study have suggested that treatment with anakinra, an interleukin-1-receptor antagonist, might be effective in patients with this illness,[10–13] but macrophage-activation syndrome still occurred despite treatment with anakinra.[14,15]

The pathogenesis of systemic-onset juvenile idiopathic arthritis remains obscure. However, interleukin 6 and soluble interleukin-6 receptor play a part as inflammatory mediators.[16] The serum interleukin-6 concentrations are related to the extent and severity of joint involvement, fever patterns, platelet counts,[17] growth retardation, and osteoporosis.[18] Transgenic mice with increased expression of human interleukin 6 were growth retarded, as are children with systemic-onset juvenile idiopathic arthritis.[19] The clinical use of tocilizumab, an anti-interleukin-6-receptor monoclonal antibody, in early trials had striking and long-lasting effects on both the systemic and articular manifestations of systemic-onset juvenile idiopathic arthritis, even in patients with severe disease that was refractory to other therapies.[20,21]

In the search to improve treatment of this difficult and debilitating childhood disease, we undertook a placebo-controlled trial of the efficacy and safety of tocilizumab (Chugai Pharmaceuticals, Tokyo, Japan) in systemic-onset juvenile idiopathic arthritis.

Methods

Patients

Patients were eligible if they were 2–19 years of age with disease onset before their 16th birthday and if they met the International League of Associations for Rheumatology classification criteria for systemic-onset juvenile idiopathic arthritis.[22] Treatment with intra-articular corticosteroids, methylprednisolone pulse treatment, immunosuppressive drugs, and disease-modifying antirheumatic drugs (DMARDs)—such as methotrexate, ciclosporin, sulfasalazine, azathioprine, and cyclophosphamide—for 2 weeks before the first administration of tocilizumab was not allowed; and treatment with TNF agents was not allowed for 12 weeks before patients started tocilizumab. Doses of oral corticosteroids had to be stable for 2 weeks before the trial.

Active disease was defined by an increase in C-reactive protein (CRP; ≥15 mg/L) concentrations and an inadequate response to corticosteroids (at ≥0·2 mg/kg prednisolone equivalent) for longer than 3 months. Clinical manifestations were carefully monitored, especially when synovitis was present with other systemic features.

Patients were excluded if they had important concurrent medical or surgical disorders, with leucopenia ($<3·5\times10^9$/L) or thrombocytopenia ($<100\times10^9$/L); cardiac disease (assessed by a paediatric cardiologist before enrolment); or developed macrophage-activation syndrome during the prestudy hospital admission. All patients were examined during screening for active infections, especially pneumonia and tuberculosis, and suspected cases were further examined by chest radiography or computed tomography.

Patients were admitted 2 weeks before the start of the trial and until the completion of the double-blind phase of the study for safety monitoring. Children were cared for by child-life specialists or they went to an in-hospital school or nursery when not accompanied by their family.

The protocols and amendments were approved by the Japanese ministry of welfare, health, and labour and the institutional review boards at every centre. The parent or legal guardian of every child gave written informed consent and the child gave assent when appropriate.

Procedures

This study consisted of three phases—an open-label lead-in phase of 6 weeks, a double-blind, randomised, placebo-controlled phase of 12 weeks, and an open-label extension phase of at least 48 weeks, and was undertaken in eight university hospitals and children's hospitals in Japan. The primary endpoints in the open-label lead-in phase of the study were the proportion of children achieving an American College of Rheumatology Pediatric (ACR Pedi) 30 response and the proportion of those showing improvements in CRP concentrations (<5 mg/L) at the end of the 6-week treatment. All patients were to receive three doses as intravenous infusions of tocilizumab at 8 mg/kg every 2 weeks. The children were assessed for improvement, defined as achievement of the ACR Pedi 30 response—ie, at least three of six ACR Pedi variables improved by at least 30% with no more than one variable worsening by more than 30%.[23] The ACR Pedi variables were physician's and patients'/parents' general assessment on a 10 cm visual-analogue scale, functional ability (childhood health assessment questionnaire, Japanese version, which has been validated and will be published), number of active joints defined by the presence of swelling or, if no swelling is present, restriction of motion accompanied by pain, or tenderness, or both, and number of joints with restriction of movement, and erythrocyte sedimentation rate (ESR). Children were also assessed for ACR Pedi 50 and 70 responses—ie, at least three of six response variables improved by at least 50% and at least 70%, respectively, with no more than one variable worsening by more than 30%.

Patients who completed the open-label lead-in phase and achieved both an ACR Pedi 30 response and CRP concentrations of less than 5 mg/L were randomly assigned to receive an infusion of tocilizumab 8 mg/kg or placebo every 2 weeks (give or take 3 days) for 12 weeks in a double-blind manner. The primary endpoint was the proportion of patients in each treatment group who completed the 12-week period and maintained an ACR Pedi 30 response and CRP concentrations of less than 15 mg/L. Patients who did not maintain an ACR Pedi 30 response or those whose CRP concentrations increased to at least 15 mg/L were withdrawn for rescue medication. Methotrexate, ciclosporin, and other DMARDs and

immunosuppressive drugs were not allowed throughout the study, with the exception of stable doses of oral corticosteroids.

ACR Pedi responses, systemic feature score,[24] and CRP concentrations were assessed every 2 weeks. Systemic features assessed were fever, rash, lymphadenopathy in cervical, axillary, and inguinal regions, and hepatomegaly, splenomegaly, and serositis. These features were scored as either absent (0 point) or present (1 point for each one of the eight features) and the score therefore could range from 0 to 8 points. CRP concentrations were measured to assess the disease response as a surrogate marker of interleukin-6 function.

Patients were monitored for safety by routine physical examinations every day during their hospital stay. Urinalysis and blood examinations—white-blood-cell and platelet counts and measurements of haemoglobin, aminotransferases, creatinine, total cholesterol, and ESR—were done every 2 weeks. CRP and ferritin concentrations were measured every week to monitor disease activity and the development of macrophage-activation syndrome. Anti-tocilizumab IgG and IgE antibodies were measured before each administration of tocilizumab. Serum tocilizumab, interleukin 6, and soluble interleukin-6-receptor concentrations were measured every 2 weeks, but results were masked to the investigators and other study personnel during the double-blind phase. Maximum body temperature was recorded daily. Patients who developed anti-tocilizumab antibodies, had grade-3 laboratory test abnormalities according to the National Cancer Institute common terminology criteria for adverse events,[25] or had important safety or compliance difficulties were withdrawn from the study.

In addition to patients who were randomised in the double-blind phase, those who did not meet the criteria for randomisation but completed the open-label lead-in phase with reductions in CRP concentrations were eligible for the open-label extension phase. All eligible patients were to receive tocilizumab 8 mg/kg every 2 weeks for at least 48 weeks. The dosing interval was adjusted according to the disease activity measured by ACR Pedi responses and CRP concentrations and it could be shortened, but not to less than 1 week. In the open-label extension phase, efficacy variables were assessed every 6 weeks and ACR Pedi variables and CRP concentrations were assessed every 2 weeks. The primary endpoint was the proportion of patients achieving an ACR Pedi 30 at the final visit. Corticosteroid-sparing effect was assessed by the reduction in corticosteroid doses, which were allowed to be adjusted during the extension phase. Safety laboratory tests were done every 2 weeks during the first 6 weeks and every 6 weeks thereafter.

All patients who left the study were required to return for a follow-up assessment 2 weeks after discontinuation of treatment. Any adverse events and laboratory abnormalities reported during the study, especially those thought to be drug-related, were followed up until resolution or stabilisation.

Statistical analysis

The method of analysis was by intention to treat. In the double-blind phase, patients were randomly (1 to 1 ratio) assigned to the two treatment groups. A dynamic allocation was done after the balance between the strata (CRP concentrations <30 mg/L or ≥30 mg/L) within the site was checked and for all patients randomly assigned up to that point. Treatment groups were assessed by the exact χ^2 test with a two-tailed significance level of 5%. Each treatment group had to include at least 20 patients to provide 90% power to detect a difference in the proportions of patients achieving both an ACR Pedi 30 response and CRP concentrations of less than 15 mg/L, so that the response was 2 (60%) of 20 patients in the tocilizumab group and 2 (10%) of 20 patients in the placebo group. Time to withdrawal was estimated by the Kaplan-Meier method and assessed by the log-rank test. Secondary endpoints included the time courses of efficacy variables. Comparisons of these variables at each time point were done with a *t* test.

Safety analysis was done for all patients who received at least one dose of the study drug. A last-observation-carried-forward approach was used for missing data for the patients who withdrew early.

This study is registered with ClinicalTrials.gov, numbers NCT00144599 (for the open-label lead-in and double-blind phases) and NCT00144612 (for the open-label extension phase).

	Open-label lead-in phase (n=56)	Double-blind phase	
		Placebo (n=23)	Tocilizumab (n=20)
Patients*			
Male	21 (38%)	8 (35%)	7 (35%)
Female	35 (63%)	15 (65%)	13 (65%)
Age (years)	8·3 (4·4)	9·3 (4·5)	8·0 (4·3)
Age group (years)			
2–5	20 (36%)	5 (22%)	9 (45%)
6–10	19 (34%)	11 (48%)	5 (25%)
11–15	13 (23%)	4 (17%)	5 (25%)
16–19	4 (7%)	3 (13%)	1 (5%)
Age at disease onset (years)†	4·3 (2·6)	5·1 (3·0)	3·9 (2·2)
Disease duration (years)	4·5 (3·6)	4·7 (4·0)	4·6 (3·5)
Number of past treatments‡	2·1 (1·0)	2·0 (1·0)	2·1 (1·0)
Study entry prednisolone-equivalent steroid dose (mg/kg per day)	0·51 (0·36)	0·46 (0·33)	0·42 (0·27)

Data are number (%) or mean (SD). *Total of percentages might not equal 100% because numbers were rounded up or down. †All patients developed the disease before their 16th birthday. ‡Disease-modifying antirheumatic drugs or immunosuppressive agents.

