Injection of Mol/MSM-1 cells into B6 × BDF1 blastocysts was able to produce germline chimeras, whereas injection into ICR blastocysts resulted in only a low percentage of chimerism. When the ES cell line derived from the 129 strain was used for gene targeting, the efficiency of germline chimera production was believed to be higher when injected into C57BL/6 blastocysts compared with those from ICR mice (Nagy et al. 2003). This was the case for the ES cell line derived from the MSM/Ms strain, although we used B6 × BDF1 recipient embryos, instead of C57BL/6 embryos, in order to obtain a larger number of embryos. The difference in body size may also be related to the low level of chimera production with ICR blastocyst injection; MSM/Ms mice have a small body size (about 10 g at 8 weeks of age), which is almost one-third that of ICR mice. The different growth rate between MSM/Ms cells and ICR cells might prevent normal development of chimeras.

The use of genetically engineered mice is now indispensable for functional analysis of the mammalian genome. Phenotypes of knockout or knockin mice are profoundly influenced by their genetic backgrounds. Most laboratory mouse strains are derived from a few suppliers and have been selected for special traits, such as susceptibility to cancer. Therefore, the phenotypes observed in laboratory mouse strains might not reflect those of wild animals, including humans. Because wild-derived mouse strains, such as MSM/Ms, have not been selected for any particular traits, mutations in MSM/Ms mice could result in different phenotypes from those caused by the same mutations in common laboratory mouse strains, thus providing new insights into gene function. Thus, the Mol/MSM-1 ES line should provide an excellent new tool for the study of functional genomics.

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ORIGINAL ARTICLE

Study of active controlled tocilizumab monotherapy for rheumatoid arthritis patients with an inadequate response to methotrexate (SATORI): significant reduction in disease activity and serum vascular endothelial growth factor by IL-6 receptor inhibition therapy

Norihiro Nishimoto · Nobuyuki Miyasaka · Kazuhiko Yamamoto · Shinichi Kawai · Tsutomu Takeuchi · Junichi Azuma · Tadamitsu Kishimoto

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Abstract We investigated the clinical efficacy and safety of tocilizumab (a humanized anti-IL-6 receptor antibody) monotherapy in active rheumatoid arthritis (RA) patients with an inadequate response to low dose methotrexate (MTX). In a multicenter, double-blind, randomized, controlled trial, 125 patients were allocated to receive either tocilizumab 8 mg/kg every 4 weeks plus MTX placebo (tocilizumab group) or tocilizumab placebo plus MTX 8 mg/week (control group) for 24 weeks. The clinical responses were measured using the American College of Rheumatology (ACR) criteria and the Disease Activity Score in 28 joints. Serum vascular endothelial growth factor (VEGF) levels were also monitored. At week 24, 25.0% in the control group and 80.3% in the tocilizumab group achieved ACR20 response. The tocilizumab group showed superior ACR response criteria over control at all time points. Additionally, serum VEGF levels were significantly decreased by tocilizumab treatment. The overall incidences of adverse events (AEs) were 72 and 92% (serious AEs: 4.7 and 6.6%; serious infections: 1.6 and 3.3%) in the control and the tocilizumab groups, respectively. All serious adverse events improved by adequate treatment. Tocilizumab monotherapy was well tolerated and provided an excellent clinical benefit in active RA patients with an inadequate response to low dose MTX.

Keywords Clinical trial · Interleukin-6 · Rheumatoid arthritis · Tocilizumab · Vascular endothelial growth factor

N. Nishimoto · J. Azuma · T. Kishimoto
Osaka University, Osaka, Japan

N. Miyasaka
Tokyo Medical and Dental University, Tokyo, Japan

K. Yamamoto
University of Tokyo, Tokyo, Japan

S. Kawai
Toho University Omori Medical Center, Tokyo, Japan

T. Takeuchi
Saitama Medical Center/School, Saitama, Japan

N. Nishimoto (✉)
Laboratory of Immune Regulation,
Graduate School of Frontier Biosciences,
Osaka University, 1-3 Yamada-oka, Suita,
Osaka 565-0871, Japan
e-mail: norihiro@fbs.osaka-u.ac.jp

Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by persistent synovitis and progressive destruction of cartilage and bone in multiple joints [1]. The affected joints exhibit hyperplasia of inflamed synovium infiltrated with a range of immunocompetent cells, which forms pannus tissue and invade cartilage and bone [2]. Angiogenesis is a characteristic histological feature of rheumatoid synovium for which vascular endothelial growth factor (VEGF) is responsible [3]. In addition, patients with RA show systemic inflammatory manifestations such as fever, fatigue, anemia, and laboratory findings, including elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), hyper-γ-globulinemia and emergence of various types of autoantibodies. These abnormalities can be explained, at least partly, by deregulated overproduction of interleukin (IL)-6, a pro-inflammatory cytokine, although the etiological causes are not fully understood.

Recently biologics targeting T cells, B cells, as well as pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1, have been successfully used in the treatment of RA. However, these therapies are not always effective. In addition, adverse reactions are also the problem for those treatments. Therefore, we need further new therapeutic agents that have a new mechanism of action and higher efficacy.

Tocilizumab is a humanized anti-human IL-6 receptor (IL-6R) monoclonal antibody that inhibits IL-6 binding to IL-6R [4]. This antibody was humanized by grafting the complementarity-determining regions from a murine anti-IL-6R antibody into human IgG1, thereby creating a functioning antigen-binding site in a reshaped human antibody and reducing the antigenicity of the antibody in human. We have demonstrated that treatment with tocilizumab improves the signs and symptoms and prevents joint damage of RA [5–8]. The objective of this clinical trial was to investigate the safety and efficacy of tocilizumab monotherapy in active RA patients with an inadequate response to MTX, an anchor drug for RA treatment, at a dose of 8 mg/week, which is approved for adult RA patients in Japan. In addition, tocilizumab effect on serum VEGF levels was also examined.

Patients and methods

Patients

Eligible patients were between 20 and 75 years old, fulfilled the American college of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for the classification of RA [9], with disease duration of more than 6 months. All candidates were treated with MTX 8 mg/week for at least 8 weeks until enrolment. They all had ≥6 tender joints (of 49 evaluated), ≥6 swollen joints (of 46 evaluated), ESR of ≥30 mm/h or CRP of ≥10 mg/l at enrolment. An inadequate response to MTX was defined as the presence of active disease, as described above. Patients were not allowed to have received prior anti-TNF agents or leflunomide (within 12 weeks prior to the first dose), plasma exchange therapy or surgical treatments (within 4 weeks prior to the first dose), DMARDs other than MTX or immunosuppressants (within 2 weeks prior to the first dose). Oral corticosteroids (prednisolone, ≤10 mg/day) were allowed if the dosage had not been changed within 2 weeks. Eligible patients had white blood cell counts $\geq 3.5 \times 10^9$/l, lymphocyte counts $\geq 0.5 \times 10^9$/l and platelet count of at least the lower limit of normal as defined by the respective local laboratory used. Patients were excluded if they had functional class IV using Steinbrocker's criteria [10], aspartate transaminase (AST), alanine transaminase (ALT) and serum creatinine ≥1.5-fold the upper limit of normal, were HBs antigen and/or HCV antibody positive, had pulmonary fibrosis or active pulmonary disease, a history of serious adverse drug reaction to MTX, concomitant pleural effusion, ascites, varicella infection, or were excessive users of alcohol on a regular basis. Patients were also excluded if they had significant cardiac, blood, respiratory system, neurologic, endocrine, renal, hepatic, or gastrointestinal disease, or had an active infection requiring medication within 4 weeks before the first dose or medical history of a serious allergic reaction. Sexually active premenopausal women were required to have a negative urine pregnancy test at the entry to the study and to use effective contraception during the study period.

Study protocol

This study was conducted at 25 sites in Japan. The study protocol was approved by the Ministry of Health, Labor and Welfare of Japan, and by the local ethical committee. Patients gave their written informed consent. This trial was registered with http://www.clinicaltrials.gov (NCT00144521). The first patient was enrolled on January 27, 2004, and the last patient exited the study on February 15, 2005.

Patients were randomly assigned to receive either tocilizumab therapy or MTX therapy as a control: tocilizumab 8 mg/kg every 4 weeks plus MTX placebo (tocilizumab group) or tocilizumab placebo plus MTX 8 mg/week (control group) for 24 weeks. The randomization was done by registering the patients to the patient registration center utilizing a centralized allocation method. The dosage of tocilizumab used in this study was chosen according to a previous dose finding study [7]. The dose of MTX was the maximum dose allowed in Japan (see Discussion). Oral corticosteroids less than 10 mg prednisolone per day were allowed, and the dose could not be increased during the study. Intra-articular injections of corticosteroid (only one joint at one treatment) and hyaluronate preparations were allowed. Use of one nonsteroidal anti-inflammatory drug (NSAID), including switching to another NSAID, was allowed. DMARDs, intravenous or intramuscular corticosteroids, plasmapheresis and surgical treatment were not allowed. Patients who received three or more doses of tocilizumab or tocilizumab placebo were able to join an open-label extension study of tocilizumab.

