groups and by 18.4% of placebo-treated patients. The next most common infectious AE, upper respiratory tract infection, was reported by 4.5% of adalimumab-treated patients and by 2.3% of placebo-treated patients. In general, the majority of infectious AEs were mild or moderate in intensity; only five AEs were considered to be severe by the investigator (acute bronchitis and two events of pneumonia in the 20 mg group and infectious bursitis and infectious dacryocystitis in the 40 mg group).

Rates of serious AEs were comparable between the adalimumab groups and the placebo group. Three serious AEs resulted in death in two patients—one patient in the adalimumab 40 mg group died from interstitial lung disease and from lung infection, which was considered possibly related to treatment, and one patient in the adalimumab 80 mg group died from cerebral hemorrhage, which was considered unrelated to treatment. There were no reports of malignancies or tuberculosis in the adalimumab treatment groups. Malignancies were reported in two patients in the placebo group during the double-blind period. There were no clinically relevant differences between treatment groups in physical examination results, vital signs, or laboratory measurements. There were some minor differences in safety profile for AAA-positive patients and AAA-negative patients, but the differences were not clinically important. The most notable difference was the rate of injection-site reactions.

Discussion

In this study of Japanese patients with moderate to severe RA, three adalimumab dosages (20, 40, and 80 mg eow) encompassed the range of effective and well-tolerated dosages shown in previous clinical studies of adalimumab in North America, Australia, and Europe. [13, 14, 17–19] For the primary efficacy endpoint—ACR20 response rates at Week 24—the adalimumab 20, 40, and 80 mg groups showed dose-dependent improvement and were statistically significantly greater than that seen in placebo-treated patients. In each adalimumab dosage group, the percentage of patients achieving an ACR 20 response increased from Week 1 through Week 12 and continued at that level through Week 24.

The results of this bridging study in Japanese patients were intended to be compared with data from a similar study in Western patients with RA conducted in Europe, Australia, and Canada [14]. Similar study designs allow for comparisons between the two study populations. Both

Table 4 Treatment-emergent adverse events

	Placebo ($n = 87$)	Adalimumab		
		20 mg ($n = 87$)	40 mg ($n = 91$)	80 mg ($n = 87$)
AE	71 (81.6)	80 (92.0)	90 (98.9)[a]	81 (93.1)[a]
Serious AE	8 (9.2)	10 (11.5)	17 (18.7)	8 (9.2)
Severe AE	5 (5.7)	3 (3.4)	4 (4.4)	5 (5.7)
Infectious AE	32 (36.8)	30 (34.5)	41 (45.1)	37 (42.5)
Serious infectious AE	1 (1.1)	4 (4.6)	6 (6.6)	3 (3.4)
Injection site reaction	2 (2.3)	27 (31.0)[a]	28 (30.8)[a]	29 (33.3)[a]
Immunologic reaction	0 (0.0)	4 (4.6)	2 (2.2)	0 (0.0)
Malignancies	2 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)
Opportunistic infection including TB	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AE leading to death	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.1)
AE leading to early withdrawal	4 (4.6)	5 (5.7)	12 (13.2)	3 (3.4)
Probable or possibly related AEs	32 (36.8)	64 (73.6)[a]	67 (73.6)[a]	61 (70.1)[a]

[a] $P < 0.05$ compared with placebo (Fisher exact test)
TB tuberculosis

studies were multicenter, double-blind, placebo-controlled, randomized trials. This study in Japanese patients is a Phase II/III study, which included 352 Japanese patients, and the Western study was a Phase III study, which included 544 patients (98.2% white). Both studies included patients with RA who had an inadequate response to one or more DMARDs and TJC $\geq$ 12, SJC $\geq$ 10, and CRP $\geq$ 2 mg/dl. This Japanese study evaluated adalimumab 20, 40, and 80 mg eow; whereas the Western study evaluated adalimumab 20 and 40 mg eow and 20 and 40 mg weekly. The 80 mg eow dosage in Japanese patients can be roughly compared with the 40 mg weekly dosage in Western patients, achieving a similar dosage over time.

