Original Article

The combined effects of iguratimod with anti-TNFα antibody on experimental arthritis models in mice

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[Objectives] The combined effects of a new antirheumatic drug, iguratimod (IGU) with anti-tumor necrosis factor (TNF)α antibody (aTNFAb) were examined in two mouse models of arthritis.

[Methods] In collagen-induced arthritis (CIA) model, arthritis was induced by immunization with type II collagen on day 0 and 21. IGU was orally administrated once daily and aTNFAb was intraperitoneally injected twice a week from day 25 to day 34. In glucose-6-phosphate isomerase (GPI)-induced arthritis model, arthritis was induced by immunization with recombinant GPI on day 0 and the treatments were started on day 5. IGU was orally administrated daily and aTNFAb was intravenously injected every other day to day 14. Efficacy was evaluated using arthritis scores, articular destruction scores, and blood biochemical analyses.

[Results] In the CIA model, the combination of IGU and aTNFAb significantly inhibited the arthritis and articular destruction more effectively than as monotherapies. Serum interleukin (IL)-1β and IL-6 levels were also reduced significantly with combined treatment. The decline in serum levels of cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase (MMP)-3 was significantly enhanced with the IGU/aTNFAb combination. In the GPI-induced arthritis model, IGU and aTNFAb lowered the arthritis scores and the combination of both treatments showed a significant effect compared with IGU alone. Regarding anti-GPI titers on day 14, IGU and/or aTNFAb showed no significant effect.

[Conclusion] In the two mouse models, the combination of IGU and aTNFAb showed greater benefit than either monotherapy, suggesting such combination therapy would be effective in clinical use.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammation of the joints and concomitant destruction of cartilage and bone. The disease is thought to be caused by an autoimmune process, thus anti-inflammatory and immunomodulating drugs have been used as therapeutic approaches. Recently, thanks to the development of therapeutic control strategies with disease-modifying anti-rheumatic drugs (DMARDs) and a new class of biologic agents, such as anti-tumor necrosis factor (TNF)-α antibody, RA patients can expect rapidly relieve their symptoms, and improve their quality of life. Furthermore, such strict therapeutic control strategies have been recommended to sustain long periods of RA remission[1]. Remission is not always possible with monotherapy, therefore the majority of RA patients are given multiple drugs. Combined therapy of anti TNF-α antibody with methotrexate (MTX), a widely used small compound, has been suggested to improve the pathology of RA[2].

Iguratimod (*N*-[3-formylamino-4-oxo-6-phenoxy-4*H*-chromen-7-yl]methanesulfonamide, IGU)[3], a small molecule with immunomodulatory and anti-inflammatory properties, has been clinically used as a novel DMARD in Japan. Using animal models of arthritis or autoimmune disease, we have found that IGU exhibits anti-inflammatory effects and improves abnormal immunological findings[4, 5]. In Japanese RA clinical trials[6], IGU has good efficacy based on the American College of Rheumatology 20% improvement criteria (ACR20 response)[7]. Moreover, patients with insufficient response to methotrexate (MTX) monotherapy exhibited a significant improvement with IGU and MTX combined therapy[8]. However, the efficacy of the combined therapy of IGU with biologic agents has not been reported, even in animal arthritis models. In the present study, we investigated the combined administration of IGU with anti-TNFα antibodies in two arthritis mouse models; collagen-induced arthritis (CIA) and glucose-6-phosphate isomerase (GPI)-induced arthritis.

Materials and methods

1) Materials

Iguratimod (IGU) was synthesized in Toyama Chemical Co., Ltd. (Tokyo, Japan). Anti-mouse TNFα monoclonal antibodies (aTNFAb) as therapeutic agent to CIA and GPI-induced arthritis were purchased from R&D Systems (Minneapolis, MN, USA) and eBioscience Inc. (San Diego, CA, USA), respectively. As control antibodies, hamster IgG or rat IgG were obtained from MP Biomedicals (Solon, OH, USA) and Jackson immunoresearch Laboratories, Inc. (West Grove, PA, USA). Bovine type II collagen was purchased from Koken (Tokyo, Japan). Recombinant GPI was prepared using a plasmid as reported previously[9]. Mouse interleukin (IL)-1β, IL-6, matrix metalloproteinase (MMP)-3 ELISA kits were purchased from R&D Systems. Cartilage oligomeric matrix protein (COMP) ELISA kit and serum amyloid A (SAA) ELISA kit were purchased from Kamiya Biomedical (Seattle, WA, USA) and Biosource International (Camarillo, CA, USA), respectively.