The clinical responses were measured using the ACR criteria as well as the Disease Activity Score in 28 joints (DAS28) and the European League Against Rheumatism (EULAR) criteria based on DAS28. Remission was defined according to EULAR definition of a DAS28 <2.6 [11]. Serum VEGF levels were also monitored. Safety was

assessed through recording of adverse events, physical examinations, and standard laboratory tests.

Statistical analysis

We determined that a sample size of 57 patients per treatment group was required to provide 90% power for detecting a significant ($P < 0.05$) difference in ACR20 response between the control group and the tocilizumab group by use of the two-side chi-square test, where ACR20 response rates in the population were assumed to be 35 and 65% in the control group and the tocilizumab group, respectively. We decided to recruit 60 patients per treatment group to allow for anticipated withdrawals. The primary end point was the ACR20 response at week 24 with the last observation carried forward (LOCF) method, using an intent-to-treat (ITT) analysis. The incidences of clinical improvements were analyzed by the chi-square test.

All statistical analyses were two-sided and P values less than 0.05 were considered significant. All patients receiving at least one dose of tocilizumab or tocilizumab placebo, and at least 4 weeks of MTX or MTX placebo administration were included in the clinical efficacy analysis.

Results

Characteristics of the patients

One-hundred and twenty-seven patients were enrolled in the study (Fig. 1). Two patients randomized to the control group withdrew before dosing (gall stone and patient's request). A total of 125 patients (64 in the control group and 61 in the tocilizumab group) received study drugs. Thirty-three patients in the control group and 54 patients in the tocilizumab group completed 24-week treatment. Withdrawal occurred in 31 patients in the control group and seven patients in the tocilizumab group. The reported reasons for withdrawal are shown in Fig. 1.

Demographics and baseline disease characteristics did not differ between the two groups (Table 1). Mean disease duration was 8.6 years. Patients had active RA, indicated by DAS28 score of 6.1 and CRP of 31 mg/l at baseline after using of MTX 8 mg/week for at least 8 weeks.

Clinical efficacy

The primary end point of the study, an ACR20 response at week 24 was 25.0% in the control group compared with 80.3% in the tocilizumab group, indicating the superiority of tocilizumab treatment ($P < 0.001$). The ACR50 and ACR70 response rates in the tocilizumab group were higher than those in the control group at all time points from week 4 onward (Fig. 2a). At the last observation, the ACR50 response rate was 10.9 and 49.2%, and the ACR70 response rate was 6.3 and 29.5% in the control group and the tocilizumab group, respectively. Additionally, the tocilizumab group showed a greater reduction in

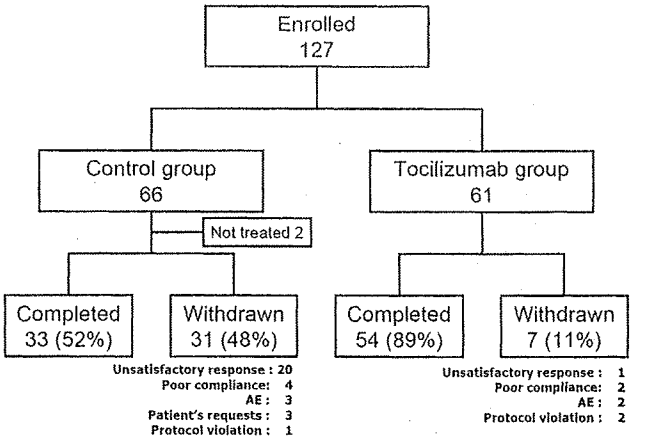

Fig. 1 Randomization, reasons for withdrawal, and numbers of patients who completed the trial. *Tocilizumab* humanized anti-interleukin-6 receptor antibody

Table 1 Patient demographics and clinical characteristics at baseline

	Control group ($n = 64$)	Tocilizumab group ($n = 61$)
Demographics		
Age (years)	50.8 ± 12.2	52.6 ± 10.6
Male:female ratio	16:48	6:55
Clinical characteristics		
RA duration (years)	8.7 ± 7.1	8.5 ± 8.4
No. of failed DMARDs, mean (range)	3.6 (1–8)	3.3 (1–8)
Functional class[a], I/II/III/IV	7:50:7:0	2:49:10:0
RA Stage[a], I/II/III/IV	3:18:17:26	1:20:22:18
Tender joint count, 0–49 scale	14.2 ± 8.6	13.8 ± 7.5
Swollen joint count, 0–46 scale	12.7 ± 7.5	12.4 ± 5.9
ESR (mm/h)	51.9 ± 24.0	51.9 ± 27.7
CRP (mg/l)	32 ± 26	30 ± 20
DAS28	6.2 ± 0.9	6.1 ± 0.9
VEGF (pg/ml)	730.8 ± 445.6	711.3 ± 417.4

Except where indicated otherwise values are mean ± SD

Tocilizumab humanized anti-interleukin-6 receptor antibody; *RA* rheumatoid arthritis; *ESR* erythrocyte sedimentation rate; *CRP* C-reactive protein; *DAS28* Disease Activity Score in 28 joints; *VEGF* vascular endothelial growth factor

[a] RA functional status determined by the American College of Rheumatology criteria. RA stage determined by Steinbrocker's criteria

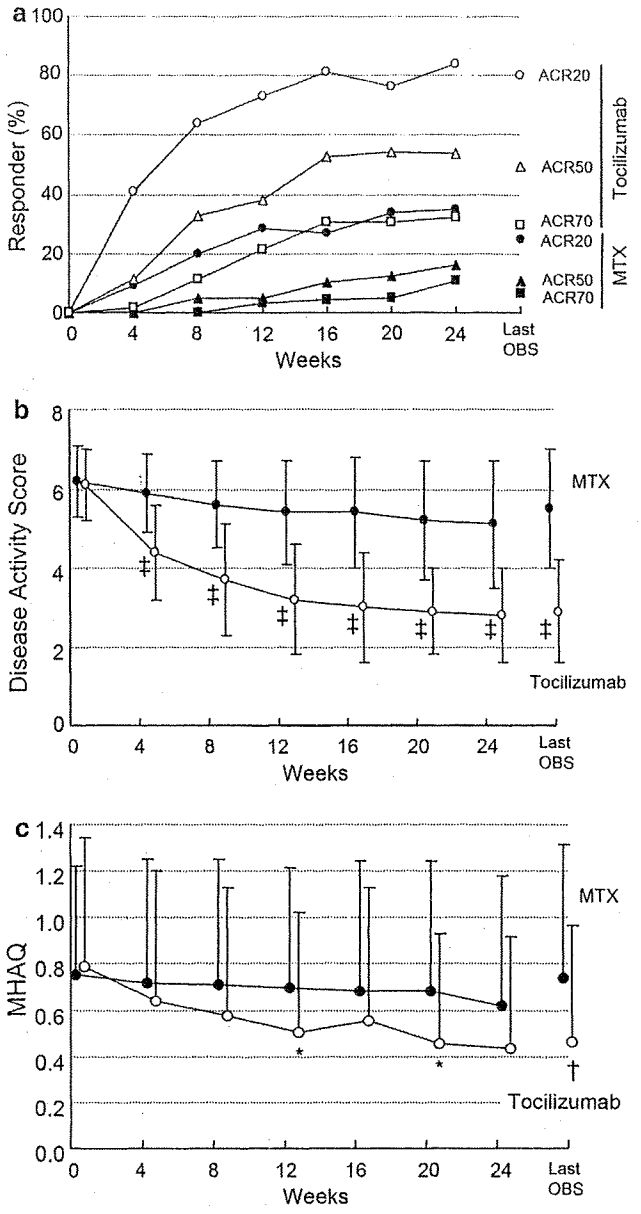

Fig. 2 Percentage of responders according to the American College of Rheumatology (ACR) improvement criteria and the Disease Activity Score in 28 joints (DAS28) as well as mean change in Modified Health Assessment Questionnaire (MHAQ) scores. Percentage of responders according to the ACR improvement criteria (**a**) and the DAS28 (**b**) according to the ITT analysis over 24 weeks. Mean change in MHAQ scores from baseline to week 24 (**c**). Last *OBS* = last observation. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ versus MTX by paired *t*-test

DAS28 than the control group did (Fig. 2b). At the last observation, the incidence of "Good" was 3.2 and 65.5%, and that of "Good or moderate" was 39.7 and 96.6% in the control group and the tocilizumab group, respectively. Similarly, remission was observed in 1.6% of the control group and 43.1% of the tocilizumab group at the last observation, and tocilizumab treatment achieved

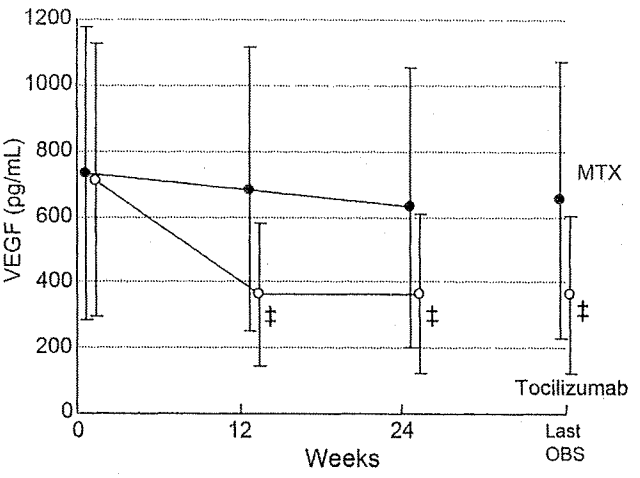

Fig. 3 Change from baseline in serum levels of VEGF. Values are the mean for each group at each time point. VEGF was measured at three points (0, 12 and 24 weeks) over the study period. *Last OBS* = last observation. ‡$P < 0.001$ versus MTX by paired *t*-test

significantly higher remission rates than MTX treatment ($P < 0.001$).