It was not practical to dose adalimumab weekly in this study conducted in Japanese patients as in the Western study, because it is generally not standard procedure for patients in Japan to self-inject their subcutaneous medications; therefore, weekly visits to receive their study drug would be inconvenient. Baseline demographics and disease characteristics were comparable, except for weight and height, which were lower in Japanese patients, as expected; TJC was also lower in Japanese patients, but not SJC.

The primary efficacy endpoints were similar—ACR20 at Week 24 (Japanese) and ACR20 at Week 26 (Western). The results of both studies found comparable ACR response rates supporting 40 mg eow as the appropriate standard dose (Fig. 4). ACR20 response rates were 19.1, 35.8, 46.0, and 53.4% in the placebo group and the adalimumab 20, 40, and 40 mg/week groups, respectively, in Western patients. Similarly, ACR20 responses were 13.8, 28.7, 44.0, and 50.6% in the placebo group and the adalimumab 20, 40, and 80 mg eow groups, respectively, in Japanese patients. Serum trough concentrations of adalimumab in both Japanese and Western patients were

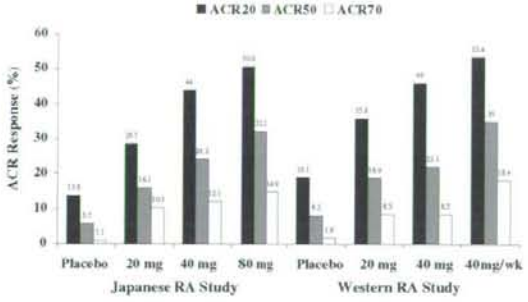

Fig. 4 Comparison of ACR response rates in Western patients with RA (Week 26) and Japanese patients with RA (Week 24)

almost constant after Week 8. However, mean steady-state serum concentrations of adalimumab in Japanese patients were lower compared with Western patients in the 40 mg group and slightly lower in the 20 mg group. There was a similar influence of AAAs on efficacy in both studies, with lower ACR20 response rates in AAA-positive patients than in AAA-negative patients, although the positivity for AAA was higher in our study compared with the Western study (40.2 [20 mg] to 44% [40 mg] versus approximate 12%) [14]. The reason for the higher percentage of AAA positivity among Japanese versus Western patients is unknown. Many of the positive samples showed low concentrations of AAA (Fig. 3b), which may explain why the overall ACR response rates are still very comparable. We could also theorize that, at some time within the 2-week dosing interval, adalimumab concentrations were sufficiently high to produce the comparable efficacy results seen in this study. Because only trough (predose) samples were tested for adalimumab concentrations, we cannot evaluate the validity of this theory within the context of this study.

Increased positivity for AAA in this study can be ascribed to monotherapy of adalimumab, as concomitant usage of MTX is known to significantly decrease the incidence of anti-human chimeric antibody against infliximab [16]. The lower incidence of AAA in the 80 mg group (26.4%) in this study compared with the 20 and 40 mg groups may be explained by the higher dose of adalimumab potentially inhibiting AAA production. Moreover, the sample size in this study is smaller than the overall population of RA patients ($N = 1062$) in which a 5% incidence of AAA has been reported [12]. Further study of the clinical importance of AAA development is warranted.

The safety profiles of adalimumab in the two studies were similar. Minor differences in safety were reported, most notably injection-site reaction, which was reported at greater percentages in Japanese patients in all adalimumab groups (67/265; 25.3%) compared with all adalimumab dose groups in Western patients (46/434; 10.6%). Almost all injection-site reactions were considered mild in intensity and were easily manageable with conservative medical treatment.