2) Animals

Male DBA/1J mice were purchased from Charles River Japan (Kanagawa, Japan). The animals were housed in an air-conditioned room settled at 23 ± 3℃ and 50 ± 20% of relative humidity, under a 12 h light-dark cycle, and fed with a commercial diet (CRF-1, Oriental Yeast; Tokyo, Japan, or Lab MR Stock®; Nosan Co., Yokohama, Japan) and received tap water *ad libitum*. The animal studies were performed in accordance with the guides for care and use of laboratory animals at Toyama Chemical Co., Ltd. and Eisai Co., Ltd.

3) nduction of models

In the CIA model, arthritis was induced by the immunization of type II collagen in male DBA/1J mice (aged 8 weeks) as described previously[4]. Briefly, the mice were immunized two times by intradermal injection with an emulsion consisting of equal volumes of Freund's complete adjuvant (FCA) (BD Diagnostic Systems, Sparks, MD, USA) and 200 μg of bovine type II collagen on day 0 and 21.

In the GPI-induced arthritis model, arthritis was induced using recombinant human GPI in male DBA/1J mice (aged 6 weeks)[10]. Mice were immunized by intradermal injection with an emulsion consisting of equal volumes of FCA (Difco, Detroit, MI, USA) and 300 μg of recombinant human GPI.

4) Treatment of CIA model

IGU (30 mg/kg/day) or vehicle was orally administered from day 25 to day 34. aTNFAb (50 μg/mouse) or hamster IgG (50 μg/mouse, as an alternative to aTNFAb) was intraperitoneally injected twice a week (day 25, day 29 and day 32). Mice were evaluated daily for incidence and severity of swelling in their paws on days 21 through 34. The clinical score was determined as follows: 0, noninvolved;

1, swelling of one or two toes, or slight swelling of the ankle; 2, swelling of one or two toes accompanied by slight swelling of the ankle, or moderate swelling of the ankle; 3, extensive swelling of the paws. The maximum score for a mouse was 12 (point 3 for each paw). The serum samples were obtained on day 35 for the measurement of IL-1β, IL-6, COMP, and MMP-3. Each protein amount was determined using ELISA kits according to the instructions provided by the reagent suppliers.

The joint observation study was performed as previously described[4]. Briefly, the paw was cut off at *ca*. 8 mm above the wrist and ankle joint and subjected to radiographic analysis using an X-ray apparatus (SOFTEX — cmbw, Softex, Tokyo) on the exposure condition of the paw samples placed on X-ray film (Fuji FR, 12 x 16.5 cm) with 36 kV and 7-8 mA for 8 sec at the distance of 64 cm. The films were inspected for the 42 joints per mouse, which are connected with the following bones: forepaw bones of carpus, five metacarpals, and four proximal phalanges (2nd to 5th); hindpaw bones of calcaneus, tarsal, five metacarpals, and four proximal phalanges (2nd to 5th). Articular lesion was scored as following; 0, negative; 0.5, positive for osteoporosis, and 0, no change; 1, partial erosion; 2, complete erosion for bone erosions. The counting of affected joints (maximum score of 105 per mouse) was performed by two persons not aware of the experimental procedures.

5) Treatment of GPI-induced arthritis model

IGU (50 mg/kg/day) or vehicle was orally administered from day 5 to day 14. aTNFAb (10,100 μg/mouse) or rat IgG (50 μg/mouse, as an alternative to aTNFAb) was intravenously injected every other day from day 5. Arthritis was evaluated by scoring the disease state (clinical index): 0 = no evidence of inflammation; 1 = subtle inflammation or localized edema; 2 = easily identified swelling but localized to either dorsal or ventral surface of paws; 3 = swelling on all aspects of paws. All four limbs were observed and graded, yielding a maximum score of 12 per mouse. The plasma samples for measuring SAA and anti-GPI titer were obtained on day 14.