Tocilizumab treatment also significantly improved MHAQ scores compared to MTX treatment (Fig. 2c). A decrease of ≥0.22 units in MHAQ scores represents significant clinical improvement and the minimum clinical important difference [12]. Such improvement was seen in 34% in the control group and 67% in the tocilizumab group at the last observation, demonstrating significant improvement in the tocilizumab group compared to the control group ($P < 0.001$).

The mean serum VEGF levels showed a marked decrease in the tocilizumab group (Fig. 3). Mean changes from baseline were −74.0 pg/ml in the control group and −346.9 pg/ml in the tocilizumab group at week 24 ($P < 0.001$).

Safety

A total of 104 adverse events occurred in 46 of 64 patients (71.9%) in the control group and 211 adverse events occurred in 56 of 61 patients (91.8%) in the tocilizumab group. Most of the adverse events were mild or moderate. Table 2 shows frequent adverse events observed in at least 5% of the patients. Nasopharyngitis was the most common adverse event in the both groups (the control group 10.9%, the tocilizumab group 18.0%).

Serious adverse events were reported in 4.7% (3 of 64 patients) and 6.6% (4 of 61 patients) in the control group and tocilizumab group, respectively. In the control group, these consisted of one event each of pneumonia, spinal compression fracture and femoral neck fracture, among which a causal relationship with the study drug could not

Table 2 Adverse events observed in at least 5% of patients

	Control group ($n = 64$)	Tocilizumab group ($n = 61$)
Nasopharyngitis	7 (10.9)	11 (18.0)
Stomatitis	0	7 (11.5)
Hyperlipidemia	1 (1.6)	4 (6.6)
Headache	2 (3.1)	4 (6.6)
Rash	2 (3.1)	4 (6.6)
Diarrhea	1 (1.6)	4 (6.6)
Upper respiratory tract inflammation	4 (6.3)	3 (4.9)

Values are the number (%) of patients

Tocilizumab humanized anti-interleukin-6 receptor antibody

be ruled out for pneumonia. In the tocilizumab group, the serious adverse events consisted of one event each of pneumonia, infectious arthritis, colonic polyp and headache, among which a causal relationship with the investigational product could not be ruled out for pneumonia and infectious arthritis. All serious adverse events improved with appropriate treatments.

Tuberculosis was not observed in this study. No tuberculosis screening or prophylactic use of any anti-tuberculosis drugs was done for this study.

Drug-related infusion reactions were reported for eight events in seven patients (11.5%) of the tocilizumab group: two with pruritus, and one each with headache, flushing, rash, arthralgia, abnormal feeling and increased blood pressure. The severity of arthralgia was moderate while all other infusion reactions were mild, and all patients continued the study.

Laboratory findings

Laboratory test abnormalities were reported in 23 and 56% of patients in the control and the tocilizumab groups, respectively. In the tocilizumab group, lipid metabolism-related changes such as an increase in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDLC) were common. Increases in TC, TG, and LDLC under non-fasting were reported in 36, 20, and 28% of the patients, respectively. Most of them were grade 1 according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Values increased until week 4 and thereafter remained constant. High-density lipoprotein cholesterol (HDLC) values were also raised in the tocilizumab group. Therefore, the atherogenic index, calculated by (TC-HDLC)/HDLC, did not change at all throughout the study period of 24 weeks.

Increases in AST, ALT, and total bilirubin were reported in 8, 11, and 0% of the patients in the control group, and 3, 5, and 2% in the tocilizumab group, respectively. These values did not continue to be increased, but became stable at week 16 in the tocilizumab group (Fig. 4). All the patients with these abnormalities in the tocilizumab group were NCI-CTC grade 1 except for 1 patient with grade 2 increase in total bilirubin. There was no patient who withdrew from this study for the reason of liver functional abnormality.

Anti-tocilizumab antibodies were not detected in this study.

Discussion

This study was a multi-center, randomized, blinded, double-dummy trial of tocilizumab in active RA patients who had an inadequate response to low dose MTX treatment in Japan. The results of this study confirmed the excellent efficacy of tocilizumab monotherapy for signs and symptoms as shown in previous studies [7, 8]. Since MTX is an anchor drug in RA treatment, it is noteworthy that tocilizumab treatment is a very efficacious treatment for the patients with an inadequate response to MTX. In addition, switching MTX therapy to tocilizumab monotherapy was safe and effective.

The dose of MTX prior to enrollment was 10–25 mg/week in CHARISMA study, which was conducted in Europe [13], while all patients in this study were treated with MTX 8 mg/week. The dosage 8 mg of MTX/week in this trial is the maximum dosage approved in Japan. The Japanese government recommends 6–8 mg/week of MTX based on the evidence from the Japanese clinical trials of MTX for RA [14, 15]. The MTX dosage used in Japan is lower than that used in Western populations treated in the EU or US. The average body weight of the patients in this study was 54 kg, and much lower than those of EU and US patients. Additionally, all patients were given folic acid in the CHARISMA study, in contrast to only 51% of the patients in this study. Considering these factors, the differences in the clinical efficacy of MTX between the CHARISMA study and this study might not be so large as expected when looking at the difference in the MTX dose.

Maini et al. demonstrated in the CHARISMA study, that adding MTX to tocilizumab increased the efficacy in terms of ACR50 and ACR70 response rates, although there was no difference in ACR20 response rates between the tocilizumab 8 mg/kg monotherapy and the tocilizumab 8 mg/kg plus MTX. Thus, the combination with MTX may be a therapeutic option, if the toxicity is not increased. In this study, however, even monotherapy with tocilizumab 8 mg/kg induced DAS28 remission at 6 months in about 40% of patients. Furthermore, since anti-tocilizumab antibodies are not detected without use of MTX, the combination with MTX is not required to suppress the emergence of anti-

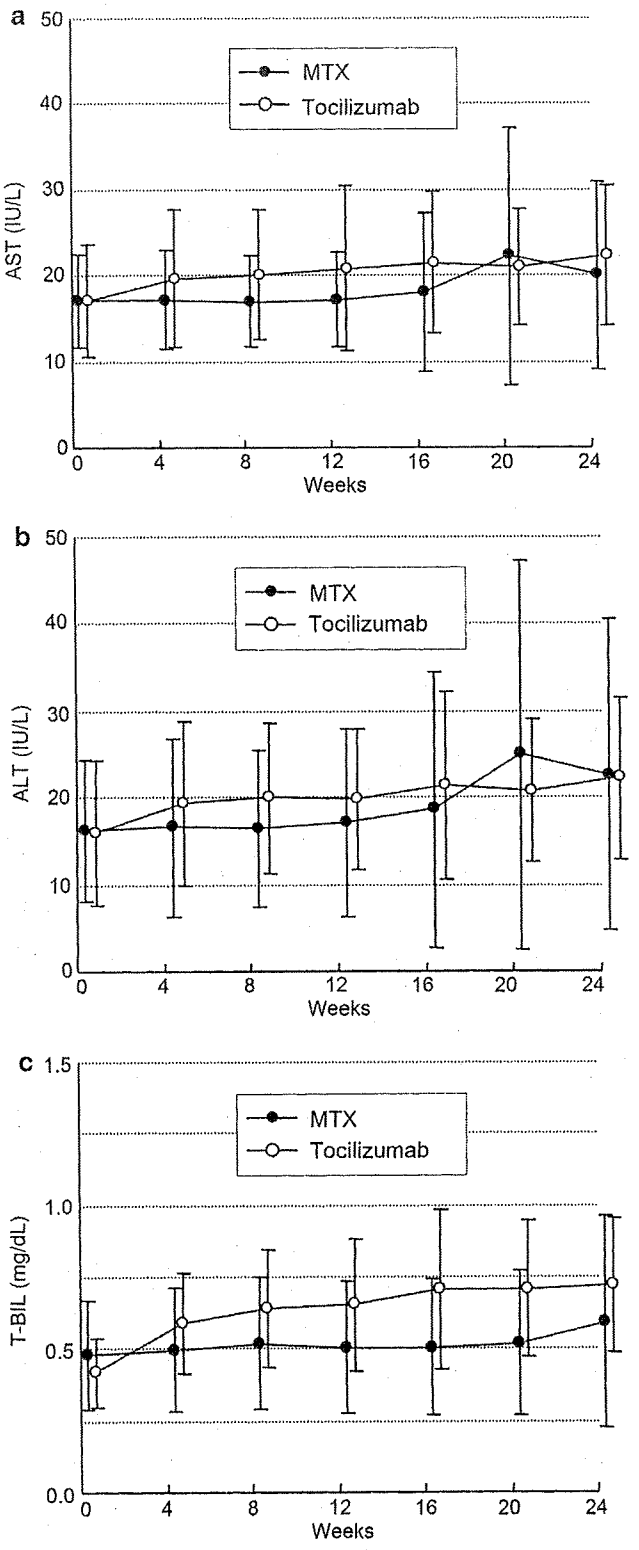

Fig. 4 Change from baseline in serum levels of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (T-BIL). Values are the mean for each group at each time point

tocilizumab antibodies as long as 8 mg/kg of tocilizumab is used. Therefore, tocilizumab will be useful for the patients who do not tolerate MTX.