Conclusions

Adalimumab 20, 40, and 80 mg eow led to significant improvement in signs and symptoms of RA in Japanese patients with moderate to severe RA. The greatest treatment responses occurred with the 40 and 80 mg adalimumab doses. Repeated doses of adalimumab 20, 40, and 80 mg were generally well-tolerated in Japanese patients. At the suggested adalimumab dosage in foreign countries (40 mg eow), similar efficacy, safety, and pharmacokinetic study results were obtained with Japanese patients with RA compared with results in Western patients. Only minor differences in the safety profile between all three adalimumab groups and the placebo group were reported in Japanese patients compared with Western patients, the most notable of which is injection-site reaction. Therefore, comparable ACR response rates and safety data support adalimumab 40 mg eow being the appropriate standard dosage to treat RA in Japanese patients.

Disclosure statement

N. Miyasaka is affiliated with Abbott Japan and Eisai.

Acknowledgments This study was sponsored by Abbott Japan Co., Ltd., Osaka, Japan, and Eisai Co., Ltd., Tokyo, Japan. The authors would like to thank Michelle L. Metelo, PharmD, for her editorial assistance in the writing of this manuscript.

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Combined insulin B:9-23 self-peptide and polyinosinic–polycytidylic acid accelerate insulitis but inhibit development of diabetes by increasing the proportion of $CD4^+Foxp3^+$ regulatory T cells in the islets in non-obese diabetic mice

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Received 21 December 2007
Available online 14 January 2008

Abstract

Insulin peptide B:9-23 is a major autoantigen in type 1 diabetes. Combined treatment with B:9-23 peptide and polyinosinic–polycytidylic acid (poly I:C), but neither alone, induce insulitis in normal BALB/c mice. In contrast, the combined treatment accelerated insulitis, but prevented diabetes in NOD mice. Our immunofluorescence study with anti-CD4/anti-Foxp3 revealed that the proportion of Foxp3 positive $CD4^+CD25^+$ regulatory T cells (Tregs) was elevated in the islets of NOD mice treated with B:9-23 peptide and poly I:C, as compared to non-treated mice. Depletion of Tregs by anti-CD25 antibody hastened spontaneous development of diabetes in non-treated NOD mice, and abolished the protective effect of the combined treatment and conversely accelerated the onset of diabetes in the treated mice. These results indicate that poly I:C combined with B:9-23 peptide promotes infiltration of both pathogenic T cells and predominantly Tregs into the islets, thereby inhibiting progression from insulitis to overt diabetes in NOD mice.
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Keywords: Insulin; Type 1 diabetes; Insulitis; Non-obese diabetic mouse; $CD4^+$ T cell; Peptide; Polyinosinic–polycytidylic acid; Regulatory T cell

Type 1 diabetes mellitus is an autoimmune disease that develops when tolerance mechanism(s) fail to control immune responses to islet-specific autoantigens, including insulin, glutamic acid decarboxylase, and heat-shock protein [1]. Insulin is an important islet autoantigen in non-obese diabetic (NOD) mice and patients with type 1 diabetes [1]. In NOD mice, insulin-autoreactive $CD4^+$ and $CD8^+$ T cells infiltrate islets of mice, and clones of these T cells can transfer diabetes to young recipients [2,3]. The B chain peptide, B:9-23, has been suggested to be a primary autoantigenic epitope in the pathogenesis of type 1 diabetes in NOD mice [4,5]. However, of interest, immunization of NOD mice with exogenous B:9-23 peptide prevents diabetes [6].

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0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.12.191

In contrast to NOD mice, we have recently found that immunization of non-diabetes prone BALB/c mice with B:9-23 peptide readily induces insulin autoantibodies, but not insulitis or diabetes [7], and that simultaneous administration of B:9-23 peptide and polyinosinic–polycytidylic acid (poly I:C, a Toll-like receptor 3 ligand), but not poly I:C alone, induces insulitis. In addition, we have also developed an experimental autoimmune diabetes model in which B:9-23 peptide induces diabetes in transgenic BALB/c mice expressing the costimulatory molecule B7.1 in their islets [8]. Disease induction is accelerated with simultaneous administration of poly I:C in this model. These data indicate that poly I:C appears to promote anti-insulin autoimmunity in BALB/c mice immunized with B:9-23 peptide.