Table 1: **Baseline demographic and disease characteristics**

	Open-label lead-in phase (n=56)			Double-blind phase						Open-label extension phase (n=50)	
	Baseline	6 weeks*	Improvement†	Placebo (n=23)			Tocilizumab (n=20)			48 weeks	Improvement†
				Baseline	6 weeks*	Last observation‡	Baseline	6 weeks*	Last observation‡		
Juvenile idiopathic arthritis core set criteria											
Number of active joints	4·0 (0–39·0)	0 (0–34·0)	73%	4·0 (0–21·0)	0 (0–13·0)	0 (0–34·0)	3·5 (0–18·0)	0 (0–4·0)	0 (0–4·0)	0 (0–4·0)	88%
Number of joints with restricted motion	0·5 (0–47·0)	0 (0–45·0)	54%	0 (0–37·0)	0 (0–41·0)	0 (0–42·0)	0·50 (0–47·0)	0 (0–45·0)	0 (0–46·0)	0 (0–62·0)	72%
Physician's global assessment of disease severity§	52·0 (18·0–100)	8·5 (0–97·0)	75%	51·0 (18·0–95·0)	5·0 (1·0–60)	14·0 (0–84·0)	51·0 (21·0–96·0)	7·50 (0–42·0)	5·50 (0–47·0)	3·5 (0–22·0)	89%
Patient's or parent's global assessment of wellbeing§	53·0 (0–90)	13·5 (0–69·0)	63%	55·0 (18·0–85·0)	10 (1·0–49·0)	39·0 (2·0–94·0)	51·5 (0–76·0)	12·0 (0–63·0)	4·5 (0–34·0)	8·5 (0–70)	75%
Score on childhood health assessment questionnaire¶	0·88 (0–3·00)	0·38 (0–3·00)	43%	0·63 (0–3·00)	0·25 (0–2·75)	0·38 (0–3·00)	0·88 (0–2·38)	0·38 (0–2·63)	0·38 (0–1·63)	0·13 (0–2·13)	67%
Erythrocyte sedimentation rate (mm/h)	44·5 (8·0–125·0)	4·0 (0–64·0)	82%	35·0 (8·0–68·0)	3·0 (1·0–13·0)	11·0 (1·0–41·0)	39·5 (8·0–103·0)	4·0 (0–9·0)	4·0 (0–7·0)	3·0 (0–12·0)	91%
Additional assessments											
Number of patients with total systemic feature score‖	1·0 (0–3·0)	1·0 (0–2·0)	34%	1·0 (0–2·0)	1·0 (0–2·0)	1·0 (0–2·0)	1·0 (0–3·0)	1·0 (0–2·0)	0·5 (0–2·0)	0 (0–1·0)	98%
0	7	23		6	11	8	1	8	10	47	
1	40	27		14	11	12	16	10	9	1	
2	8	6		3	1	3	2	2	1	0	
3	1	0		0	0	0	1	0	0	0	
White-blood-cell count ($\times 10^3$ per μL)	12·4 (4·9–30·8)	8·4 (2·5–21·9)	29%	12·8 (6·4–21·0)	8·1 (3·5–14·1)	12·2 (4·4–24·6)	11·2 (4·9–16·1)	6·6 (2·5–10·4)	7·4 (4·1–11·6)	6·4 (3·4–37·4)	36%
C-reactive protein (mg/L)	43·5 (16·0–190·0)	0·5 (0–99·0)	90%	38·0 (17·0–131·0)	0·2 (0–1·0)	15·0 (0–101·0)	35·0 (16·0–190·0)	0·1 (0–1·0)	0·1 (0–21·8)	0·1 (0–2·0)	99%

Data are median (range), unless otherwise indicated. *Measurements at the end of open-label lead-in phase or at withdrawal. †Improvement between baseline and the end of open-label lead-in phase. ‡Measurements at the end of double-blind phase or at withdrawal. §Score on a visual-analogue scale could range from 0 mm (best) to 100 mm (worst). ¶Score could range from 0 (best) to 3 (worst). ‖Systemic feature score includes febrile episode, rheumatoid rash, lymphadenopathy, hepatosplenomegaly, and serositis; score could range from 0 (best) to 8 (worst).

Table 2: **Measurement of disease activity and improvement from baseline**

Role of the funding source

The sponsor of the study supplied the study medication and was responsible for data processing and management, statistical analysis, and reporting of serious adverse events. All authors had full access to the study data on request. The corresponding author had final responsibility to submit the report for publication.

Results

Table 1 summarises the baseline demographic and clinical characteristics of the 56 patients who took part in the open-label and double-blind phases of the study. All patients had onset of systemic-onset juvenile idiopathic arthritis before their 16th birthday (range 6 months to 12 years). All patients had previously received oral corticosteroids. Most patients had previously received at least two DMARDs or immunosuppressive drugs, or both, such as methotrexate and ciclosporin.

Patients had moderate disease activity at entry in the open-label lead-in phase of the study, despite background corticosteroid treatment (table 2) as shown by the ESR, CRP concentrations, systemic feature score (median 1, range 0–3), and fever (>38°C, present in 49 [88%] of 56 patients). The baseline demographic characteristics of patients in the placebo and active treatment groups during the double-blind phase showed minor but not significant differences in baseline disease severity (table 2). The distribution of ACR Pedi 30, 50, and 70 responses at completion of the open-label lead-in phase was similar in the placebo and tocilizumab groups, and median ESR values and CRP concentrations were low and much the same in both groups in the double-blind phase.

Figure 1 shows the trial profile. Six patients were withdrawn during the open-label lead-in phase: three developed anti-tocilizumab IgE antibodies, two had serious adverse events (one anaphylactoid reaction, one gastrointestinal haemorrhage), and one because of absence of efficacy. Six patients did not meet the response criteria for randomisation—CRP concentrations (<5 mg/L) and ACR Pedi 30 response—for the double-blind phase of the study. In the double-blind phase, one patient in the tocilizumab group had to be excluded from the efficacy analysis because the study mask for this patient was broken by

mistake and pharmacokinetic data were unexpectedly unmasked; therefore, 43 patients were included in the efficacy analysis. One patient was withdrawn from each treatment group in the double-blind phase because of adverse events (figure 1).

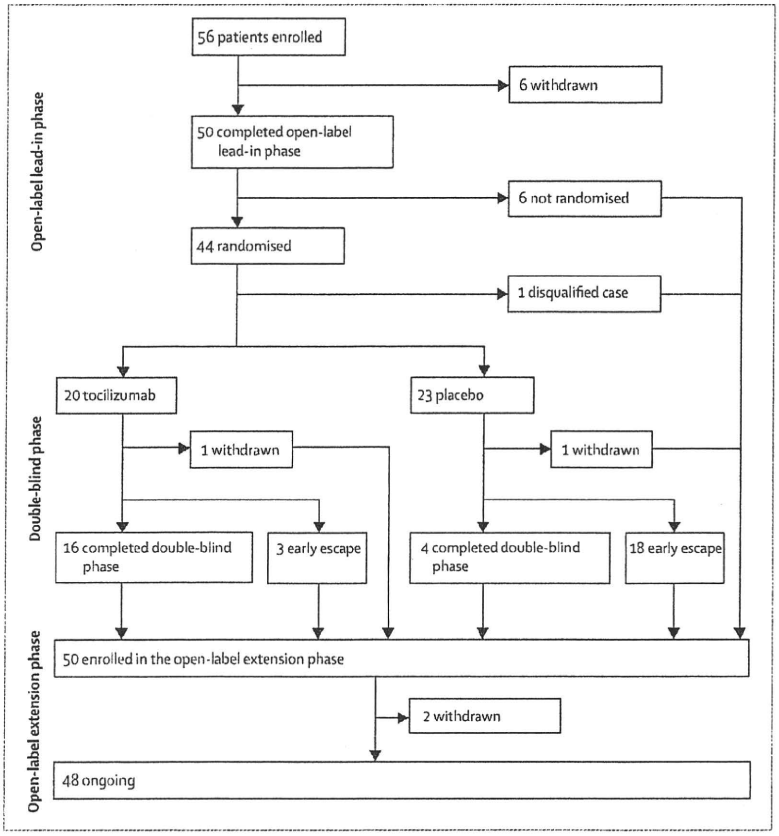

Figure 1: Trial profile

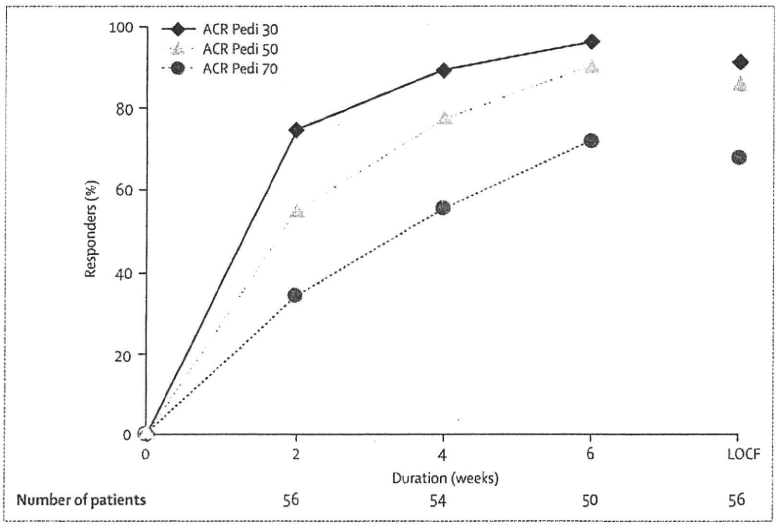

Figure 2: Time courses of American College of Rheumatology Pediatric (ACR Pedi) 30, 50, and 70 responses in initial open-label lead-in phase
LOCF=last observation carried forward.

In the open-label extension phase, patients randomly assigned in the double-blind phase and six patients who were not randomly assigned but completed the open-label lead-in phase were given tocilizumab. These six patients had limited treatment options and showed benefit to some extent in the open-label extension phase. All patients started tocilizumab treatment in the open-label extension phase immediately after they left or completed the initial phases. Two of 50 patients continuing tocilizumab treatment were withdrawn during the open-label extension phase because of adverse events—anaphylactoid reaction in one and development of anti-tocilizumab antibodies in the other.