SAA was measured by an ELISA kit according to manufacture-derived protocol. Anti-GPI antibody titer was measured by a following ELISA system; 96-well plates were coated with the solution of GPI (2 μg/ml) and incubated overnight at 4℃. After washing, diluted samples were added, and incubated 1 h at room temperature. Then, anti mouse IgG-peroxidase (ICN Biomedicals Inc., Costa Mesa, CA, USA) was added (diluted at 1:1000) and incubated for 1 h at room temperature. To detect the signals, 3,3',5,5'-tetramethylbenzidine (TMB) solution was added and read 450 nm absorbance by a plate reader (SPECTRA MAX 250, Molecular Devices Co., Sunnyvale, CA, USA).

6) Statistical analysis

All results are expressed as the means with S.E.M., and the statistical differences between two groups were made by Student's *t*-test, Aspin-Welch test or Mann-Whitney *U*-test. The multiple comparisons were assessed by non-parametric or parametric Dunnett test. Data were analyzed with statistical programs (SAS release 8.2 or 9.2; SAS Institute Japan Ltd., Tokyo, Japan and Stat Preclinica 1.2; Takumi Information Technology, Tokyo, Japan). In all tests, $p<0.05$ (two-tails) was considered to be significant.

Results

1) Combined effect on arthritis in CIA model

The changes in arthritis score of each group were shown in Fig.1A. Before grouping (day 25), 56.7% of mice evoked arthritis in their paws and the grouping was done so that the incidence in each group became in similar values of 50 to 60%. At a day of final observation (day 35), average score of vehicle-treated control group was 9.0. IGU at 30 mg/kg/day or aTNFAb at 50 μg/mouse decreased the arthritis score compared with the control group. Combined treatment of IGU with aTNFAb enhanced the reduction of arthritis score and showed a significant inhibition compared with the control group. The score of articular destruction including osteoporosis and bone erosion at day 35 was presented in Fig.1B. Treatment with IGU or aTNFAb showed 46% or 35% decrease in the score respectively, but the effects were not significant from control. Combination of IGU with aTNFAb significantly reduced the score compared with control.

2) The effects on serum IL-1β and IL-6 levels in CIA model

Fig.2 showed serum IL-1β and IL-6 levels at day 35. The IL-1β level of control group was 10 times higher than that of normal group. IL-6 levels were undetectable (<3.9 pg/ml) in 7 of 8 mice of normal group, but those in control group were prominently increased with onset of CIA. Although IGU lowered the IL-1β and IL-6 levels, no significant effects were observed. aTNFAb decreased IL-1β level

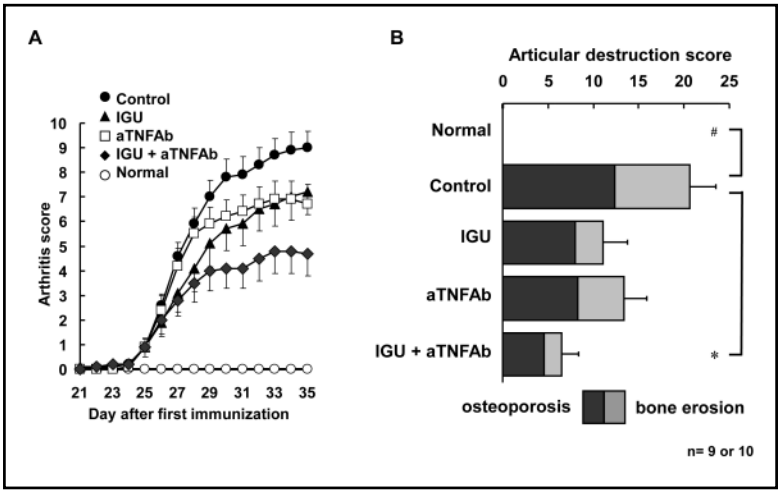

Fig.1 Effects of IGU in combination with aTNFAb on the development of arthritis and articular destruction in mice with CIA

(A) Mice were immunized with type II collagen twice (day 0 and 21). Severity of arthritis was graded on a scale of 0 to 3 for each paw as described in Materials and methods, and arthritis score was expressed as a sum of scores of four paws. The data represent the mean with S.E.M. (n = 9 or 10).

(B) The evaluation of articular destruction in paws on day 35 was performed as described in Materials and methods. Articular destruction score is shown as a combination of osteoporosis and erosion score. The data represent the mean with S.E.M. of the score (n = 9 or 10). #p<0.05, significant difference from Control by Mann-Whitney U-test. * p<0.05, significant difference from Control by non-parametric Dunnett test.