Since the previous report has demonstrated that poly I:C treatment confers significant protection against diabetes in NOD mice [9], we are left with the question of whether combination treatment of NOD mice with B:9-23 peptide and poly I:C would promote or prevent diabetes. We here first showed that treatment with B:9-23 peptide and poly I:C unexpectedly (or surprisingly) accelerated insulitis, but prevented diabetes in young NOD mice. We first thought that TGF-β might mediate diabetes protection despite acceleration of insulitis, because it has been reported that the TGF-β producing T cell clone which was found to react to insulin B:9-23 peptide protects NOD mice from disease induced by adoptive transfer of diabetogenic spleen cells [10]. We therefore injected TGF-β monoclonal antibody (2g7, a generous gift from Dr. Sylvaine You, INSERM U580 Hopital NECKER, Paris) to NOD mice after B:9-23 peptide immunization [11]. These studies however showed that TGF-β neutralization did not influence the disease inhibition by B:9-23 peptide and poly I:C in NOD mice (our unpublished data).

Another possibility is naturally arising $CD4^+CD25^+$ regulatory T cells (Tregs). They develop in the thymus and in the periphery, and actively maintain immunological self-tolerance. Tregs have been shown to be essential for regulation of several autoimmune diseases, including type 1 diabetes [12]. The transcriptional factor Foxp3 has been identified to be essential for development and function of Tregs [13]. Insulin-specific Tregs induced by *in vivo* immunization and *in vitro* restimulation with B:9-23 peptide efficiently prevent diabetes induced by adoptive transfer of diabetogenic T cells [14]. In patients with type 1 diabetes, it has been reported that regulatory T cell markers such as Foxp3 mRNA is upregulated in peripheral blood mononuclear cells stimulated with insulin *in vitro* [15]. In this study, we evaluated whether Tregs play a role in the aforementioned action of combined B9-23 peptide and poly I:C.

Materials and methods

Mice. Female NOD mice, 3–4 weeks of age, were purchased from Clea Japan (Tokyo, Japan). All mice were kept under specific pathogen-free conditions at the Laboratory Animal Center for Biomedical Research of Nagasaki University, and were housed in an air conditioned room with a 12-h light-darkness cycle. Animal care and all experimental procedures were performed in accordance with the Guideline for Animal Experimentation of Nagasaki University with approval of the Institutional Animal Care and Use Committee.

Injection of B:9-23 peptide, poly I:C or anti-CD25 antibody. Mouse proinsulin II B chain-derived peptide B:9-23 (SHLVEALYLVCGERG), synthesized and HPLC-purified to greater than 95% homogeneity, was purchased from SIGMA Genosys (Hokkaido, Japan). Polyinosinic–polycytidylic acid sodium salt (poly I:C) was from Sigma–Aldrich KK (Tokyo, Japan). Anti-CD25 antibody was purified from ascites of mice intraperitoneally (ip) injected with hybridoma PC61 (a generous gift from Dr. K. Yui at Nagasaki University) using a HiTrap™ protein G HP column (Amersham, Piscataway, NJ).

B:9-23 peptide (100 μg/mouse) in incomplete Freund's adjuvant (IFA) was subcutaneously injected into the scruff of NOD mice at 4 weeks of age (day 1). Poly I:C (7.5 μg/g body weight) was ip administered on days 1–5 and 8–12. PC61 was ip injected at the indicated time point.

Monitoring diabetes by blood glucose levels. The blood glucose levels of mice were monitored every other week with a Glutest-Ace meter (Sanwa Kagaku, Nagoya, Japan) starting at 12 weeks of age to determine the development of diabetes following B:9-23 peptide and/or poly I:C injection without PC61. In the study with PC61, the blood glucose levels were monitored twice a week starting at 4.5 weeks of age. Mice with blood glucose levels above 250 mg/dl for two consecutive measurements were considered diabetic.