All 56 patients enrolled in the open-label lead-in phase were included in the efficacy analysis. Figure 2 shows the proportion who had ACR Pedi 30, 50, and 70 responses. At the last observation, the ACR Pedi 30, 50, and 70 response rates were seen in 51 (91%), 48 (86%), and 38 (68%) of 56 patients, respectively. 48 (86%) of 56 patients had an improvement in their actual CRP concentrations to less than 5 mg/L and this reduction took place within 2 weeks of starting tocilizumab. Overall 44 (79%) of 56 patients achieved both an ACR Pedi 30 and CRP concentrations of less than 5 mg/L at week 6. Moreover, every ACR Pedi variable showed a sustained response to tocilizumab treatment. Similar to CRP concentrations, median ESR rapidly fell within 2 weeks, joint counts and childhood health assessment questionnaire decreased by week 4, and general assessments continued to improve until week 6 (table 2). The proportion of patients with the systemic feature score of at least 1 decreased from 49 (88%) of 56 patients to 33 (59%) of 56 patients during the lead-in open-label phase (table 2).

Four (17%) of 23 patients in the placebo group and 16 (80%) of 20 patients in the tocilizumab group ($p<0·0001$) completed the 12-week double-blind phase and maintained an ACR Pedi 30 response and CRP concentrations of less than 15 mg/L. Similarly, four (17%) of 23 patients had an ACR Pedi 50 response and CRP concentrations of less than 15 mg/L in the placebo group compared with 16 (80%) of 20 patients in the tocilizumab group; and three (13%) of 23 patients had an ACR Pedi 70 response and CRP concentrations of less than 15 mg/L in the placebo group versus 15 (75%) of 20 patients in the tocilizumab group. Figure 3 shows the time to early escape. Duration of sustained efficacy was increased in the tocilizumab group compared with the placebo group ($p<0·0001$). The median time to early escape was 4·9 weeks in the placebo group, but longer than 12 weeks in the tocilizumab group.

Both CRP concentrations and ESR remained low in the tocilizumab group, but increased in the placebo group after patients entered the double-blind phase. Median values for both indices on the last observation day were lower in the tocilizumab group than in the placebo (table 2).

Figure 4 shows the changes in ACR Pedi 30, 50, and 70 responses from the end of the open-label lead-in phase to the last study visit of the double-blind phase. More patients in the placebo group than in the tocilizumab group lost their response or had a reduction in their response; patients in the tocilizumab group showed further improvement in ACR Pedi 70 with continued treatment.

Four patients on placebo completed the double-blind phase of the study despite having undetectable serum tocilizumab concentrations 3–5 weeks after randomisation; these patients remained responders at the end of the double-blind phase. Three of these patients had mild disease at study entry, which might have been the reason for their lasting response to tocilizumab treatment during the open-label lead-in phase.

At the 48-week analysis in the open-label extension phase, 48 (96%) of 50 patients were still receiving tocilizumab. Median duration of treatment for the 50 patients from the initial open-label lead-in phase was 61·1 (range 8·7–98·9) weeks; 48 of these patients completed 48-week assessments. The numbers of patients who achieved ACR Pedi 30, 50, and 70 responses at 48 weeks were 47 (98%), 45 (94%), and 43 (90%), respectively. The median absolute change from baseline in ESR at week 48 was −34 (−121 to −7) mm/h and median percentage change was −93·2% (−100·0% to −78·6%). The median absolute change from baseline in CRP concentrations at the same time point was −43·1 (−190·0 to −16·0) mg/L and median percentage change was −99·7% (−100·0% to −95·1%).

Haemoglobin concentrations and platelet counts showed improvement after patients started tocilizumab. Median haemoglobin concentration increased from 111 (range 74–151) g/L at baseline to 124 (73–179) g/L at week 48. Median platelet count decreased from $41·8\times10^{10}$ ($16·8\times10^{10}$ to $86·2\times10^{10}$) per L at baseline to $30·2\times10^{10}$ ($13·1\times10^{10}$ to $55·6\times10^{10}$) per L at week 48. All 48 patients with 48-week efficacy data were given stable doses of oral corticosteroids throughout the initial open-label lead-in and double-blind phases and during the open-label extension phase, 33 (69%) and 22 (46%) were able to reduce their doses by at least 30% and at least 50%, respectively. Figure 5 shows the efficacy responses in each treatment group during the double-blind phase and open-label extension phase. The efficacy response of 21 patients who met the rescue criteria in the double-blind phase improved immediately after they resumed tocilizumab infusion in the extension phase. Patients lost their response to tocilizumab during placebo treatment in the double-blind phase but regained it once tocilizumab treatment was restarted in the open-label extension phase.

No deaths or cases of macrophage-activation syndrome occurred during the lead-in and double-blind phases of the study. Two serious adverse events were reported during the open-label lead-in phase: one anaphylactoid

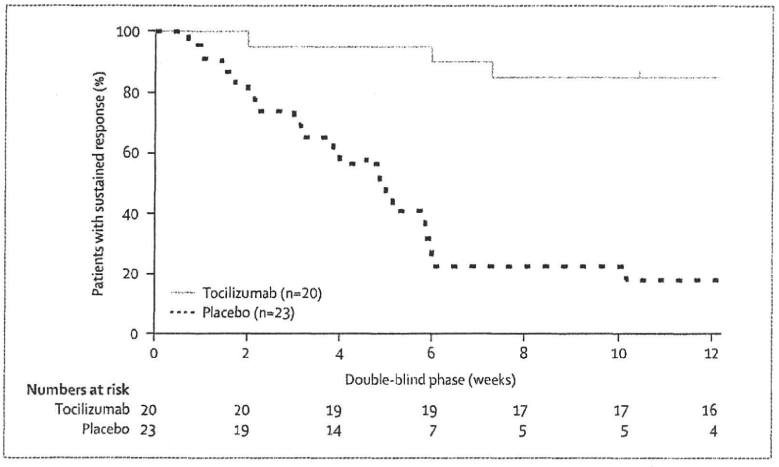

Figure 3: Time course of early escape for rescue medication

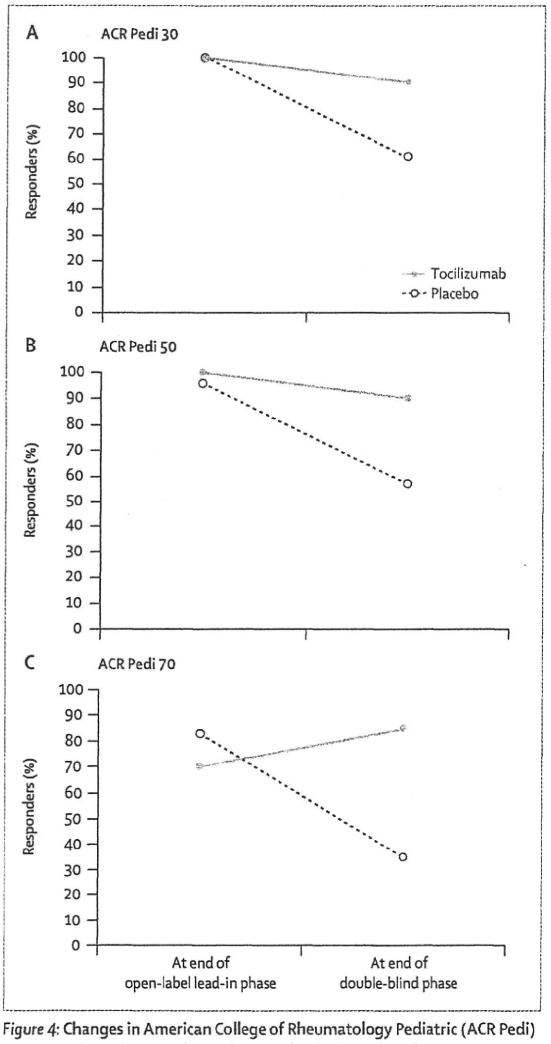

Figure 4: Changes in American College of Rheumatology Pediatric (ACR Pedi) responses from the open-label phase to the double-blind phase

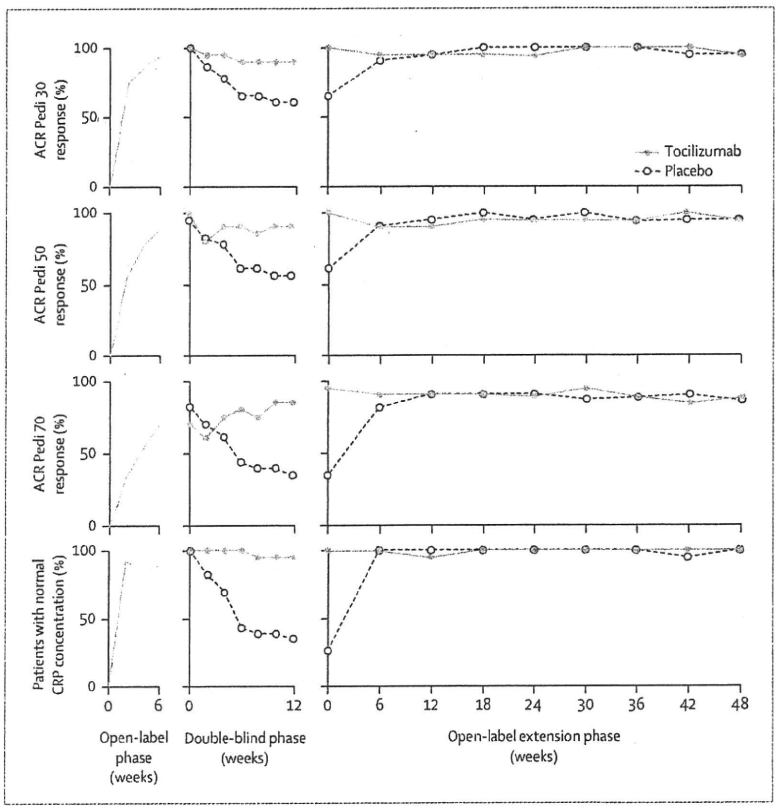

Figure 5: **Efficacy responses during the double-blind and open-label extension phases**
CRP=C-reactive protein. ACR Pedi=American College of Rheumatology Pediatric.

reaction in a patient who tested negative for IgE-type anti-tocilizumab antibodies and previously had had allergic reactions to aspirin and infliximab, and one case of gastrointestinal haemorrhage from diffuse acute or chronic colonic ulceration in a patient with a history of chronic diarrhoea and rectal bleeding.