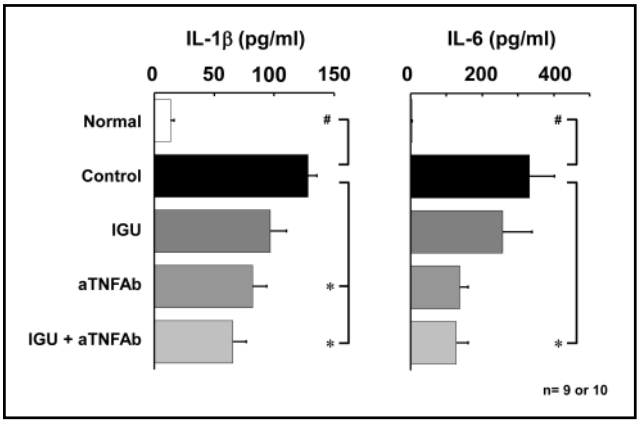

Fig.2 Effects of IGU in combination with aTNFAb on serum IL-1β and IL-6 levels in mice with CIA

The serum IL-1β and IL-6 level on day 35 was determined using mouse IL-1β and IL-6 ELISA kits, respectively. The data represent the mean with S.E.M. (n = 9 or 10). #p<0.05, significant difference from Control by Aspin-Welch test. *p<0.05, significant difference from Control by parametric Dunnett test.

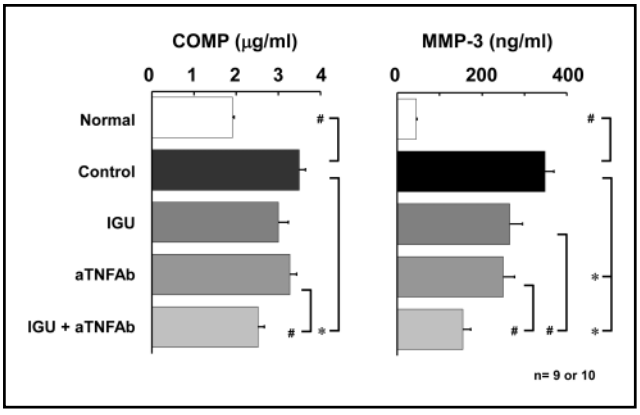

Fig.3 Effects of IGU in combination with aTNFAb on serum COMP and MMP-3 levels in mice with CIA

The serum COMP and MMP-3 level on day 35 was determined using mouse COMP and MMP-3 ELISA kits, respectively. The data represent the mean with S.E.M. (n = 9 or 10). #p<0.05, significant difference by Aspin-Welch test. *p<0.05, significant difference from Control by parametric Dunnett test.

significantly versus control but not IL-6 level (p=0.061). Combined treatment of IGU with aTNFAb augmented the decline of IL-1β and IL-6 levels.

3) The effects on serum COMP and MMP-3 levels in CIA model

As shown in Fig.3, serum COMP level of control group was doubled compared to normal group and IGU or aTNFAb had no significant effect on the level. Significant reduction of the level was observed by combined treat-

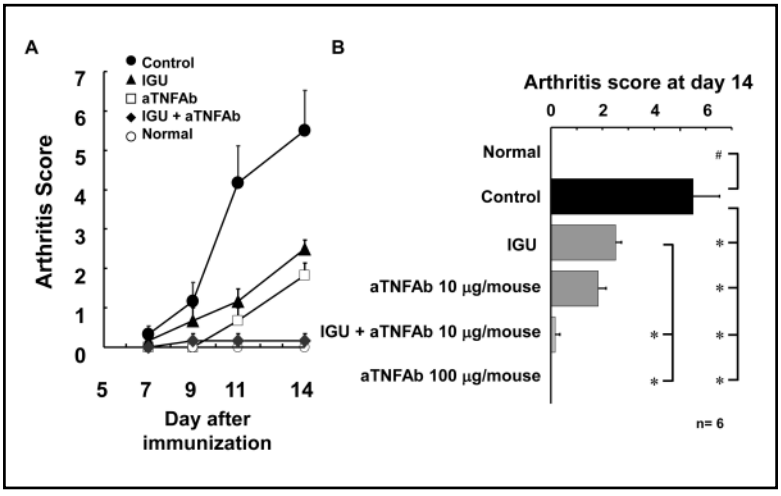

Fig.4 Effects of IGU in combination with aTNFAb on the development of arthritis in mice with GPI-induced arthritis

Mice were immunized with GPI on day 0 and the paws were observed every two or three days. Severity of arthritis was graded on a scale of 0 to 3 for each paw as described in Materials and methods, and arthritis score was expressed as a sum of scores of four paws. The data represent the mean with S.E.M. (n = 6). #p<0.05, significant difference from Control by Student's t-test. *p<0.05, significant difference from Control by parametric Dunnett test.