Histology. Pancreata were obtained at 8 weeks of age after the administration of B:9-23 peptide and/or poly I:C. Pancreata, thyroid tissues, and salivary glands were obtained at 6 weeks of age after the administration of PC61 with or without B:9-23 peptide and poly I:C. Each section of the tissues was histologically analyzed by fixing in 10% formalin and staining with hematoxylin and eosin. A minimum of 20 islets from each mouse were microscopically observed by two different observers for the presence of insulitis, and the levels of insulitis were scored according to the following criteria; 0, no lymphocyte infiltration; 1, islets with lymphocyte infiltration in less than 25% of their area; 2, 25–50% of the islet area infiltrated; 3, 50–75% of the islet area infiltrated; 4, more than 75% infiltrated or small retracted islets.

Immunohistochemistry. The primary antibodies used were rabbit anti-mouse Foxp3 Ab [16] (the final concentration of 2.5 μg/ml) and FITC hamster anti-mouse CD4 (H129.19) (BD Biosciences Pharmingen, San Diego, CA) (1:250 dilution). The secondary antibodies were Alexa Fluor 555 goat anti-rabbit IgG (0.5 μg/ml) and Alexa Fluor 488 goat anti-hamster IgG (BD Biosciences Pharmingen) (1:250 dilution).

Pancreata and pancreatic draining lymph nodes (PLNs) (for four mice in each group) were embedded in Tissue-Tek OCT compound (Miles, Elkhart, IN), frozen in liquid nitrogen, and cut by a cryostat into 6- to 7-μm-thick sections. The sections were fixed with cold acetone for 10 min at 4 °C and use for immunofluorescence. We used an already established double-immunofluorescence staining protocol for FoxP3 and CD4 [16]. After blocking non-specific reactions and endogenous biotin activity using Blocking One (Nacalai Tesque, Kyoto, Japan), the samples were incubated with each primary Abs for 1 h at room temperature and subsequently with secondary Abs for 1 h at room temperature, and then fixed for 10 min at 4 °C in PBS containing 4% paraformaldehyde. All sections were analyzed with a confocal laser scan microscope LSM5Pascal (Carl Zeiss, Germany). The number of $Foxp3^+CD4^+$ cells was counted in four non-consecutive microscopic fields within the T cell areas of islets and PLN.

Statistical analysis. Group differences were analyzed with the Turkey HSD test, and differences between Kaplan–Meier survival curves were estimated by the log rank test using Dr. SPSS II for Windows software (SPSS Inc., Chicago, IL). P values less than 0.05 were considered statistically significant. Insulitis levels were analyzed by Ridit analysis, and levels of t higher than 1.96 or lower than −1.96 were considered statistically significant.

Results

Administration of B:9-23 peptide in combination with poly I:C significantly accelerated the development of peri-insulitis but prevented development of diabetes

We first evaluated the influence of administration of exogenous B:9-23 peptide and/or poly I:C on development of insulitis and diabetes in NOD mice at 4 weeks of age. As determined by life table analysis, B:9-23 peptide alone or in combination with poly I:C significantly suppressed development of diabetes, as compared to the PBS-treated control group ($P < 0.0005$ and $P < 0.001$, respectively). Injection of poly I:C alone at 4 weeks of age slightly inhibited development of diabetes, but this inhibition was insignificant ($P = 0.1$) (Fig. 1A).

The pancreata from NOD mice at 8 weeks of age (4 weeks after the beginning of treatment) were used for histological analysis. Unexpectedly, the levels of insulitis were significantly increased by administration of B:9-23 peptide alone or poly I:C alone, compared with the control group ($T = 4.304$ or $T = 6.183$, respectively). Simultaneous administration of B:9-23 peptide and poly I:C further accelerated development of insulitis ($T = 10.77$) (Fig. 1B).