This double-blind study of tocilizumab also confirms the previous finding that IL-6 receptor inhibition improves serum VEGF levels of RA patients [16]. Serum VEGF levels markedly decreased during tocilizumab therapy compared to the treatment with MTX. VEGF is produced by macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells, synovial lining cells in synovium [17], neutrophils in synovial fluid [18], and peripheral blood mononuclear cells [19] from patients with RA. VEGF is a potent angiogenic factor and thought to be responsible for the angiogenesis necessary to oxygenate the hypertrophic synovial tissues of the affected joints of RA patients [20, 21]. VEGF also induces vascular permeability and mediates inflammation [22–24]. Therefore, the decrease in VEGF may be an important part of the mechanism how IL-6 receptor inhibition with tocilizumab exerts its therapeutic efficacy in RA. Since serum VEGF levels correlate with disease activity scores and radiographic progression in RA patients [16, 25], the dramatic decrease in VEGF also underlines the efficacy of tocilizumab. The normalization of VEGF by blockade of IL-6 function alone indicates that IL-6 is essential for the VEGF production in this disease.

Tocilizumab monotherapy was generally well tolerated. There was no specific type of infection related to tocilizumab therapy. There is no indication for an increased risk of reactivation of latent tuberculosis, which is often a problem in anti-TNF therapy [26]. In this study patients did not receive prophylactic medication nor were they screened for latent or active tuberculosis at the time of screening.

The increase in TC observed is in concordance with observations in previous studies of tocilizumab [7, 8]. This may be secondary to the improvement in inflammation. Furthermore, there was no cardiovascular adverse event related to the increase in TC. However, further investigation will be required to evaluate whether or not tocilizumab might increase the risk for developing ischemic heart diseases.

The mean value elevations of liver functional tests (AST, ALT and total bilirubin) were seen in the tocilizumab group as well as in the control group, but they were within normal range. Liver functional tests abnormalities were more frequently observed in the control group than in the tocilizumab group. Moreover, most of them were grade 1 according to the NCI-CTC. These abnormalities were clinically not significant and no hepatitis was observed. Therefore, tocilizumab monotherapy appears to be as tolerable as MTX in terms of liver function.

Conclusion

This study demonstrates that tocilizumab monotherapy in patients with active RA who had an inadequate response to

low dose MTX treatment in Japan has an excellent efficacy with a positive benefit-risk ratio.

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Conflict of interest statement NN has served as a consultant to and received honoraria from Chugai Pharmaceutical, the manufacturer of tocilizumab. NN also works as a scientific advisory board of Hoffmann-La Roche. TK holds a patent for tocilizumab. The other authors have no competing interests.

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Research article

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Interactions among type I and type II interferon, tumor necrosis factor, and β-estradiol in the regulation of immune response-related gene expressions in systemic lupus erythematosus

Hooi-Ming Lee[1], Toru Mima[1], Hidehiko Sugino[1], Chieko Aoki[1], Yasuo Adachi[1], Naoko Yoshio-Hoshino[1], Kenichi Matsubara[2] and Norihiro Nishimoto[1]

[1]Laboratory of Immune Regulation, Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamada-Oka, Suita City, Osaka 565-0871, Japan
[2]DNA Chip Research Incorporated, 1-1-43 Suehirocho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Corresponding author: Norihiro Nishimoto, norihiro@fbs.osaka-u.ac.jp

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Abstract

Introduction Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterized by various clinical manifestations. Several cytokines interact and play pathological roles in SLE, although the etiopathology is still obscure. In the present study we investigated the network of immune response-related molecules expressed in the peripheral blood of SLE patients, and the effects of cytokine interactions on the regulation of these molecules.

Methods Gene expression profiles of peripheral blood from SLE patients and from healthy women were analyzed using DNA microarray analysis. Differentially expressed genes classified into the immune response category were selected and analyzed using bioinformatics tools. Since interactions among TNF, IFNγ, β-estradiol (E2), and IFNα may regulate the expression of interferon-inducible (IFI) genes, stimulating and co-stimulating experiments were carried out on peripheral blood mononuclear cells followed by analysis using quantitative RT-PCR.

Results Thirty-eight downregulated genes and 68 upregulated genes were identified in the functional category of immune response. Overexpressed IFI genes were confirmed in SLE patient peripheral bloods. Using network-based analysis on these genes, several networks including cytokines – such as TNF and IFNγ – and E2 were constructed. TNF-regulated genes were dominant in these networks, but *in vitro* TNF stimulation on peripheral blood mononuclear cells showed no differences in the above gene expressions between SLE and healthy individuals. Co-stimulating with IFNα and one of TNF, IFNγ, or E2 revealed that TNF has repressive effects while IFNγ essentially has synergistic effects on IFI gene expressions *in vitro*. E2 showed variable effects on IFI gene expressions among three individuals.

Conclusions TNF may repress the abnormal regulation by IFNα in SLE while IFNγ may have a synergistic effect. Interactions between IFNα and one of TNF, IFNγ, or E2 appear to be involved in the pathogenesis of SLE.

Introduction

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterized by multiple organ damage, high titers of autoantibodies, and various clinical manifestations [1]. Numerous disorders in the immune system and abnormalities in cytokine productions have been described in patients with SLE. The exact pathological mechanisms are still obscure, however, and the roles of the cytokines are not well understood. High levels of TNF, type I interferon, and type II interferon in the sera of patients with SLE have been reported [2-4]. On the other hand, an impaired production of IL-12 by T lymphocytes from SLE patients *in vitro* has also been

aRNA: amino allyl RNA; Ct: cycle threshold; E2: β-estradiol; FcγR: Fcγ receptor; GBP: guanylate binding protein; HLA: human leukocyte antigen; IFI: interferon-inducible; IFIT: interferon-induced protein with tetratricopeptide repeats; IFN: interferon; IL: interleukin; IRF7: interferon regulatory factor 7; ISG15: interferon-stimulated gene, 15 kDa; MAPK: mitogen-activated protein kinase; MHC: major histocompatibility complex; NFκB: nuclear factor of kappa light polypeptide; OAS1: 2',5'-oligoadenylate synthetase 1; OASL: 2',5'-oligoadenylate synthetase-like; PBMC: peripheral blood mononuclear cell; PCR: polymerase chain reaction; RT: reverse transcription; SLE: systemic lupus erythematosus; TLR: Toll-like receptor; TNF: tumor necrosis factor.

observed [5,6]. Cytokines are pleiotropic in their biological activity, and it is known that our immunity is regulated by highly sophisticated cytokine networks. Comprehending the pathological roles of these abnormally induced cytokines and immunoregulatory networks of cytokines in SLE patients is therefore important so that appropriate treatment can be offered.

The microarray is a powerful tool to exhaustively investigate the gene expressions of autoimmune diseases that have complex pathogenesis and heterogeneous manifestations, such as SLE. So too are the various databases and bioinformatics tools such as gene ontology analysis, which can functionally categorize genes, or network-based analysis to investigate molecule interactions [7]. These tools have proven useful to further analyze the enormous data from microarray analysis, providing several new findings [8].

Most microarray analyses in SLE have been performed using peripheral blood mononuclear cells (PBMCs) while recent studies provide strong evidence that IFN-related genes are overexpressed in SLE patients [9-13]. In the present study, to investigate the abnormal immune system in SLE, we focused on genes in the functional category of immune response differentially expressed in SLE patients compared with healthy individuals. Our results using SLE whole blood showed definite overexpression of IFN-regulated genes in this category. As molecules in the immune response category are always communicating with each other, we performed a network-based analysis to identify aberrant regulations or interactions among differentially expressed molecules observed in this study. We also investigated the effect of interactions between IFNα and one of TNF, IFNγ, or β-estradiol (E2) on the expression of these molecules.