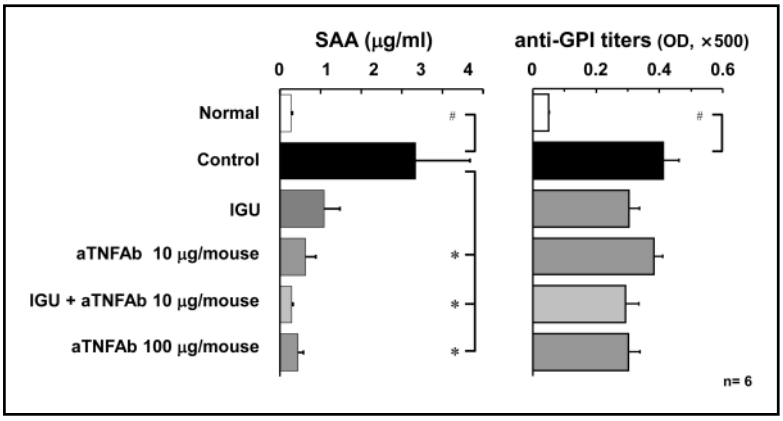

Fig.5 Effects of IGU in combination with aTNFAb on SAA level and anti-GPI titers in mice with GPI-induced arthritis

(A) SAA level on day 14 was determined using mouse SAA ELISA kit. The data represent the mean with S.E.M. (n = 6). #p<0.05, significant difference from Control by Student's t-test. *p<0.05, significant difference from Control by parametric Dunnett test.

(B) The anti-GPI titers was determined using GPI ELISA, detailed in Material and Methods. #p<0.05, significant difference from Control by Student's t-test

ment of IGU with aTNFAb in comparison with control. The serum MMP-3 level in control group was eight times as high as that in normal group. There was no significant effect between IGU and control in MMP-3 level, whereas aTNFAb significantly declined the level versus control. Moreover, IGU in combination with aTNFAb reduced the MMP-3 level significantly compared with all other arthritis groups.

4) Combined effect on arthritis in GPI-induced arthritis model

Time courses of arthritis score in each group were shown in Fig.4A and the scores on day 14 were in Fig.4B. In vehicle-treated control group, the arthritis score was increased from day 7 to day 14. IGU at 50 mg/kg/day or aTNFAb at 10 μg/mouse significantly lowered the arthritis in their paws. There was significant reduction with combined treatment of IGU with aTNFAb in the arthritis score compared with control and monotherapy of IGU at day 14. aTNFAb at 100 μg/mouse was almost completely inhibited the arthritis score.

5) The effects on SAA level and anti-GPI titers in GPI-induced arthritis model

The SAA level and anti-GPI titers at day 14 were presented in Fig.5. Treatment with aTNFAb diminished the SAA level significantly compared with control. Combination of IGU with low dose of aTNFAb also decreased the SAA level significantly, which was nearly equal to that in the monotherapy of a high dose aTNFAb. On the other hand, all treatments including combination of IGU with aTNFAb did not show any significant effects on the anti-GPI titers.

Discussion

We investigated the efficacy of combined treatment of

IGU with aTNFAb on two arthritis models in mice. One is CIA model that has been generally utilized for pharmacological evaluation of anti-inflammatory/anti-rheumatic drugs[11] and another is GPI-induced arthritis model in which the therapeutic effects of biologics have been known to be similar to those in patients with RA[10]. In our previous study using the CIA model, administration of IGU was started on the day of first immunization (day 0) and IGU at 30 mg/kg/day significantly suppressed the incidence and severity of paw swelling on day 34[12]. Furthermore, we have found that treatment with IGU at 30 mg/kg/day improved in the arthritis score by 38% when administration was started on day 21[4]. In order to examine the therapeutic efficacy, IGU was administrated from day 25 when the onset of CIA was observed in more than half of mice. Administration of IGU at the same dose resulted in approximately 20% inhibition, commensurating with our previous study results[4].