Additionally, during the double-blind phase, two adverse events needed patients to be withdrawn from the study; one had infectious mononucleosis associated with striking increases in liver enzymes (aspartate aminotransferase 527 IU/L, alanine aminotransferase 676 IU/L, and lactate dehydrogenase [LDH] 874 IU/L) and neutropenia (963 cells per μL) 2 weeks after the fifth dose of tocilizumab. Laboratory values returned to normal 3 weeks after the onset of Epstein–Barr virus infectious mononucleosis and the patient resumed tocilizumab in the open-label extension phase. The other patient had herpes zoster during placebo administration in the double-blind phase when serum tocilizumab concentrations were below the limit of quantification. Herpes zoster was treated with aciclovir and the patient resumed tocilizumab administration in the open-label extension phase.

Most of the adverse events that arose during both the open-label lead-in and double-blind phases were mild or moderate in severity and typical of those noted with other biological agents in similar settings.[19,23] Adverse events frequently reported were symptoms of upper-respiratory-tract infections and gastroenteritis, but not of tuberculosis. In the double-blind phase, the occurrence of gastroenteritis was similar in the tocilizumab group (one [5%] of 21 patients) and placebo (one [4%] of 23 patients) groups, whereas the frequency of upper-respiratory-tract infection was increased in the placebo group (four [17%] of 23 patients) versus the tocilizumab group (two [10%] of 21 patients). Ten patients had mild infusion reactions during the open-label lead-in phase. Development of anti-tocilizumab IgE antibodies was noted in four patients.

In the open-label extension phase of the study, 13 serious adverse events were noted, which included bronchitis, gastroenteritis, and an anaphylactoid reaction (leading to patient withdrawal). The cases of bronchitis (n=2) and gastroenteritis (n=2) resolved with antibiotic treatment. The most common adverse events were nasopharyngitis (33 [59%]), upper-respiratory-tract infection (19 [34%]), gastroenteritis (16 [29%]), and bronchitis (14 [25%] of 56 patients). Increases in alanine aminotransferase (16 [29%]), aspartate aminotransferase (12 [21%]), and LDH (10 [18%]) were noted; increases of at least grade 2 in alanine aminotransferase and aspartate aminotransferase were recorded in 12 and eight patients, respectively. Transaminases tended to increase early during tocilizumab administration and then to subside during continuation of treatment. Mild increases, mostly within the normal range, in total cholesterol were noted. Tuberculosis was not reported.

Discussion

At the end of the open-label lead-in phase, the ACR Pedi 30, 50, and 70 response rates showed that tocilizumab had excellent and rapid effectiveness against systemic-onset juvenile idiopathic arthritis. After 6 weeks, the patients who did not respond—as defined in terms of both ACR Pedi 30 response and reduced CRP concentrations—were generally younger with a shorter disease duration and more severe inflammation than those who did respond to treatment. Patients who remained on tocilizumab in the double-blind phase had sustained improvements in clinical measures of effectiveness and wellbeing, whereas most of those in the placebo group needed rescue treatment.

The design of this study was chosen on the basis of counsel with the Japanese Pharmaceuticals and Medical Devices Agency because to do a standard placebo-controlled trial when there is preliminary evidence of effectiveness with a new drug would be ethically unsound.[6] The withdrawal design has the disadvantage that as patients are withdrawn for rescue treatment, the numbers of controls decrease. Therefore, the primary endpoint in the double-blind phase inevitably has to be time to early escape. However, in the open-label

extension phase the primary endpoint was the ACR Pedi 30 response rate, which was measured against baseline rather than a control population.

Active joints and systemic symptoms were not included in the inclusion criteria because refractory patients often received high-dose corticosteroids and the manifestation of joint disease and systemic features would be less obvious. To force these patients to reduce their corticosteroid dose to make their actual disease activity visible would be unethical. A necessity to start a mid to high dose of corticosteroids for a specific time and the failure to suppress inflammation as evidenced by CRP concentrations are good indicators of active disease in this patient population.

Masked assessors of joint disease were not used in this study, which might have biased the results. Since the joint assessments were done by trained paediatric rheumatologists who followed standardised methods, these assessors were thought to be unnecessary. However, interpretation of the results has some limitations.

Laboratory indicators of acute-phase reactants changed rapidly—within 2 weeks after the first infusion of tocilizumab. Median white-blood-cell, neutrophil, and platelet counts decreased in patients on active treatment, as previously described for patients with polyarticular juvenile idiopathic arthritis in response to etanercept,[6] but interleukin-6-receptor inhibition rapidly returned body temperature to normal and increased the median haemoglobin concentration. These findings accord with de Benedetti and colleagues'[18] hypotheses that interleukin 6 is causally related to the spiking fever and anaemia of systemic-onset juvenile idiopathic arthritis.

Common adverse events were gastrointestinal, nasopharyngeal, and upper-respiratory-tract infections, but they were mild. There might have been a potential absence of acute-phase reactions in response to infections because of inhibition of interleukin-6 signalling by tocilizumab. However, a mild increase in CRP concentration during infections suggested incomplete inhibition of interleukin-6 signalling or overwhelmed inflammatory responses.

Two serious adverse events—anaphylactoid reaction and gastrointestinal haemorrhage—were noted in the open-label lead-in phase. Gastrointestinal haemorrhage was presumably caused by the long-term high-dose corticosteroid treatment since the patient had previously had two similar episodes of gastrointestinal haemorrhage.

Aminotransferase concentrations tended to increase early in the tocilizumab administration, generally within the first 3–6 months and are possibly related to the pathological process unique to systemic-onset juvenile idiopathic arthritis, treatment methods such as steroid tapering, or biological effect of interleukin 6 on liver, or both.

A comparison of the time courses of ACR Pedi responses in the placebo and tocilizumab groups during the double-blind phase could not be made easily because the number of patients in the placebo group decreased rapidly because of withdrawal. Four patients in a phase II long-term study remain in remission without tocilizumab treatment or any other medications.[26] Although spontaneous disease remission can occur in these patients, the possibility exists that the interleukin-6-receptor inhibition induced long-lasting secondary changes in inflammatory and immune processes, leading to disease remission.

Effectiveness data in the extension phase were calculated against the baseline of the open-label lead-in phase because of the absence of a transition lag between the double-blind and open-label extension phases. Because the placebo periods were mostly 2–6 weeks, all patients in the double-blind phase can be discussed as one group in the extension study. The response rates at 48 weeks were almost the same between the groups.

By week 48, ESR and CRP concentrations had decreased from baseline. After completion of the initial open-label lead-in and double-blind phases, corticosteroid doses were reduced by at least 50% in most patients. Since the complications related to corticosteroid use—including growth retardation and osteoporosis—are still major problems in these children with persistent disease, the corticosteroid-sparing effect might lead to substantial benefit in the treatment of systemic-onset juvenile idiopathic arthritis.

The extension study showed that tocilizumab can be used to treat systemic-onset juvenile idiopathic arthritis and patients maintained a good response rate in terms of ACR Pedi data without flares in disease; tocilizumab had a good tolerability profile, which was much like that of other biological agents.[6]

Macrophage-activation syndrome remains the most devastating and life-threatening complication in the disease course of refractory systemic-onset juvenile idiopathic arthritis. The cause of this disorder is unknown, but the decrease has arisen after introduction of several pharmacological agents, and often follows infections.[27] Thus, macrophage-activation syndrome could develop during tocilizumab treatment.

Tocilizumab could play an important part in the treatment of systemic-onset juvenile idiopathic arthritis because interleukin 6 is directly implicated in the pathogenesis of this disease[16,17] and because tocilizumab needs less frequent administration than does anakinra. Uncontrolled studies suggest that anakinra could be effective in the treatment of both systemic symptoms and arthritis in patients with systemic-onset juvenile idiopathic arthritis, but confirmatory studies are needed. Methotrexate and anti-TNF treatments are thought to be less beneficial in this arthritis than in other subtypes of juvenile idiopathic arthritis.

Two important issues related to this class of products—namely malignant diseases and autoimmunity—were not clearly assessed because of the small sample size and the short follow-up. Longer

follow-up with a larger patient population than that in this study is needed to address these issues.

Thus, the results of this placebo-controlled and open-label extension study with tocilizumab in children with systemic-onset juvenile idiopathic arthritis show a sustained clinical improvement and a favourable risk-benefit profile. The findings of this study might represent a step forward in the control of a disease that has previously proved to be difficult to manage.

Contributors

The study protocol was developed by the authors and clinical study investigators in collaboration with the study sponsor. All data discussed in this report were interpreted by the authors in collaboration with the study sponsor. The report was written by the corresponding author in consultation with the other authors and the sponsor. The authors participated in the study procedures, including patient enrolment, screening, clinical assessments, and follow-up.

Conflict of interest statement

NN received a consulting fee from the sponsor and works as a scientific advisory board member of Hoffman-La Roche, which developed tocilizumab in collaboration with the sponsor. TK holds a patent for tocilizumab for treatment of inflammatory disorders, including rheumatoid arthritis and Castleman's disease. The other authors declare that they have no conflict of interest.

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Abnormal expression of the genes involved in cytokine networks and mitochondrial function in systemic juvenile idiopathic arthritis identified by DNA microarray analysis

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ABSTRACT

Objectives: Systemic juvenile idiopathic arthritis (sJIA) is a rheumatic disease in childhood characterised by systemic symptoms and a relatively poor prognosis. Peripheral leukocytes are thought to play a pathological role in sJIA although the exact cause of the disease is still obscure. In this study, we aimed to clarify cellular functional abnormalities in sJIA.

Methods: We analysed the gene expression profile in peripheral leukocytes from 51 patients with sJIA, 6 patients with polyarticular type JIA (polyJIA) and 8 healthy children utilising DNA microarrays. Gene ontology analysis and network analysis were performed on the genes differentially expressed in sJIA to clarify the cellular functional abnormalities.