Materials and methods

Patients and healthy individuals

Eleven patients (all women, median age 35 years, range 27 to 72 years) with SLE fulfilled by the diagnostic criteria of the American College of Rheumatology [14] and six healthy women were enrolled in the present study after obtaining their written informed consent. The study was approved by the Ethical Committee of Osaka University Medical School for clinical studies on human subjects.

The majority of the SLE patients (n = 10) were treated with <20 mg/day prednisolone. Three of these 10 patients were treated with one of cyclosporine, azathioprine, or methotrexate in combination with prednisolone, respectively. The remaining patient was treated with >20 mg/day prednisolone.

The median disease activity score of SLE patients based on the SLE Disease Activity Index 2000 instrument [15] was 10 (range 6 to 24). Two patients had very active states (SLE Disease Activity Index 2000 score >12) while the other patients had active states (SLE Disease Activity Index 2000 score = 4 to 12). The median of the assessment based on the BILAG index [16] was 4 (range 1 to 13).

Meanwhile, the median of the total white blood cells for the patients was 6,160 (range 4,840 to 12,230). The median of the total number (proportion) of neutrophils was 4,919 (80.0%) (range 3,640 to 9,674, 75.2% to 90.1%), and that of lymphocytes was 838 (11.8%) (range 480 to 1,517, 6.6% to 20.5%).

GeneChip microarray and data analysis

Peripheral blood was collected directly into PAXGene tubes (Qiagen, Valencia, CA, USA). Total RNA was extracted using the PAXGene Blood RNA kit with the optimal on-column DNase digestion (Qiagen). Amino allyl RNA (aRNA) was synthesized from 1 μg total RNA using the Amino Allyl MessageAmp™ aRNA kit (Ambion, Austin, TX, USA). Five micrograms of aRNA from each sample (11 SLE patients and six healthy control individuals) and the equivalent quantity of reference aRNA from a mixture of RNA extracted from peripheral blood of 12 healthy women were subjected to Cy3 and Cy5 labeling, respectively. Both labeled aRNAs were mixed in equal amounts and were hybridized with the oligonucleotide-based DNA microarray AceGene (HumanOligoChip30K; DNA Chip Research, Yokohama, Japan), which contained about 30,000 human genes.

The microarrays were scanned using ScanArray Lite (PerkinElmer, Boston, MA, USA) and the signal values were calculated using the DNASIS Array (Hitachi Software Engineering, Tokyo, Japan) according to the manufacturer's instructions. The intensities of no-probe spots were used as the background. The median and standard deviation of background levels were calculated. Genes whose intensities were less than the median plus two standard deviations of the background level were identified as null. The Cy3/Cy5 ratios of all spots on the DNA microarray were normalized by the global ratio median. Genes with at least 80% good data across each group of samples were selected for further analysis. The microarray data have been deposited in NCBIs Gene Expression Omnibus [GEO:GSE12374].

Gene ontology and network-based analysis

Genes identified to be differentially expressed by >10% according to the microarray analysis with a median signal intensity difference of at least 100 between the SLE patient and healthy individual groups (in order to reduce errors pertaining to low-level expression at close to noise level) were functionally categorized using Expression Analysis Systematic Explorer version 2.0 bioinformatics software [17,18]. Interactions among the differentially expressed genes in the functional category of immune response were investigated through the use of Ingenuity Pathway Analysis version 5.5 [19]. Networks generated by less than five uploaded genes were excluded from the analysis.

Stimulation of peripheral blood mononuclear cells

To assess TNF signaling, PBMCs from six patients diagnosed with SLE and from three healthy individuals were utilized. All PBMCs used in the experiments were isolated from heparinized whole blood using a Ficoll-Paque™ Plus (GE Healthcare Biosciences, Uppsala, Sweden) gradient centrifugation according to the manufacturer's recommendations. The cells were incubated in RPMI 1640 with 10% heat-inactivated fetal bovine serum and TNF (20 ng/ml) in a carbon dioxide incubator at 37°C for 24 hours.

To examine the effects of interactions between IFNα and one of TNF, IFNγ, or E2 on interferon-inducible (IFI) genes, we performed co-stimulating experiments on PBMCs. The PBMCs isolated from three healthy women were cultured with 20 ng/ml TNF, 15 ng/ml IFNγ, 2 ng/ml E2, and 500 U/ml IFNα or null, and were co-stimulated with TNF and IFNα, with IFNγ and IFNα, or with E2 and IFNα. PBMCs were cultured at a final concentration of 1.5×10^6 cells/ml.

TNF [GenBank:CAA26669] and IFNγ [GenBank:AAB59534] were purchased from R&D Systems (Minneapolis, MN, USA). IFNα [GenBank:NP_000596] and E2 were purchased from PBL Biomedical Laboratories (Piscataway, NJ, USA) and Sigma (St Louis, MO, USA), respectively.

Preparation of cDNA and quantitative RT-PCR

Total RNA from the PBMCs was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. One microgram of RNA was reverse-transcribed into cDNA using 2.5 μM random hexamers and 125 units MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA) in a 100 μl reaction mixture. Four microliters of the twofold-diluted cDNA products were amplified in a 25 μl reaction mixture containing TaqMan Universal Master Mix and each TaqMan probes (Applied Biosystems). The assay identification numbers for the probes are presented in Table 1.

The real-time PCR was performed in a 96-well optical plate with the Applied Biosystems 7500 real-time PCR system under the following cycling conditions: 2 minutes at 50°C (one cycle), 10 minutes at 95°C (one cycle), 15 seconds at 95°C and 1 minute at 60°C (40 cycles). For each gene (performed in duplicate for each sample), cycle threshold (Ct) values were determined from the linear region of the amplification plot and were normalized by subtracting the Ct value of GAPDH (generating a ΔCt value). The response to the cytokines or E2 was determined by subtracting the ΔCt value for the time-matched control from the ΔCt value for the stimulated sample (ΔΔCt value). The fold change was subsequently calculated using the formula $2^{-\Delta\Delta Ct}$ (where ΔΔCt was converted to an absolute value).

Statistical analysis

The unpaired Mann-Whitney test was used to determine statistically significant differences in the mRNA expression levels between the SLE patient and healthy individual groups. The criterion for the statistical significance was $P < 0.05$.

Results

Immune response-related genes identified by gene ontology analysis

Thirty-eight downregulated genes and 68 upregulated genes were categorized into the functional category of immune response. Most of the 68 upregulated genes were interferon regulated – including 17 IFI genes such as interferon-induced protein with tetratricopeptide repeats (IFIT) 1, 2',5'-oligoadenylate synthetase 1 (OAS1), 2',5'-oligoadenylate synthetase-like (OASL), interferon-stimulated gene, 15 kDa (ISG15), and interferon regulatory factor 7 (IRF7) that have been reported as overexpressed in the PBMCs of SLE.

Network-based analysis on the downregulated or upregulated genes in the functional category of immune response

There were two networks represented by the downregulated genes. Twenty-three out of the 38 downregulated genes were included in the first network, including p38 mitogen-activated protein kinase (MAPK) complex and NFκB complex depicted at the center of Figure 1a. p38 MAPK is phosphorylated in response to inflammatory cytokines including IL-1β [20] and TNF. Phosphorylated p38 MAPK contributes to the activation of NFκB, which regulates the gene expression of various cytokines, chemokines and adhesion molecules [21]. Although TNF was not identified in this network, we found that most of the molecules were TNF-regulated – including cell surface antigens (CD40, CD14, CD1C), chemokine (C-C motif) receptor 7, and acute phase proteins such as serum amyloid A_1 and apelin. These data, together with a previous report of increased TNF levels in the serum of SLE patients [2], suggested that an abnormality in TNF signaling might exist. Meanwhile, a cluster of MHC class II genes consisting of HLA-DRA, HLA-DQA1, HLA-DQB1, and CD74 (also known as HLA-DRG) were also identified in this network. The second network, composed of nine downregulated genes, implied that there were interactions among TNF, IFNγ, IL-2, IL-4, and E2 (Figure 1b).