Administration of B:9-23 peptide with poly I:C increased the frequency of $CD4^+Foxp3^+$ regulatory T cells in the islets, but not in the PLNs

To further characterize insulitis enhanced by B:9-23 peptide and poly I:C, the frequency of Tregs in the islets and PLNs was determined by CD4 and Foxp3 staining as a marker of Tregs. We found that approximately 8% of $CD4^+$ T cells were Foxp3-positive both in the islets and the PLNs in 6 weeks old control mice (Figs. 2A and 3A, B). Combined treatment of B:9-23 peptide with poly I:C increased the proportion of $CD4^+Foxp3^+$ T cells in the islets up to ~17% ($P < 0.0005$) (Figs. 2A and 3A), but did not change the percentage in the PLNs (Fig. 3B). These results indicate that the combination of B:9-23 peptide and poly I:C enhanced insulitis, but concomitantly increased proportion of Tregs in T cells infiltrated into the islets.

Depletion of Tregs induced extremely rapid onset of insulitis and diabetes in mice treated with B:9-23 peptide and poly I:C

To clarify the role played by infiltrating $Foxp3^+CD4^+$ Tregs, we attempted to deplete Tregs by using PC61, a widely used means to examine the function of Tregs. In our preliminary experiment, 500 μg/mouse PC61 efficiently depleted $CD25^+$ cells (data not shown). A single administration of PC61 into mice at 4 weeks of age accelerated the spontaneous development of diabetes ($P = 0.0046$ vs. PC61(−) PBS) (Fig. 4A). Surprisingly, pre-treatment with PC61 of mice at 3.5 weeks of age abrogated the disease inhibition induced by B:9-23 peptide and poly I:C, and instead greatly accelerated the onset of diabetes. Thus, disease developed in 35% and 45% mice vs. 0% in non-depleted mice at age of 6 and 10, respectively, weeks ($P < 0.005$) (Fig. 4B).

Pre-treatment with PC61 also decreased the percentage of $Foxp3^+$ T cells among the $CD4^+$ T cells infiltrated into the islets in untreated NOD mice ($P = 0.002$ vs. PC61(−)

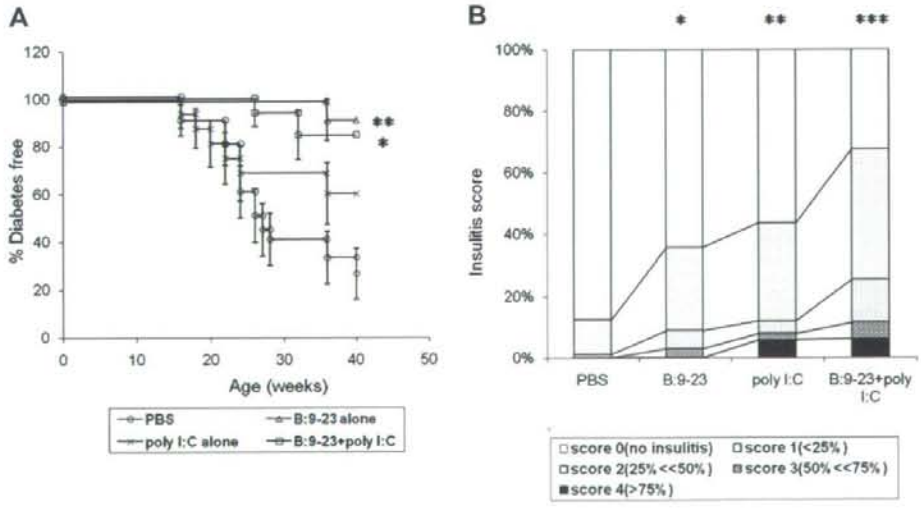

Fig. 1. (A) Life table analysis for development of diabetes following administration of B:9-23 peptide and/or poly I:C in NOD mice. Open triangles, B:9-23 peptide alone ($n = 10$); x, poly I:C alone ($n = 16$); open squares, B:9-23 peptide + poly I:C ($n = 16$); open circles, PBS ($n = 19$). $^*P < 0.001$; $^{**}P < 0.0005$. (B) Levels of insulitis at 8 weeks of age determined by Ridit analysis. A level of $T > 1.96$ was regarded as a significant increase. A level of $T < -1.96$ was regarded as a significant suppression. $^*T = 4.304$, $^{**}T = 6.183$, and $^{***}T = 10.77$.