Result: A total of 3491 genes were differentially expressed in patients with sJIA compared to healthy individuals. They were functionally categorised mainly into a defence response group and a metabolism group according to gene ontology, suggesting the possible abnormalities in these functions. In the defence response group, molecules predominantly constituting interferon (IFN)γ and tumour necrosis factor (TNF) network cascades were upregulated. In the metabolism group, oxidative phosphorylation-related genes were downregulated, suggesting a mitochondrial disorder. Expression of mitochondrial DNA-encoded genes including cytochrome *c* oxidase subunit 1(MT-CO1) and MT-CO2 were suppressed in patients with sJIA but not in patients with polyJIA or healthy children. However, nuclear DNA-encoded cytochrome *c* oxidases were intact.

Conclusion: Our findings suggest that sJIA is not only an immunological disease but also a metabolic disease involving mitochondria disorder.

Juvenile idiopathic arthritis (JIA) is one of the most common autoimmune diseases in childhood. It arises before 16 years of age and is accompanied by arthritis lasting more than 6 weeks. JIA is classified into seven subclasses; these include systemic JIA (sJIA) and articular type JIA, which also includes oligoarticlar and polyarticular type JIA (polyJIA).[1–3] While patients with articular type JIA show a localised inflammation of the joints, patients with sJIA are also characterised by systemic inflammatory symptoms such as spiking fever, skin rash, pericarditis and hepatospienomegaly. Growth disorders are frequently associated with the condition, leading to permanent disability in patients with sJIA. Moreover, about 10% of patients with sJIA progress to macrophage activation syndrome (MAS), which is often fatal.[4 5] Thus, sJIA may be a distinct disease from articular type JIA.[6]

In sJIA, cytokine regulation disorders have been suggested to be involved in the pathogenesis, although the exact causes of the disease is still obscure. Interleukin (IL)18 is increased in serum from patients with sJIA and correlates with clinical symptoms.[7] Aberrant induction of proinflammatory cytokines such as tumour necrosis factor (TNF), IL1 and IL6 is also observed in patients with sJIA[8–10] and therapies targeting these cytokines successfully control the disease activity.[9 11 12] These cytokines activate the immunocompetent cells, including lymphocytes and neutrophils in the affected joints as well as in peripheral blood.[13 14] Therefore, analysis of the functional abnormalities in peripheral leukocytes may be helpful for understanding the pathological condition of sJIA. Since the immune system is regulated by exquisite mechanisms in cell–cell communications and cytokine networks, such abnormalities may be reflected not in the expression of the small number of independent genes, but in the deviated expression of a substantial number of genes in some networks. Thus, an exhaustive analysis using DNA microarrays could be amenable to finding such deviated expressions in the multiple genes of such networks.

In this study, we investigated the functional abnormalities of peripheral blood cells using bioinformatics analysis on the genes detected by DNA microarrays differentially expressed in patients with sJIA compared to healthy individuals.

METHODS

Patients and healthy individuals

The analysis using patient peripheral blood in this study was approved by the Ethical Committee of Osaka University Medical School and all the attending institutes. Each patient's parent or legal guardian gave written informed consent. Younger patients with sufficient intellectual maturity to understand what was proposed signed and dated a separately designed written informed assent form in addition to the informed consent form signed by their parent or guardian. A total of 51 patients with sJIA and 6 patients with polyJIA fulfilled the

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Table 1 Clinical data for 51 patients with systemic juvenile idiopathic arthritis (sJIA) and 6 patients with polyarticular JIA (polyJIA)

	sJIA (n = 51)	polyJIA (n = 6)
Age, median (range) years	8 (2–19)	13.5 (6–19)
Female:male ratio	33:18	4:2
Duration of JIA, median (range) years	3.3 (0.4–16.2)	7.5 (0.7–17.2)
CRP, median (range) mg/dl	4.3 (1.6–19)	4.3 (1.1–8.2)
Erythrocyte sedimentation rate	46 (8–125)	43 (29–83)
Fever:		
<37.5	18 (35.3%)	6 (100%)
37.5 to 38	14 (27.5%)	0 (0%)
>38	19 (37.2%)	0 (0%)
White blood cells, median (range)	12 300 (4920–30 700)	9050 (5200–13 200)
Proportion of neutrophils, median (range)	70.2% (38.4–96)	65.0% (40.6–90.5)
Proportion of lymphocytes, median (range)	20.5% (3.2–52.5)	23.6% (6–49.3)
Proportion of monocytes, median (range)	5.3% (0.6–12)	7.1% (3–9.7)
Number of active joints, median (range)	4 (0–39)	21 (5–27)
Doctor global assessment of disease severity, median (range)	52 (18–100)	72.5 (52–90)
Patient or parent global assessment of over all well-being, median (range)	52 (18–90)	83 (76–100)
Score on Child Health Assessment Questionnaire, median (range)	0.88 (0–3)	1.94 (0.25–2.88)
Dose of corticosteroids, median (range) mg/kg	0.37 (0.03–1.84)	0.17 (0.12–0.18)
Dose of MTX, median (range) mg/week	7.5 (0–20)	6 (0–15)
Number of concomitant DMARDs, median (range)	2 (0–5)	1 (0–2)

Eligible patients were 2 to 19 years of age and fulfilled the ILAR classification for JIA. Active joints were defined as joints with swelling not due to deformity or in joints without swelling, limitation of motion plus pain and/or tenderness. Dose of corticosteroid is converted into prednisolone.
CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; ILAR, International League of Associations of Rheumatology; MTX, methotrexate.

diagnostic criteria of the International League of Associations of Rheumatology (ILAR).[1] For the healthy controls, we obtained blood samples at their regular health assessment from eight healthy children whose informed assent was obtained and whose parent gave written informed consent.

Sample proceeding and microarray hybridisation

Peripheral blood was collected directly into PAXGene tubes (Qiagen, Valencia, California, USA). Total RNA was extracted using the PAXgene Blood RNA Kit with the optional on-column DNase digestion. Amino allylRNA (aRNA) was synthesised from 1 μg of total RNA using an Amino Allyl MessageAmp aRNA kit (Ambion, Austin, Texas, USA). aRNA, 5 μg from each sample, and the equivalent quantity of reference aRNA from a mixture of RNA from peripheral blood of 45 healthy adults were subjected to Cy3 and Cy5 labelling, respectively. Both labelled aRNA were mixed in equal amounts and hybridised with an oligonucleotide-based DNA microarray, AceGene (HumanOligoChip30K, DNA Chip Research, Kanagawa, Japan), which contained about 30 000 human genes.

Data acquisition and analysis

The microarrays were scanned using ScanArray Lite (PerkinElmer, Boston, Massachusetts, USA) and signal values were calculated using DNASIS Array (Hitachi Software Engineering, Tokyo, Japan). The median and SD of background levels were calculated and the genes whose intensities were less than median plus 2SD of background levels were identified as null. The Cy3/Cy5 ratios of all spots on the DNA microarrays were normalised by the global ratio median normalisation method. Genes with at least 80% good data across each group of samples were selected for further analysis.

Gene ontology analysis

Genes identified to be differentially expressed by more than 0.2 base 2 logarithm (about 10%) according to the microarray analysis and a median signal intensity difference of at least 100 between each type of JIA and healthy group (to reduce errors pertaining to low-level expression close to noise level) were analysed for significant functional clusters of genes using Expression Analysis Systematic Explorer (EASE) V. 2.0 (http://david.niaid.nih.gov/david/ease.htm).

Network analysis

Network analyses were conducted using Ingenuity Pathways Analysis (IPA) V. 4.2 (Ingenuity Systems; http://www.ingenuity.com) to investigate biological interaction networks of the genes categorised as the over-represented "defence response group.

Quantification of the gene expression level by real-time PCR

To quantify the gene expression level in peripheral blood cells, we performed real-time quantitative PCR analysis using commercially available assays on demand probe primer sets (Applied Biosystems, Foster City, California, USA).

Statistical analysis

The unpaired Mann–Whitney U test was used to determine the statistical significant difference in the mRNA expression levels between the sJIA and healthy groups or between the polyJIA and healthy groups. The Pearson product-moment correlation coefficient was used to determine the index of correlation. The criterion for statistical significance was $p<0.05$.

Table 2 Deviated functional categories in overexpressed genes of systemic juvenile idiopathic arthritis (sJIA)

GO biological process	List (626 genes) Hits	Population (13 802 genes) Hits	EASE score	GO accession no.
Response to stimulus:				
Response to external stimulus	90	1539	8.69E-03	GO:0009605
Response to biotic stimulus	57	963	3.13E-02	GO:0009607
Defence response	52	887	4.58E-02	GO:0006952
Metabolism:				
Protein biosynthesis	43	650	1.28E-02	GO:0006412
Glycoprotein metabolism	12	132	3.72E-02	GO:0009100
Protein amino acid glycosylation	11	117	3.95E-02	GO:0006486
Protein complex assembly	11	110	2.74E-02	GO:0006461
Establishment of localisation:				
Transport	119	2049	2.82E-03	GO:0006810
Intracellular transport	38	613	4.55E-02	GO:0046907
Intracellular protein transport	30	438	2.62E-02	GO:0006886
Cell motility	24	342	3.81E-02	GO:0006928
Organisation physiological process:				
Excretion	7	40	8.69E-03	GO:0007588
Digestion	7	56	4.02E-02	GO:0007586
Cell organisation and biogenesis:				
Mitochondrion organisation and biogenesis	3	7	3.69E-02	GO:0007005

EASE, Expression Analysis Systematic Explorer software, V. 2.0; GO, Gene Ontology database.