Our analysis found only four networks represented by the upregulated molecules. The first network, constructed by 25 upregulated molecules, was the network with p38 MAPK, NFκB, and TNF receptor depicted at the center of Figure 2a. A cluster of the Toll-like receptor (TLR) family (that is, TLR1, TLR2, TLR4, and TLR5) and another cluster of Fcγ receptors (FcγRs) were identified in this network. The two clusters were indirectly connected through p38 MAPK and NFκB, suggesting there may be functional interactions among these molecules through this pathway. This network was overlapped with

Table 1

Assay identification numbers for probes

Probe	Identification number
CD40	Hs01002913_m1
CD1C	Hs00233509_m1
CD14	Hs00169122_g1
Chemokine (C-C motif) receptor 7 (CCR7)	Hs00171054_m1
IL12B	Hs00233688_m1
IL-4 receptor (IL4R)	Hs00166237_m1
Prostaglandin E synthase (PTGES)	Hs00610420_m1
Interferon-induced protein with tetratricopeptide repeats 1 (IFIT1)	Hs01911452_m1
Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3)	Hs00382744_m1
Interferon-induced protein with tetratricopeptide repeats 5 (IFIT5)	Hs00202721_m1
Interferon, alpha-inducible protein 6 (IFI6)	Hs00242571_m1
Interferon, gamma-inducible protein 16 (IFI16)	Hs00194261_m1
Interferon, alpha-inducible protein 27 (IFI27)	Hs00271467_m1
Interferon, gamma-inducible protein 30 (IFI30)	Hs00173838_m1
Interferon-induced protein 35 (IFI35)	Hs00413458_m1
Interferon induced transmembrane protein 1 (IFITM1)	Hs01652522_g1
Interferon-stimulated gene, 15 kDa (ISG15)	Hs00192713_m1
Interferon regulatory factor 7 (IRF7)	Hs00242190_g1
2',5'-oligoadenylate synthetase 1 (OAS1)	Hs00242943_m1
2',5'-oligoadenylate synthetase-like (OASL)	Hs00388714_m1
Guanylate binding protein 1 (GBP1)	Hs00266717_m1
Guanylate binding protein 2 (GBP2)	Hs00269759_m1
IL8RA	Hs00174146_m1
C-type lectin domain family 4, member E (CLEC4E)	Hs00372017_m1
TNFα-induced protein 6 (TNFAIP6)	Hs00200180_m1

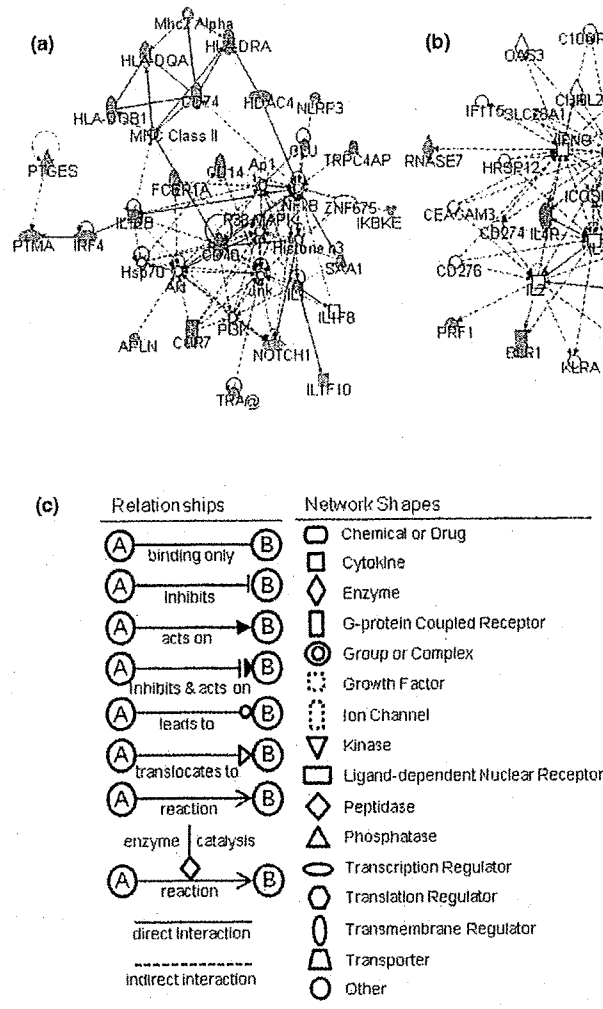
the fourth network, whose central molecules were IFNγ and E2 (Figure 2d). There were nine IFI molecules found in the first and fourth networks. The second network was represented by Akt and a calcium ion at the center (Figure 2b), while the third network was mainly attributed to TNF (Figure 2c). We found that two IFI molecules were included in the second network, and that seven out of the 14 upregulated molecules that constructed the third network were IFI molecules.

Gathering the above results, TNF, IFNγ, and E2 were depicted by both downregulated and upregulated molecules in the networks. As most of the genes in the immune response were TNF regulated, we performed stimulating experiments on the PBMCs of SLE patients and healthy individuals to assess the TNF regulation on the immune response-related molecules in SLE. On the other hand, although the expression of IFNα was not upregulated and was not depicted in networks related to TNF, IFNγ, or E2, IFI molecules were found ranging over the four networks. Furthermore, it has been reported that there exist elevated levels of type I interferon in the SLE serum. Type I interferon therefore appears to have complicated interactions with various cytokines and E2. This encouraged us to further examine the effects of interactions between IFNα and one of TNF, IFNγ, or E2 on IFI gene expression.

Gene expression profiles of peripheral blood mononuclear cells by TNF stimulation for SLE patients and healthy individuals

Seven downregulated genes (CD40, CD1C, CD14, chemokine (C-C motif) receptor 7, IL12B, IL-4 receptor, and prostaglandin E synthase) and 12 upregulated genes (IFIT1, IFIT3, IFIT5, ISG15, IRF7, OASL, OAS1, guanylate binding protein

Figure 1

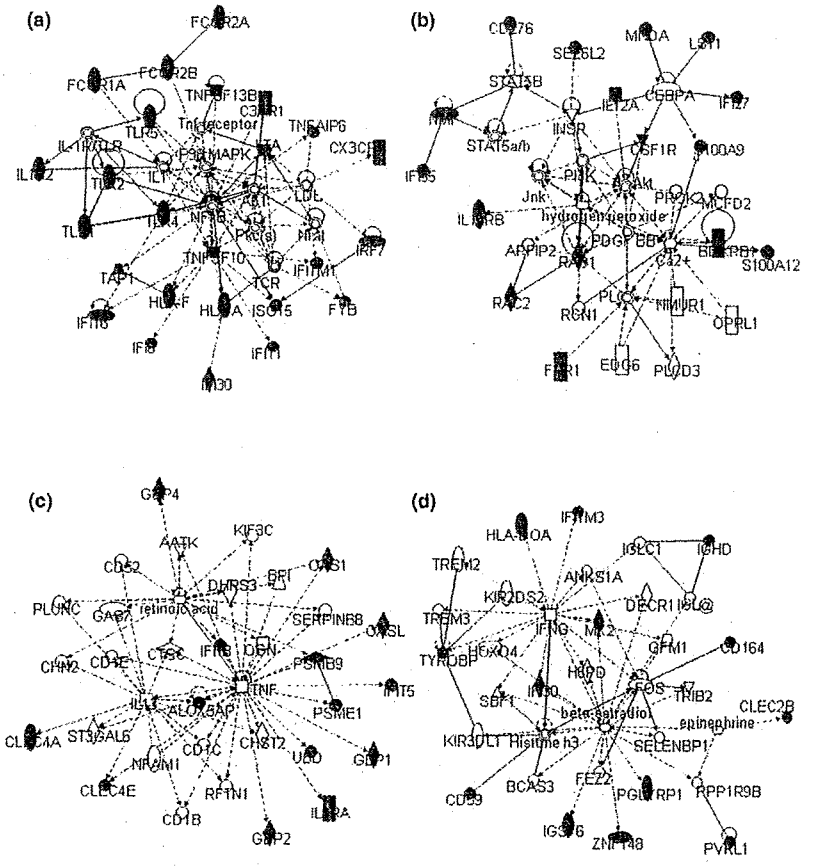


Network-based analysis of downregulated genes in the functional category of immune response. (a) Network 1 and **(b)** Network 2 constructed by downregulated genes. **(c)** Network graphical representation. Genes or gene products are represented as individual nodes whose shapes represent the functional class of gene products. The biological relationship between the two nodes is represented as an edge (line). All edges are supported by at least one reference from the literature stored in the Ingenuity Pathways Knowledge Base (IPKB). Genes in colored nodes were found over-represented in the functional category of immune response. Genes in uncolored nodes were not found over-represented but were depicted by the computationally generated networks on the basis of evidence stored in the IPKB indicating a strong biologic relevance to that network.

(GBP) 1, GBP2, IL8RA, C-type lectin domain family 4 member E, and TNFα-induced protein 6), all of which were TNF regulated, were selected and their mRNA expressions upon TNF stimulation were measured by quantitative RT-PCR. All of the genes selected showed essentially the same responses to TNF stimulation on PBMCs independent of the individual (Figure 3). CD40, IL12B, prostaglandin E synthase, C-type lectin domain family 4 member E, and TNFα-induced protein 6 were upregulated, while CD1C, IFIT1, IFIT3, OAS1, and IL8RA were downregulated upon TNF stimulation in both SLE patients and healthy individuals.