On the other hand, the efficacy of aTNFAb on CIA model arthritis score has been known to be weak in comparison with anti-IL-1 α/β antibodies, which showed an almost complete inhibition[13-15]. Our present study is consistent with the result of Williams et al[16]. also showing that aTNFAb partially reduced both the arthritis and articular destruction scores.

Combination of IGU with aTNFAb yielded a significant decrease in arthritis scores, serum levels of inflammation cytokines (IL-1β and IL-6), and MMP-3 in the CIA model. These findings revealed an enhanced effect of IGU and aTNFAb on the inflammatory responses. Moreover, X-ray joint destruction scores were also reduced by combined treatment in the CIA model, and linked to an apparent effect on the cartilage disruption marker, COMP. In this way, add-on treatment of IGU with aTNFAb showed good efficacy in the CIA model, where aTNFAb monotherapy is known to be less effective.

Next, for examining the combined effects of IGU and aTNFAb, the GPI-induced arthritis model was selected since the B cell activation in response to GPI has been reported to be involved in the pathogenesis[17]. In agreement with the previous report[10], present study showed that aTNFAb monotherapy markedly alleviated the arthritis in this model, IGU (50 mg/kg/day) and aTNFAb achieved comparable reduction in arthritis scores. Moreover, combined administration of IGU with aTNFAb almost completely inhibited the onset of arthritis. The SAA levels, a serum marker of systemic inflammation, supported the beneficial effect on arthritis score.

A remarkable high level of anti-GPI titer was observed in the control group on day 14. However, IGU and/or aTNFAb did not significantly alter the titers. Schubert et al. had reported that anti-GPI titers were not indicative of the arthritis-susceptibility of the mouse strain, and there was no induction of arthritis when the purified anti-GPI IgG of arthritic mice was transferred to other arthritis-susceptible recipient mice[18]. These results suggested that anti-GPI antibody is not sufficient for the pathogenesis of GPI-induced arthritis. The above-cited experiment had shown that treatment with various antibodies, including aTNFAb, lowered the arthritis score but only tended to suppress the production of anti-GPI antibodies[10]. Our present study with aTNFAb monotherapy demonstrated similar effects regarding the titers.

We had reported that inhibition of immunoglobulin production by IGU was one of its pharmacological features[19]. Our previous study showed that anti-type II collagen antibody levels were decreased by administration of IGU at 100 mg/kg/day from day 0 in the CIA model, but not at 30 mg/kg/day[20]. Consequently, a significant reduction in anti-GPI titer level was expected with IGU at 50 mg/kg/day, but such inhibition could not be observed in total immunoglobulin level to GPI. Class-specific antibody measurement of anti-GPI titers may be required to detect any clear effects of IGU on the titers.

In conclusion, IGU in combination with aTNFAb effectively suppressed arthritic paw lesions in the CIA and GPI-induced arthritis models. Articular destruction was also inhibited by the combined treatment in the CIA model. Many known factors are involved in human RA pathology, therefore, combined therapy of multiple drugs often brings robust effects in RA. In our current study, the complementary mechanisms of IGU and aTNFAb including immunoglobulin production suppression and inflammatory cytokine reduction yielded additional efficacy versus monotherapy alone.

Source of funding

None

Conflict of interests

K.T., J.S. and M.M. are employees of Toyama Chemical Co. Ltd. Y.I. and K.M. are employees of Eisai Co. Ltd. T.S. and I.M. were not compensated for their work on this manuscript.