Table 3 Deviated functional categories in underexpressed genes of systemic juvenile idiopathic arthritis (sJIA)

GO biological process	List (1641 genes) Hits	Population (13 802 genes) Hits	EASE score	GO accession no.
Immune system process:				
Antigen processing	9	32	3.02E-02	GO:0019882
Antigen processing, exogenous antigen via MHC class II	7	18	1.46E-02	GO:0019886
Metabolism:				
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	441	3429	2.61E-02	GO:0006139
Transcription from Pol II promoter	86	477	7.37E-05	GO:0006366
Regulation of transcription from Pol II promoter	48	254	1.28E-03	GO:0006357
RNA metabolism	80	460	4.33E-04	GO:0016070
RNA processing	74	430	9.78E-04	GO:0006396
RNA splicing	26	122	4.31E-03	GO:0008380
mRNA metabolism	30	172	3.24E-02	GO:0016071
mRNA processing	27	154	4.04E-02	GO:0006397
Phosphorylation	95	645	1.98E-02	GO:0016310
Oxidative phosphorylation	13	45	5.14E-03	GO:0006119
ATP synthesis coupled electron transport	11	36	7.56E-03	GO:0042773
Mitochondrial electron transport, NADH to ubiquinone	8	29	4.92E-02	GO:0006120
Regulation of translation	16	74	2.59E-02	GO:0006417
Regulation of translational initiation	10	34	1.53E-02	GO:0006446
Aromatic compound metabolism	24	121	1.49E-02	GO:0006725
Aromatic amino acid family metabolism	11	46	4.07E-02	GO:0009072
Aromatic amino acid family biosynthesis	7	23	4.75E-02	GO:0009073
Aromatic compound biosynthesis	9	33	3.58E-02	GO:0019438
Protein folding	25	134	2.57E-02	GO:0006457
Cell organisation and biogenesis:				
Organelle organisation and biogenesis	69	435	1.05E-02	GO:0006996
Cytoskeleton organisation and biogenesis	58	351	8.57E-03	GO:0007010
Cytoplasm organisation and biogenesis	77	519	3.05E-02	GO:0007028
Cell development:				
Neurogenesis	68	455	3.63E-02	GO:0022008

EASE, Expression Analysis Systematic Explorer software, V. 2.0; GO, Gene Ontology database; MHC, major histocompatability complex.

Table 4 Deviated functional categories in overexpressed genes of polyarticular JIA (polyJIA)

GO biological process	List (455 genes) Hits	Population (13 802 genes) Hits	EASE score	GO accession no.
Response to stimulus:				
Response to biotic stimulus	50	963	1.49E-03	GO:0009607
Defence response	46	887	2.46E-03	GO:0006952
Response to stress	40	872	3.12E-02	GO:0006950
Immune system process:				
Immune response	42	792	2.66E-03	GO:0006955
Humoral immune response	12	175	3.00E-02	GO:0006959
Metabolism:				
Biosynthesis	62	1199	3.89E-04	GO:0009058
Macromolecule biosynthesis	55	1002	2.15E-04	GO:0009059
Protein biosynthesis	42	650	4.85E-05	GO:0006412
ATP biosynthesis	7	29	3.06E-04	GO:0006754
Oxidative phosphorylation	6	45	1.56E-02	GO:0006119
ATP synthesis coupled electron transport	5	36	2.97E-02	GO:0042773
Coenzyme metabolism	10	103	6.81E-03	GO:0006732
NADPH metabolism	3	10	4.08E-02	GO:0006739
Carbohydrate catabolism	10	88	2.39E-03	GO:0016052
Glycosaminoglycan catabolism	3	5	1.01E-02	GO:0006027
Glucose catabolism	7	57	1.08E-02	GO:0006007
Ubiquitin-dependent protein catabolism	11	122	6.96E-03	GO:0006511
Establishment of localisation:				
Ion transport	32	637	1.91E-02	GO:0006811
Proton transport	11	70	9.09E-05	GO:0015992
Cellular physiological process:				
Regulation of cell proliferation	17	269	1.65E-02	GO:0042127
Regulation of biological process:				
Regulation of biological process	19	354	4.52E-02	GO:0050789

EASE, Expression Analysis Systematic Explorer software, V. 2.0; GO, Gene Ontology database.

RESULTS

Demographic and clinical characteristics of the patients

The demographic and clinical characteristics of patients with sJIA and patients with polyJIA analysed in this study are summarised in table 1.

Patients with sJIA who had been treated with corticosteroids (continued treatment for 3 months or longer at a dose of 0.2 mg/kg or more as prednisolone equivalent; 44 patients) but who failed to respond adequately or in whom treatment could not be continued or the dose could not be increased due to adverse reactions (7 patients) were enrolled in this study. All the patients had active disease in terms of C-reactive protein (CRP) of >1.5 mg/dl or erythrocyte sedimentation rate (ESR) of >30 mm/h in spite of the appropriate therapies as shown in table 1. The patients with polyJIA failed to respond adequately to the treatment with prednisolone at a dose of 0.2 mg/kg or less and/or disease-modifying antirheumatic drugs (DMARDs). They all had five or more active arthritis joints and CRP of >1.0 mg/dl or ESR of >30 mm/h despite the appropriate therapy. They had been treated with oral corticosteroids and DMARDs. Patients with sJIA received significantly higher doses of corticosteroids than patients with polyJIA ($p<0.05$). There

Table 5 Deviated functional categories in underexpressed genes of polyarticular JIA (polyJIA)

GO biological process	List (1016 genes) Hits	Population (13 802 genes) Hits	EASE score	GO accession no.
Metabolism:				
Transcription from Pol II promoter	47	477	3.51E-02	GO:0006366
Regulation of transcription from Pol II promoter	28	254	3.39E-02	GO:0006357
tRNA aminoacylation for protein translation	14	106	4.70E-02	GO:0006418
Cellular physiological process:				
Establishment and/or maintenance of cell polarity	5	13	1.21E-02	GO:0007163
Divalent and trivalent inorganic cation transport	15	106	2.31E-02	GO:0015674
Iron ion homeostasis	5	18	3.88E-02	GO:0006879
Organismal physiological process:				
Perception of sound	15	82	2.45E-03	GO:0007605
Response to stimulus:				
Response to DNA damage stimulus	25	229	4.99E-02	GO:0006974
Cell development:				
Axonogenesis	8	46	4.93E-02	GO:0007409

EASE, Expression Analysis Systematic Explorer software, V. 2.0; GO, Gene Ontology database.

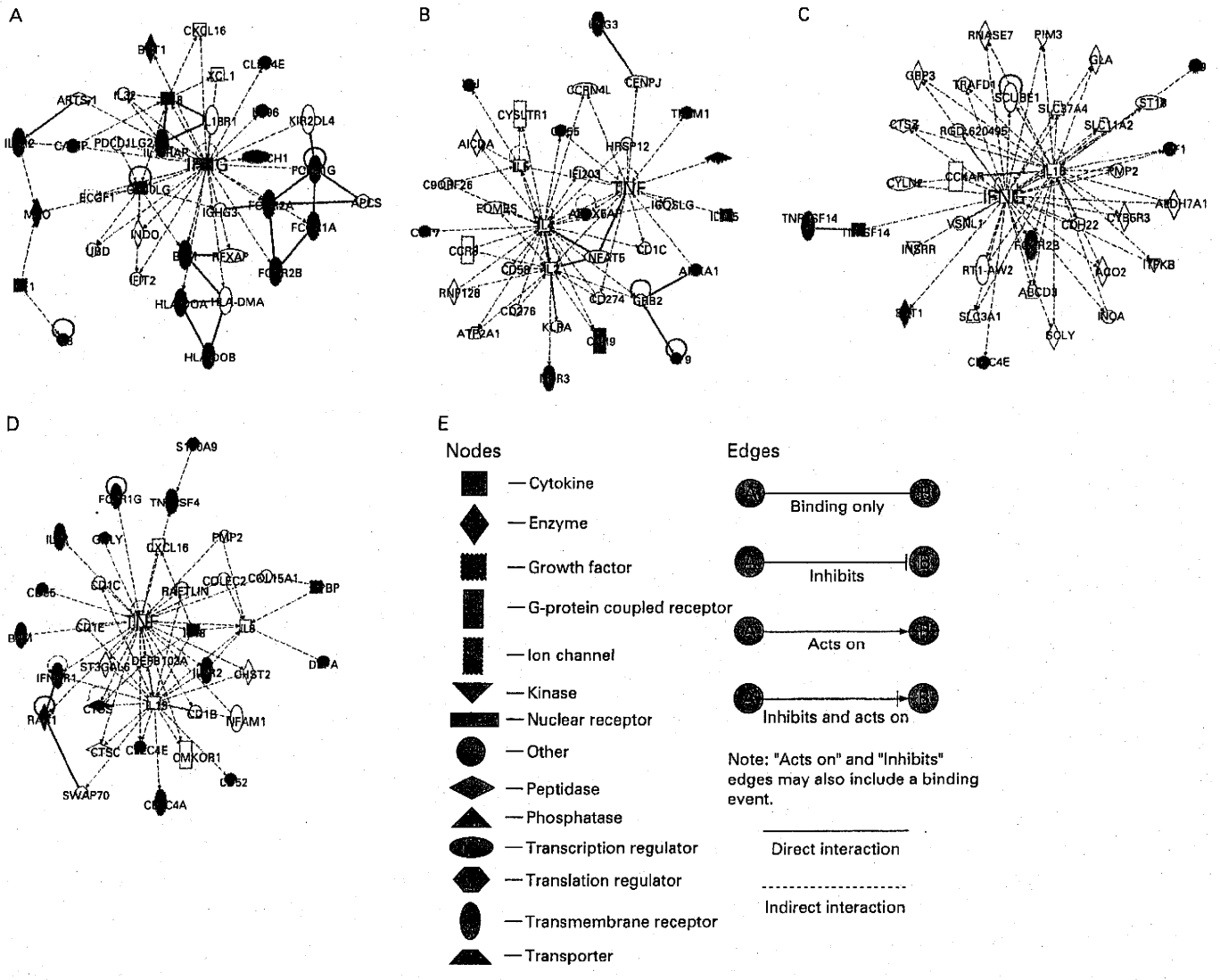

Figure 1 Network-based analysis on defence response genes. Ingenuity Pathways Network analysis, a web-delivered application (http://www.ingenuity.com), enables us to discover, visualise and explore biological interaction networks.[31] This analysis displays up to 35 genes in a single network. We chose networks containing more than 10 genes of that group as a meaningful network in systemic juvenile idiopathic arthritis (sJIA). For comparison, networks of polyarticular JIA (polyJIA) that included the same central molecule in sJIA were described. A,B. Defence response regulated by tumour necrosis factor (TNF) and interferon (IFN)γ in sJIA. C,D. Defence response regulated by TNF and IFNγ in polyJIA. E. explanation of the symbols. Genes are represented as individual nodes whose shapes represent the functional class of the gene products. Genes in coloured nodes were found in the over-represented category of defence response. Genes in uncoloured nodes were not found in the over-represented category of defence response and were depicted in the computationally generated networks on the basis of evidence stored in the Ingenuity Pathways Knowledge Base indicating a strong biological relevance to that network.

was no statistically significant difference in the methotrexate (MTX) doses between patients with sJIA and patients with polyJIA. The white blood cell counts and the proportion of neutrophils in patients with sJIA were higher than normal, but these were not significantly different between patients with sJIA and patients with polyJIA.