The *in vivo* gene expression profiles of SLE, however, were different from the results of *in vitro* PBMC stimulation by TNF. For example, CD40 was downregulated *in vivo* but was upregulated upon TNF stimulation *in vitro*. Meanwhile, IFI genes such as IFIT1, IFIT3, OAS1, ISG15 and IRF7, and IL8RA were upregulated *in vivo*, but IFIT1, IFIT3, OAS1 and IL8RA were downregulated, while ISG15 and IRF7 showed almost no response to TNF *in vitro*. These data suggest that other soluble factors might be involved in the regulation on the gene expression. Indeed, high levels of interferon in SLE serum have been suggested to cause overexpression of IFI genes [22]. Interestingly, we not only found that TNF had repressive

Figure 2



Network-based analysis of upregulated genes in the functional category of immune response. **(a)** Network 1, **(b)** Network 2, **(c)** Network 3, and **(d)** Network 4 constructed by upregulated genes.

effects on IFI genes IFIT1, IFIT3, IFIT5, ISG15, and IRF7 expression, but that the effect was significantly stronger on SLE patients' PBMCs than those of healthy individuals. This result may be caused by the differences in the baseline expressions where IFI genes were overexpressed *in vivo* in SLE patients.

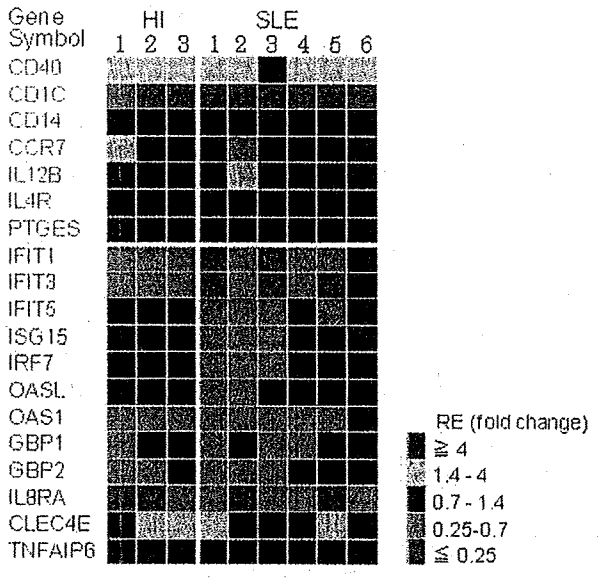
Repressive effect of TNF on interferon-inducible gene expressions in peripheral blood mononuclear cells *in vitro*

The expression of 15 IFI genes (IFIT1, IFIT3, IFIT5, IFI6, IFI16, IFI27, IFI30, IFI35, interferon-induced transmembrane protein 1, ISG15, IRF7, OAS1, OASL, GBP1, and GBP2) in PBMCs upon stimulation were measured. All of these genes were upregulated upon IFNα stimulation, while only some were upregulated by IFNγ (data not shown). On the other hand, TNF also showed a repressive effect on the expressions of most IFI genes in PBMCs *in vitro* in this experiment.

The relative expressions of three of the representative genes (that is, IFIT1, IFIT3, and IFI27) from three women are shown in Figure 4. A remarkable suppression was observed through the TNF and IFNα co-stimulating experiment (Figure 4a). On the other hand, there was synergism between IFNγ and IFNα on IFI gene expressions, although with some exceptions like IFIT1 (Figure 4b). IFIT1 was downregulated upon IFNγ and IFNα co-stimulation, unlike stimulation with IFNα alone. E2 showed no significant or consistent interaction with IFNα for most of the IFI genes. Inconsistent responses to E2 stimulation, however, were observed among the three healthy donors on IFI27. E2 tended to downregulate IFI27 expression in one donor but upregulated expression in the other two donors (Figure 4c).

To test a hypothesis that TNF decreases IFI gene expression through suppressing IFNα production, we examined the effect of TNF or IFNα on IFNα mRNA expression. Its expression was too low to be measured and there were no significant changes in TNF, IFNα, or TNF + IFNα 24-hour-stimulated PBMCs.

Figure 3



Effect of TNF stimulation on gene expression in healthy individuals and systemic lupus erythematosus patients. Peripheral blood mononuclear cells (PBMCs) from six systemic lupus erythematosus (SLE) patients and three healthy individuals (HI) were isolated and stimulated for 24 hours in the absence and presence of 20 ng/ml TNF. The relative mRNA expressions (RE) compared between TNF-stimulated and non-stimulated control individuals were measured using quantitative RT-PCR. The RE of seven downregulated genes (highlighted in green) and 12 upregulated genes (highlighted in red) are designated by five colors as shown. See Table 1 for gene identification.

Discussion

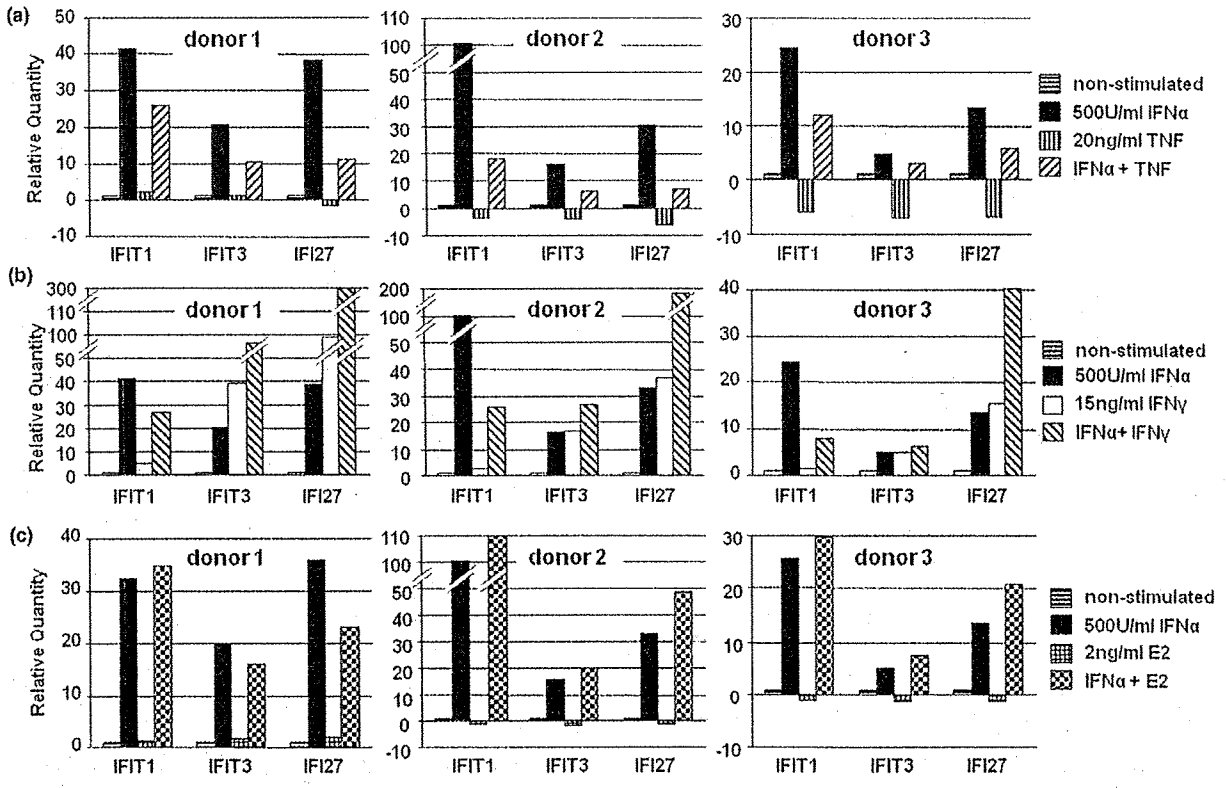
To identify the molecules involved in the aberrant immune system of SLE, we compared the gene expression profiles of peripheral blood between SLE patients and healthy individuals using microarray technology followed by gene ontology analysis. Most previously reported SLE studies utilizing microarray analysis have used PBMCs, but in the present study we used whole blood from SLE patients to exhaustively analyze the gene expression profiles of immune response-related molecules *in vivo*. Despite an additional proportion of granulocytes (mainly neutrophils), our results showed that there was an overexpression of several interferon-regulated genes. This result was in agreement with a previous report showing that peripheral blood from SLE patients had remarkably homogeneous gene expression patterns with an overexpression of IFI genes [10], and confirms the involvement of interferon in SLE.

Since the immune system is regulated by an elaborate network, interactions among the downregulated genes and the upregulated genes of the immune response category were further investigated by utilizing network-based analysis. A cluster of the TLR family (that is, TLR1, TLR2, TLR4, and TLR5) and another cluster of FcγRs were upregulated and depicted in the same network, which had p38 MAPK and NFκB at the center. Our finding that FcγR genes were overexpressed in the peripheral blood of SLE patients is novel, although the overexpression of TLR genes has been recently reported [23]. Furthermore, this is the first report showing that these clusters possibly interact with each other through p38 MAPK and NFκB signaling pathways in a network, and consequently contribute to SLE. Indeed, it has been shown that FcγRIIb is a gene susceptible to SLE both in humans and mice [24]. Means and Luster have reported that a functional interaction between TLR9 and CD32 (also known as FcγRIIa) may be involved in the pathogenesis of SLE, and they also have suggested the possibility that TLR7 may activate cells through similar pathways [25]. Although in our study overexpression of DNA-recognizing TLR9, which has been suggested to be triggered by immune complexes containing DNA in SLE [26,27], was not statistically significant according to the rank test, seven out of the 11 SLE patients showed upregulated expressions of TLR9. In addition, TLR1, TLR2, TLR4, and TLR5 – which serve to recognize bacterial components such as lipopolysaccharide or lipopeptides [28,29] – were also upregulated. Our network-based analysis therefore suggested the hypothesis that the interaction between TLRs and FcγRs is involved in the pathogenesis of SLE.