References

1) Smolen JS, Landewé R, Breedveld FC, Dougados M, Emery P, Gaujoux-Viala C, et al: EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis. 2010; 69: 964-975.
2) Baranauskaite A, Raffayová H, Kungurov NV, Kubanova A, Venalis A, Helmle L, Srinivasan S, Nasonov E, Vastesaeger N, et al: Infliximab plus methotrexate is superior to methotrexate alone in the treatment of psoriatic arthritis in methotrexate-naive patients: the RESPOND study. Ann Rheum Dis. 2012; 71: 541-548
3) Tanaka K: Iguratimod (T-614): A novel disease modifying anti-rheumatic drug. Rheumatol. Reports. 2009; 1: 11-15.
4) Inaba T, Tanaka K, Takeno R, Nagaki H, Yoshida C, Takano S: Synthesis and antiinflammatory activity of 7-methanesulfonylamino-6-phenoxychromones. Antiarthritic effect of the 3-formylamino compound (T-614) in chronic inflammatory disease models. Chem Pharm Bull (Tokyo). 2000; 48: 131-139.
5) Aikawa Y, Tanuma N, Shin T, Makino S, Tanaka K, Matsumoto Y: A new anti-rheumatic drug, T-614, effectively suppresses the development of autoimmune encephalomyelitis. J Neuroimmunol. 1998; 89: 35-42.
6) Hara M, Abe T, Sugawara S, Mizushima Y, Hoshi K, Irimajiri S, et al: Efficacy and safety of iguratimod compared with placebo and salazosulfapyridine in active rheumatoid arthritis: a controlled, multicenter, double-blind, parallel-group study. Mod Rheumatol. 2007; 17: 1-9.
7) Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al: American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum. 1995; 38: 727-735.
8) Ishiguro N, Yamamoto K, Katayama K, Kondo M, Sumida T, Mimori T, Soen S, Nagai K, Yamaguchi T, Hara M, et al: Concomitant iguratimod therapy in patients with active rheumatoid arthritis despite stable doses of methotrexate: a randomized, double-blind, placebo-controlled trial. Mod Rheumatol. 2013; 23 :430-439.
9) Matsumoto I, Lee DM, Goldbach-Mansky R, Sumida T, Hitchon CA, Schur PH, Anderson RJ, Coblyn JS, Weinblatt ME, Brenner M, Duclos B, Pasquali JL, El-Gabalawy H, Mathis D, Benoist C: Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. Arthritis Rheum. 2003; 48: 944-954.
10) Matsumoto I, Zhang H, Yasukochi T, Iwanami K, Tanaka Y, Inoue A, Goto D, Ito S, Tsutsumi A, Sumida T: Therapeutic effects of antibodies to tumor necrosis factor-alpha, interleukin-6 and cytotoxic T-lymphocyte antigen 4 immunoglobulin in mice with glucose-6-phosphate isomerase induced arthritis. Arthritis Res Ther. 2008; 10: R66
11) Wooley PH: Collagen-induced arthritis in the mouse. Methods Enzymol. 1988; 162: 361-373.
12) Tanaka K, Shimotori T, Taniguchi Y, Eguchi M, Abe C: Pharmacological studies on T-614, a novel anti-inflammatory agent: effect on typeII collagen-induced arthritis in DBA/1J mice and spontaneous arthritis in MRL/l mice. Int J Immunotherapy. 1993; 9: 69-78.
13) Joosten LA, Helsen MM, van de Loo FA, van den Berg WB: Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice. A comparative study using anti-TNF alpha, anti-IL-1 alpha/beta, and IL-1Ra. Arthritis Rheum. 1996; 39: 797-809.
14) Joosten LA, Helsen MM, Saxne T, van De Loo FA, Heinegard D, van Den Berg WB: IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation. J Immunol. 1999; 163: 5049-5055.
15) Williams RO, Marinova-Mutafchieva L, Feldmann M, Maini RN: Evaluation of TNF-alpha and IL-1 blockade in collagen-induced arthritis and comparison with combined anti-TNF-alpha/anti-CD4 therapy. J Immunol. 2000; 165: 7240-7245.
16) Williams RO, Feldmann M, Maini RN: Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. Proc Natl Acad Sci U S A. 1992; 89: 9784-9788.
17) Bockermann R, Schubert D, Kamradt T, Holmdahl R: Induction of a B-cell-dependent chronic arthritis with glucose-6-phosphate isomerase. Arthritis Res Ther. 2005; 7: R1316-R1324
18) Schubert D, Maier B, Morawietz L, Krenn V, Kamradt T: Immunization with glucose-6-phosphate isomerase induces T cell-dependent peripheral polyarthritis in genetically unaltered mice. J Immunol. 2004; 172:

4503-4509.
19) Tanaka K, Yamamoto T, Aikawa Y, Kizawa K, Muramoto K, Matsuno H, Muraguchi A: Inhibitory effects of an anti-rheumatic agent T-614 on immunoglobulin production by cultured B cells and rheumatoid synovial tissues engrafted into SCID mice. Rheumatology (Oxford). 2003; 42: 1365-1371.
20) Urata N, Yamamoto M, Taniguchi Y, Aikawa Y, Funaki J, Tanaka K: Effect of a disease-modifing antirheumatic drug iguratimod (T-614) on chronic arthritis in experimental animals. Jpn Pharmacdol Ther. 2007; 35: 571-582.