Gene ontology analysis on the genes differentially expressed in patients with JIA identified by DNA microarray analysis

DNA microarray analysis revealed that 3491 genes were differentially expressed in patients with sJIA compared to healthy children with statistical significance: 1273 out of 3491 genes were upregulated and the remaining 2218 genes were downregulated. Similarly, 2406 genes were differentially expressed in polyJIA: 691 genes out of 2406 genes were upregulated and the remaining 1715 genes were downregulated.

To identify the aberrant cellular functions in peripheral leukocytes from patients with sJIA and patients with pdgJIA, gene ontology analysis was performed on the genes differentially expressed in sJIA and pdgJIA using EASE.

The tables 2–5 list EASE results. EASE performs theme discovery, defined as the identification of functional categories that describes a statistically significant number of genes in a list with respect to the number of genes described by the functional categories in the population of genes from which the list is derived. We conducted EASE analysis using the Gene Ontology (GO) database (http://www.geneontology.org/GO.database.shtml) terms for the "biological process" category. An EASE score (Fisher exact test) represents the probability that an over-representation of certain functional category occurs by chance.[15] Categories with an EASE score of less than 0.05 are listed. A hierarchical relationship between biological processes was

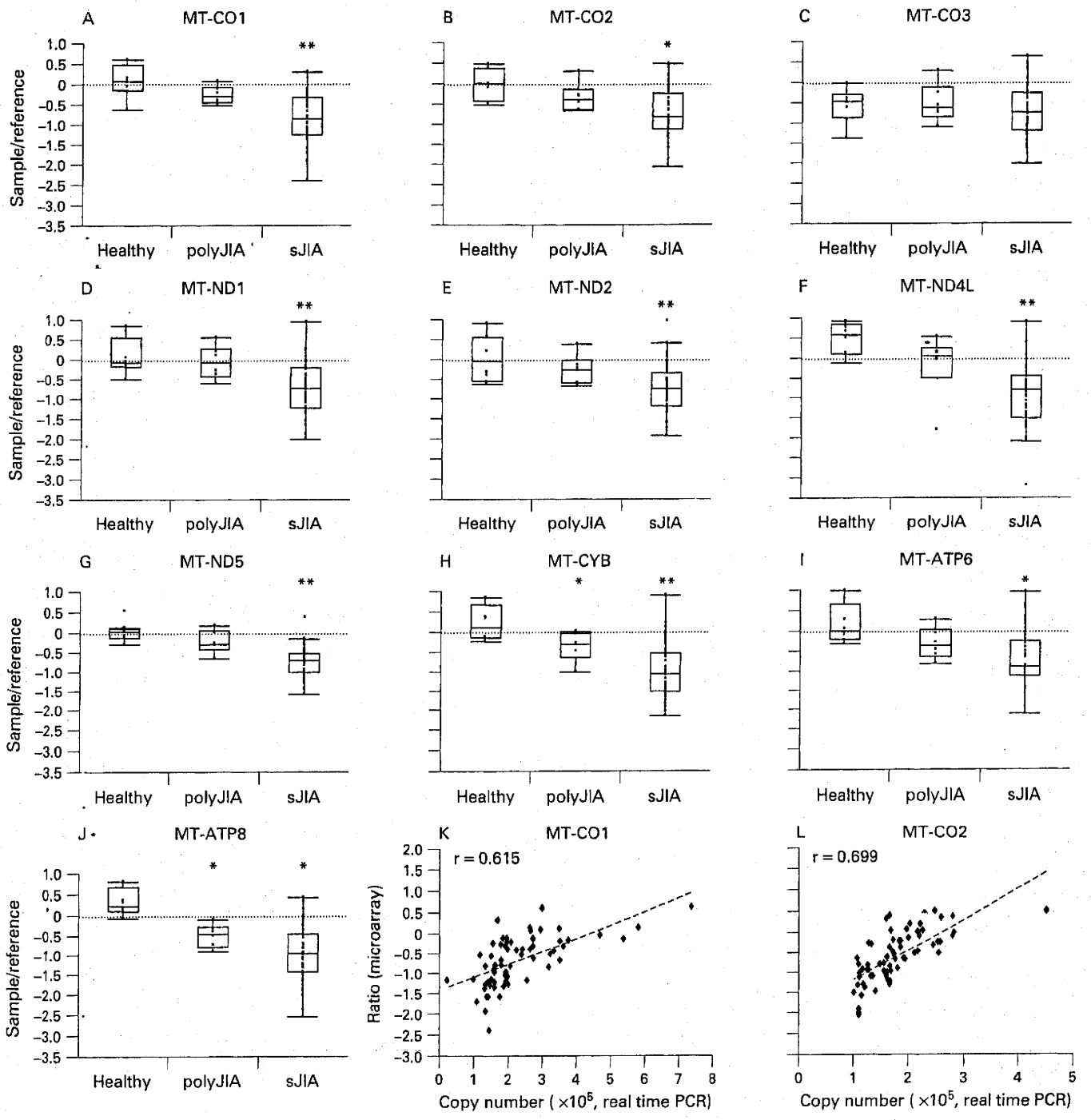

Figure 2 Decrease in the expression of mitochondrial DNA-encoded genes in systemic juvenile idiopathic arthritis (sJIA). A–J. Expression levels of mitochondrion DNA were represented. All data represents microarray data. All genes except for mitochondrially encoded cytochrome c oxidase subunit 3 (MT-CO3) were significantly downregulated in sJIA. Mitochondrially encoded cytochrome *b* (MT-CYB) and mitochondrially encoded ATP synthase 8 (MT-ATP8) were significantly downregulated in polyarticular JIA (polyJIA). * $p<0.05$ for sJIA vs healthy or polyJIA vs healthy (Mann–Whitney U test). ** $p<0.05$ for sJIA vs healthy and sJIA vs polyJIA. Boxes contain the 50% of values falling between the 25th and 75th percentiles, the horizontal line within the box represents the median value and the "whiskers" are the lines that extended from the box to the highest and lowest values, excluding outliers. K,L. Correlation between the mitochondrial gene expression data of microarray and quantitative PCR. For TaqMan PCR assay, cDNA synthesised from 16 ng of total RNA were used to measure the gene expression with a TaqMan Universal PCR Master Mix reagent, commercially available assays on demand probe primer sets (MT-CO1: Hs0259684_g1, MT-CO2: Hs02596865_g1) (Applied Biosystems) and 7500 Real Time PCR System (Applied Biosystems). For quantification standard of either MT-CO1 or MT-CO2, plasmid DNA including a PCR product of each gene was used. The vertical axis shows the expression levels of microarray, and the horizontal axis the copy number determined by quantitative PCR. Microarray data of MT-CO1 and MT-CO2 significantly correlated with the data of quantitative PCR. Index of correlation of MT-CO1 was 0.615, and of MT-CO2 was 0.699.

arranged by manual operation based on term lineage of GO. A category including too many genes was omitted. Number of genes refers to genes categorised in each GO differentially expressed in JIA. "List" refers to the total number of genes either upregulated or downregulated in each type of JIA that are annotated in the EASE system. "Population" reports all genes that are annotated in the EASE system. The total number of genes in "Population" was 13 802. "Hits" shows the number of genes in the list that belong to the respective gene category. Table 2 shows GO categories significantly over-represented in upregulated genes of sJIA. Among 1273 genes upregulated in sJIA, 626 genes were annotated in the EASE system because they included genes whose functions were unknown as well as the mRNA of those expected to be expressed according to the whole genome sequence. Table 3 shows GO categories significantly over-represented in downregulated genes of sJIA. Among 2218 genes downregulated in sJIA, 1641 genes were annotated in the EASE system. Table 4 shows GO categories significantly over-represented in upregulated genes of polyJIA. Among 691 genes upregulated in polyJIA, 455 genes were annotated in the EASE system. Table 5 shows GO categories significantly over-represented in downregulated genes of polyJIA. Among 1715 genes downregulated in polyJIA, 1016 genes were annotated. in the EASE system. Broad (high level) biological process included more specific (lower level) biological process.

The EASE analysis identified five major functional categories: response to stimulus, metabolism, establishment of localisation, physiological process and cell organisation and biogenesis in the upregulated genes in sJIA (table 2). In the category of response to stimulus, there were three subcategories with a statistically significant number of genes: response to external stimulus, response to biotic stimulus and defence response. These were independently categorised in gene ontology. A total of 52 genes in either the response to external stimulus or the response to biotic stimulus group also belonged to the defence response group. The remainder was categorised mainly in the response to abiotic stimulus group, which was not independently over-represented. Therefore, the functional category of defence response was predominantly over-represented in response to stimulus. Similar findings were also observed for polyJIA (Table 4).

In the category of metabolism, a subcategory of protein biosynthesis was over-represented in sJIA. Of 43 upregulated genes in the protein biosynthesis group, 20 genes were related to ribosomes (18 ribosomal proteins and 2 tRNA synthetases) and 11 genes were related to glycosylation. These genes were essential for various kinds of protein synthesis. Therefore, protein synthesis appeared to be upregulated in sJIA. The remaining genes were not classified into over-represented subcategories that might help to understand their functional abnormality. This category was also over-represented in polyJIA. Of the 42 upregulated genes in this category, 23 were related to ribosomes (21 ribosomal proteins and 2 tRNA synthetases) and 6 genes were related to glycosylation. The remaining genes in polyJIA were not classified into any over-represented subcategories, similar to sJIA.

In the category of cell organisation and biogenesis, a subcategory of mitochondrion organisation and biogenesis was over-represented. Although the population of this category of EASE consists of only seven genes, three of them were upregulated in sJIA. These three genes were SLC25A4, nuclear respiratory factor 1 (NRF1) and optic atrophy 1 (OPA1). SLC25A4 is induced at stress or damage in mitochondria. NRF1 and OPA1 are necessary for replication of the mitochondrial genome as well as synthesis of new mitochondrial components. This category was over-represented only in sJIA.