We additionally found that networks whose central molecule was TNF, IFNγ, or E2 were represented by both the downregulated genes and the upregulated genes in the functional category of immune response. This observation suggested that TNF, IFNγ, or E2 may be involved in the abnormal expressions of both downregulated and upregulated genes in the immune response. Indeed, the elevated level of some cytokines such as TNF and interferon in the sera of SLE patients has been reported [2,4,30,31]. Although our data did not show a significant increase in the gene expressions of TNF, IFNγ, or IFNα in themselves according to rank test, more than one-half of the SLE patients' individual data showed an increase in the TNF gene expression in our study (data not shown). For IFNα, the expression was not increased in the peripheral blood but it may be produced at the other site. Siegal and colleagues have demonstrated that purified interferon-producing cells were $CD4^+CD11c^-$ type 2 dendritic cell precursors, which produce 200 to 1,000 times more IFNα than other blood cells after a microbial challenge [32]. E2 is enzymatically synthesized in the ovary, and therefore does not transcript and cannot be detected in peripheral blood in the present study. There is, however, a 10 to 15 times higher frequency of SLE in women during childbearing years, probably due to an estrogen hormonal effect [33]. We therefore believe these results are a good reason to further investigate E2 involvement in SLE pathogenesis.

Concerning the interaction between cytokines, to our knowledge this is the first report showing that TNF has a repressive effect on IFI genes *in vitro*. Although the exact mechanisms of

Figure 4



Effect of cytokines or β-estradiol on the expressions of interferon-inducible genes. Peripheral blood mononuclear cells from three healthy donors were cultured with the indicated cytokines for 24 hours. RNA was analyzed by quantitative RT-PCR as described in Materials and methods. Relative expression of the indicated genes – interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), interferon-induced protein with tetratricopeptide repeats 3 (IFIT3), and interferon alpha-inducible protein 27 (IFI27) – compared with their nonstimulated cultures is shown. Each bar represents the mean value of duplicate wells as compared with the nonstimulated control. Downregulated genes were arbitrarily assigned a negative value.

the IFI gene product involvement in SLE pathogenesis are still poorly understood, we suspect that the elevated expression of TNF in SLE reduces the overexpression of IFI genes. Since serum levels of both TNF and IFNα were reportedly elevated in SLE, as mentioned above, it is possible that the increased serum TNF level in SLE is an outcome to compensate the immune system balance altered by IFNα in SLE. Consider that patients with rheumatoid arthritis or Crohn's disease under TNF-blocking therapies can develop autoantibodies to nuclear antigens [34]; therapeutic TNF blockades could thus lead to an exacerbation of certain autoimmune diseases such as SLE and to provoke lupus-like manifestations. Palucka and colleagues reported recently that blocking TNF signaling increases the production of IFNα by plasmacytoid dendritic cells and induces an IFN signature in the blood of arthritis patients [35]. This may be another mechanism for TNF inhibitor to induce the IFN signature. We confirmed that there was no significant effect, however, of TNF on IFNα gene expression in the PBMCs in our experiment. Furthermore, the 500 units/ml IFNα we used for stimulation is obviously a higher amount than endogenously produced IFNα. TNF therefore appeared to directly suppress IFI gene expression in PBMCs. We suggest that the direct suppressive effect of TNF on the IFN signature induced by IFNα, at least, exists in the network regulation of cytokines *in vivo*.

The results of the co-stimulating experiments did not show any strong evidence of a functional interaction between E2 and IFNα on the expression of IFI genes. Inconsistent gene expression patterns were observed in the co-stimulating experiments, possibly due to the hormonal effects of the women donors. The modulation of estrogens on humoral immune response seems to be greatly dependent on its physiological concentration, and E2 is a versatile hormone that plays a wide variety of roles in our body [36]. We therefore cannot exclude the possibility that E2 also plays a significant role in the pathophysiology of SLE.

Conclusion

TNF may have a counter effect on the abnormal regulation of IFNα on the immune response-related gene expressions, while IFNγ may have a synergistic effect with IFNα in SLE. Interactions between IFNα and one of TNF, IFNγ, or E2 had a suggested involvement in the pathogenesis of SLE.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

H-ML and TM contributed equally to this work. H-ML performed data analysis, interpretation of the microarray studies, sample preparation, stimulating and co-stimulating experiments, RNA purification, quantitative RT-PCR assays, and drafted of manuscript. TM performed data analysis, interpretation of the microarray studies, and patient recruitment. HS assisted with data analysis. CA performed labeling and scanning of the microarrays. YA assisted with data analysis. NY-H assisted with data analysis. KM assisted in microarray data acquirement. NN designed the study, enrolled patients, and assisted with data analysis and interpretation. All authors read and approved the final manuscript.

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Clinical value of blocking IL-6 receptor

Toru Mima and Norihiro Nishimoto

Laboratory of Immune Regulation, Wakayama Medical University, Wakayama City, Wakayama, Japan

Correspondence to Norihiro Nishimoto, MD, Laboratory of Immune Regulation, Wakayama Medical University, 105 Saito Bio Innovation Center, 7-7-20 Saito-Asagi, Ibaraki City, Osaka 567-0085, Japan
Tel: +81 72 646 8039; fax: +81 72 646 8140;
e-mail: norichan@wakayama-med.ac.jp

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Purpose of review

Interleukin-6 (IL-6) is a multifunctional cytokine that regulates inflammatory response and immune reaction. Overproduction of IL-6 is pathologically involved in inflammatory autoimmune diseases such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis, and therefore, blocking IL-6 activity is one of therapeutic options for these diseases. Tocilizumab is a humanized anti-IL-6 receptor (IL-6R) antibody and inhibits IL-6 activity. There is now accumulating evidence that tocilizumab is therapeutically effective for patients with RA and other inflammatory autoimmune diseases. This article reviews the clinical value of blocking IL-6R.

Recent findings

Tocilizumab, as monotherapy and in combination with methotrexate, has been shown to be effective for RA patients with insufficient efficacy to methotrexate or other disease-modifying antirheumatic drugs. These findings of tocilizumab have been expanded to patients refractory to tumor necrosis factor inhibitors. Tocilizumab also retards the progression of structural joint damage. Furthermore, a 5-year long-term safety and efficacy has been shown. Tocilizumab is also a promising therapeutic option for other rheumatic diseases such as systemic-onset juvenile idiopathic arthritis, adult-onset Still's disease, and Takayasu arteritis.

Summary

Blocking IL-6R with tocilizumab represents a promising new treatment for RA and other inflammatory diseases. Large registry data will warrant the safety profile of tocilizumab.

Keywords

interleukin-6, joint destruction, juvenile idiopathic arthritis, rheumatoid arthritis, tocilizumab

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Introduction

In the last decade, biological agents represented by tumor necrosis factor (TNF) inhibitors have been introduced into the therapy for patients with rheumatoid arthritis (RA). These powerful agents have sifted the therapeutic paradigm of RA, and clinical remission has become a realistic goal of RA therapy [1]. However, the powerful TNF inhibitors are not always effective for every patient. In addition, the majority of responders experience only a partial clinical improvement in their disease. Therefore, we still need another therapeutic option having high efficacy and safety. Interleukin-6 (IL-6) is another target molecule the biological activities of which should be inhibited for the treatment of RA.

IL-6 is a multifunctional cytokine with various biological activities such as induction of inflammatory response, regulation of immune reaction, and hematopoiesis [2]. IL-6 induces the proliferation and differentiation of T cells as well as the terminal differentiation of B cells, including autoantibody-producing cells. Thus, overproduction of IL-6 augments the autoimmune reaction. Recently, the pathogenic significance of T helper (Th) cells that produce IL-17 (and the related cytokine, IL-17F), IL-6, and TNF, but not IL-4 or interferon-γ, was focused on autoimmune diseases. A subset of $CD4^+$ T cells that produce IL-17 and are distinct from Th1 and Th2 are called Th17 cells [3–5]. They have been shown to have crucial roles in the induction of inflammation and autoimmune diseases [6]. Interestingly, transforming growth factor-β (TGFβ), in the presence of IL-6, was reported to induce the differentiation of pathogenic Th17 cells [7,8]. By contrast, TGFβ, in the absence of IL-6, induces naturally occurring $CD4^+CD25^+$ forkhead box P3 $(FOXP3)^+$ T regulatory (Treg) cells, which inhibit autoimmunity and protect against tissue injury [9,10]. Treg cells can also suppress Th1, Th2 as well as Th17 cells. In these reciprocal developmental pathways for the generation of pathogenic effector Th17 cells and Treg