In addition, the categories of establishment of localisation and organisation-physiological process were also over-represented in sJIA, suggesting possible abnormalities in these functions.

The EASE analysis identified four major functional categories: immune system process, metabolism, cell organisation and biogenesis and cell development in the downregulated genes in sJIA (table 3). In the category of metabolism, the subcategories related to RNA metabolism, including a transcription from Pol II promoter and a RNA processing group, were over-represented, while the categories related to DNA metabolism were not over-represented (table 3). Most of the genes in the transcription from Pol II promoter group were transcription factors. Simultaneously, some genes related to transcription factors in this category were upregulated. Different expression patterns of transcription factors indicated the change of protein production, suggesting the activation of peripheral leukocytes.

The category of oxidative phosphorylation was also over-represented in sJIA. Genes categorised in oxidative phosphorylation were related to the phosphorylation of ADP to ATP that accompanies the oxidation of a metabolite through the operation of the respiratory chain, and were downregulated in sJIA (table 3). Of 13 genes downregulated in this category, 9 were encoded by mitochondrial DNA.

In polyJIA, the genes related to ATP synthesis were upregulated (table 4). These were all encoded by the nuclear genome and included NADH dehydrogenase (ubiquinone) 1β subcomplex (NDUFB) 1, NDUFB2, NDUFB3 and NDUFB6.

In addition, the categories of cell organisation and biogenesis and cell development were also identified to be over-represented in sJIA, suggesting possible abnormalities in these functions.

A network-based analysis of the genes in the defence response category

Network-based analysis was conducted of the molecules categorised in defence response to identify the relationship among these molecules and the centred molecules in the networks. Two networks were found to consist of 10 or more upregulated genes in the defence response group in sJIA. One was the network in which IFNγ was central (fig 1A). The other network was mainly attributed to TNF (fig 1B). Networks involving TNF and IFNγ were also identified in polyJIA (fig 1C,D) although the IFNγ network cascade consisted of only seven upregulated molecules in polyJIA (fig 1C). There were some differences in the molecules constituting the TNF and IFNγ networks between sJIA and polyJIA. It is noteworthy that IL18 was seen in both types of JIA (fig 1A,D). IL18 was contained in the sJIA IFNγ network and in the polyJIA TNF network.

Decrease in the expression of mitochondrial DNA-encoded genes in sJIA

In the category of oxidative phosphorylation, identified to be significantly over-represented in the downregulated genes in sJIA, most of the genes were encoded by mitochondrial DNA. Expression levels of these mitochondrial DNA-encoded genes are shown in fig 2. Except for mitochondrially encoded cytochrome *c* oxidase subunit 3 (MT-CO3), all of the genes showed a significant decrease in sJIA compared with healthy children. Furthermore, MT-CO1, mitochondrially encoded NADH dehydrogenase 1 (MT-ND1), MT-ND2, MT-ND4L,

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MT-ND5 and mitochondrially encoded cytochrome *b* (MT-CYB) were significantly downregulated in sJIA compared with those in polyJIA as well as healthy individuals. The expression levels of these genes were not influenced by the corticosteroid or MTX doses. In addition, no relation was observed between the gene expression and the proportion of either neutrophils or lymphocytes. Another mitochondrial DNA-encoded gene, MT-CO3 was downregulated but, statistically, was not significantly lower in sJIA compared to healthy children. MT-CO3 expression in healthy children was significantly lower than that of healthy adults. The expression levels of MT-CO1 and MT-CO2 determined by quantitative PCR were well correlated with those by microarray, thus verifying the data.

There was no significant decrease in the expression of cytochrome *c* oxidase (COX) subunits encoded by the nuclear genome in sJIA such as COX subunit IV isoform 1 (COX4I1), COX5A, COX5B, COX6A1, COX6B2, COX6C, COX7A1, COX7A2, COX7B, COX7C or COX8A. Therefore, the expression of COX subunits encoded by nuclear genome was intact.

DISCUSSION

Using DNA microarray technology, we analysed the gene expression profiles of peripheral blood to identify the molecules involved in the pathogenesis of sJIA. It was found that thousands of genes were differentially expressed in patients with sJIA. Such a considerably large number of affected genes reflect that various types of cell, including lymphocytes, neutrophils and macrophages, probably contribute to the pathogenesis. Since overproduction of proinflammatory cytokines such as TNF and IL6 is reportedly involved in the disease, these soluble factors may affect the peripheral leukocytes and alter the gene expression profiles in the cells. Indeed, the genes in the defence response category constituted TNF and IFNγ network cascades. IL18 was also identified to be involved in the network cascade in sJIA. This finding confirmed previous reports that IL18 plays a pathological role in sJIA.[7] Interestingly, upregulation of some molecules in the TNF and IFNγ cascades were also observed in polyJIA, where IL18 was also involved. Although it is not clear whether or not the pathogenic antigens are common between sJIA and polyJIA, TNF, IFNγ and IL18 all seem to play common pathological roles between the two types of JIA. According to network analysis, however, the IFNγ cascade was dominant in sJIA, while TNF cascade was dominant in polyJIA. This finding might explain the fact that anti-TNF therapy was very effective for patients with polyJIA but far less so in patients with sJIA.[16] Although TNF and IFNγ network cascades were depicted in both types of JIA, the surrounding molecules in the TNF and IFNγ network cascades were not always the same. Such a difference may cause the different clinical features between the two types of JIA and be useful for the differential diagnosis.

Like defence response abnormalities, metabolic abnormalities were also evident in sJIA. In the oxidative phosphorylation group, which was over-represented only in sJIA, most of the mitochondrial molecules encoded by mitochondrial DNA were suppressed while those encoded by genomic DNA were not. Corticosteroid treatment might affect the gene expression in this category because the corticosteroid doses in sJIA were higher than those in polyJIA. However, the corticosteroid dose did not correlated with the expression of mitochondrial genes. Overall, there were no over-represented categories correlated to any other drug therapies. In addition, the increase in the proportion of neutrophils and the decrease in the proportion of lymphocytes might affect the gene expression because the mRNA from whole blood cells was analysed in this study. However, there was no correlation between the gene expression and the proportion of either neutrophils or lymphocytes. Therefore, the decrease in the expression of various genes, including key genes such as MT-CO1 and MT-CO2, should be characteristic of sJIA. MT-CO1 and MT-CO2 are subunits of COX, which is the terminal component of the mitochondrial respiratory chain and transfers electrons from reduced cytochrome *c* to molecular oxygen. The mammalian COX is an enzymatic complex of inner mitochondrial membrane composed of 13 subunits. Three of them (MT-CO1, 2 and 3) are encoded by mitochondria DNA and the remaining 10 subunits (COX4, 5A, 5B, 6A, 6B, 6C, 7A, 7B, 7C and 8) are encoded by genomic DNA. MT-CO1 and MT-CO2 form the catalytic centre of the enzyme while MT-CO3 and 10 other subunits play structural and regulatory roles.[17] Thus, MT-CO1 and MT-CO2 are key subunits of COX. Indeed, mutations in either MT-CO1 or MT-CO2 have been reported to induce severe reduction of COX activity and mitochondrial damage.[18 19] Moreover, NRF1[20] and OPA1,[21] of which the expression is induced by the synthesis of new mitochondrial components, and SLC25A4,[22] of which expression is induced by mitochondrial damage, were upregulated only in sJIA. These findings strongly support the existence of mitochondrial damage in sJIA. Since TNF induces mitochondrial damage,[23] over-production of TNF may lead to the downregulation of mitochondrial DNA-encoded genes.

MAS is a life-threatening complication observed in sJIA but not in polyJIA. While the aetiology of MAS is unknown, it is thought to be partly caused by IFNγ released from activated T cells and proinflammatory cytokines, particularly TNF, from activated macrophages.[24] Our data clearly showed that IFNγ and TNF cascades were activated. Therefore, patients with active disease are likely to develop MAS. Mitochondrial damage may also contribute to the development of MAS because MAS is often successfully treated by ciclosporin A (CsA), which suppresses cytokine release from activated T cell and stabilises the mitochondrial membrane.[25] CsA is more effective for MAS than methylprednisolone, which has no efficacy in stabilising the mitochondrial membrane.[26] Therefore, stabilisation of the mitochondrial membrane may be important to control MAS. Because TNF induces mitochondrial damage through the instability of mitochondrial membrane,[23] inhibition of TNF may protect mitochondrial damage. Success in the treatment of MAS by TNF blocker supports this idea.[27 28] However, reports that MAS has been observed in some patients with sJIA during treatment with TNF blockers suggest that factors other than TNF may contribute to mitochondrial damage and consequently to MAS.[29 30] Further study will be required to know the exact mechanisms causing mitochondrial damage and MAS.

Our data indicates that sJIA is not only an immunological disease but also a metabolic disease involving mitochondrial disorder. This study also encouraged us to use bioinformatics tools together with microarray analysis to study autoimmune diseases of which the aetiology or the pathological conditions are not clear.

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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

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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

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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

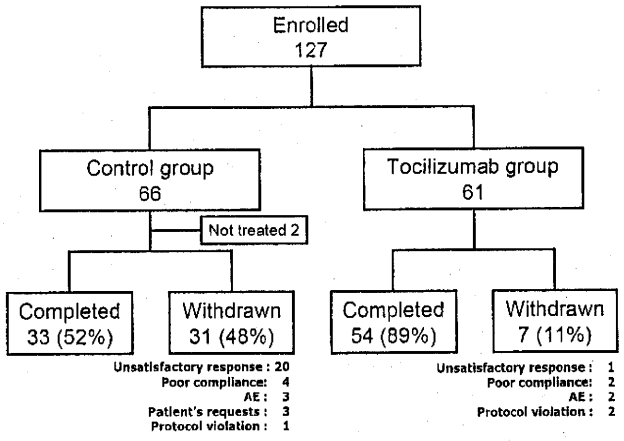

Fig. 1 Randomization, reasons for withdrawal, and numbers of patients who completed the trial. *Tocilizumab* humanized anti-interleukin-6 receptor antibody

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